

Mycoplasma bovis Infections in Young Calves

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KEYWORDS

- *Mycoplasma bovis* • Calves • Respiratory disease
- Pneumonia • Otitis media

Mycoplasma bovis was first isolated from a case of severe mastitis in a US dairy cow in 1961, almost half a century ago.¹ It is now recognized as a worldwide pathogen of intensively farmed cattle and in recent years has emerged as an important cause of disease in young dairy and veal calves in North America and Europe. Pneumonia, otitis media, and arthritis are common manifestations of *M bovis* infection in young calves, and have been collectively termed *Mycoplasma bovis*-associated disease (*MbAD*). *Mycoplasma bovis* also continues to be an important cause of mastitis in adult cows²⁻⁵ and respiratory disease and arthritis in stocker and feeder cattle.⁶⁻¹⁰ Readers are referred to recent reviews of mycoplasmal mastitis^{5,11} and *MbAD* in feedlot cattle^{10,12} in North America; this article will focus on the clinical aspects of *M bovis* infections in young calves.

ETIOLOGY AND PATHOPHYSIOLOGY

Mycoplasma bovis belongs to the class Mollicutes (from the Latin *mollis*, soft; *cutis*, skin), a group of bacteria so named because they lack cell walls and are instead enveloped by a complex plasma membrane. They are also characterized by their tiny physical size and correspondingly tiny genomes (0.58 to 2.2 megabases).¹³ Perhaps as a direct consequence of the limited biosynthetic capacity of their small genome, mycoplasmas usually form an intimate association with host cells to obtain the growth and nutritional factors necessary for their survival.¹⁴ Mycoplasmas typically inhabit mucosal surfaces, including those of the respiratory, urogenital, and gastrointestinal tracts; the eyes; and the mammary glands.¹⁴ Their individual relationship with the host varies from primary or opportunistic pathogens to commensals.

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Interactions between mycoplasmal pathogens and their hosts are much more complex than might be expected from their small genome and structural simplicity. Mycoplasmas can induce a broad range of immunomodulatory events by direct effects on macrophages, neutrophils, and lymphocytes, and by indirect effects through induction of cytokine secretion from these and other cells (eg, epithelial cells).¹⁴ *Mycoplasma bovis* is no exception, and this pathogen is very effective at evading and modulating the host immune response, and the immune response contributes to the pathogenesis of *MbAD*.^{15–19} The complicated relationship between mycoplasmas and their hosts means that many aspects of these interactions are poorly understood, even for the host-pathogen relationships for which there is a large body of research data. For *M bovis* infections, little is known about the factors that contribute to development of disease or to the production of an effective immune response.

Of those microbial factors that may contribute to *M bovis* pathogenesis, perhaps the best characterized is a large family of immunodominant variable surface lipoproteins (Vsp).^{20–24} Surface lipoprotein variation in mycoplasmas is thought to be a means of adapting to varying environmental conditions, including the host response, and may be important in determining the chronic nature of many mycoplasmal infections.²⁵ The members of the Vsp family in *M bovis* undergo high-frequency phase and size variation, providing a vast capacity for antigenic variation.^{21,22,24} Some *M bovis* Vsp have been shown to contain adhesive domains,²⁴ and others may play a role in biofilm formation;²⁶ however, the expression of particular Vsp has not been associated with disease severity, the site of infection, or with genotype.^{27,28} It is important to recognize that Vsp expression is not a stable feature of any particular population of *M bovis* cells; instead, an *M bovis* population varies in Vsp expression over time.²⁹ Although variation in *M bovis* surface antigens likely contributes to immune evasion,³⁰ the precise roles that these highly immunogenic lipoproteins play in pathogenesis remain to be determined.

Typical of respiratory mycoplasmal pathogens, *M bovis* appears to be well adapted to colonize the upper respiratory tract (URT), where it may remain for long periods of time without causing clinical disease.^{3,31} Disease occurs when host and/or pathogen factors result in replication and dissemination to other sites (eg, from the URT to the lower respiratory tract [LRT] or middle ear), and/or as a result of a detrimental host inflammatory response. Hematologic dissemination from sites of infection can occur, with the joints being a frequent site of secondary colonization.^{32,33}

Dissemination of a bacterial infection to the middle ear can occur by several possible routes, including extension of external ear infections via the tympanic membrane, colonization of the oropharynx and extension into the tympanic bulla via the eustachian (auditory) tube, or by hematogenous spread.³⁴ In pigs, otitis media caused by *Mycoplasma hyorhinis* occurs by extension of URT infections to the middle ear via the eustachian tube.^{35,36} In experimentally infected neonatal calves, *M bovis* colonization of the eustachian tubes occurred in almost all calves that had nasopharyngeal colonization, suggesting that ascending infection of the eustachian tube is the primary route by which *M bovis* enters the middle ear.³⁷

As with other mycoplasmal respiratory infections,³⁸ innate responses and local humoral responses, especially phagocytosis and killing by alveolar macrophages facilitated by opsonization with specific antibody, are important in protection from *MbAD*. However, strong adaptive immune responses that develop after infection often fail to resolve the infection or prevent *MbAD*.³⁹ Modulation of the immune response by *M bovis*, including the widespread activation of macrophages and excessive recruitment of neutrophils and lymphocytes to sites of infection, appear to contribute to the development of *MbAD*.^{39–41} The immune response in *M bovis*-associated respiratory disease has been recently reviewed,^{12,15} and will not be covered in detail in this article.

THE IMPORTANCE OF MYCOPLASMA-ASSOCIATED CALF DISEASE

Evidence for Mycoplasmas as Etiologic Agents of Calf Disease

Mycoplasma bovis

It is now well established that *M bovis* plays a causal role in respiratory disease, otitis media, and arthritis in young calves. There are a number of reports of respiratory disease outbreaks where *M bovis* was the predominant bacteria isolated from lungs of affected calves.^{42–47} In addition, although bovine pneumonia rarely involves a single infectious agent, experimental infection studies have shown that inoculation with *M bovis* alone can cause pneumonia in calves.^{33,48,49} Seroconversion to *M bovis* is associated with increased respiratory disease rates⁵⁰ as well as decreased weight gain and increased number of antibiotic treatments in feedlot calves.^{7,51} However, as with most bovine respiratory pathogens, colonization alone is not always sufficient to cause disease. *M bovis* can be isolated from the URT, trachea, and LRT of calves without clinical disease or gross lesions,^{31,52–54} although its presence in the LRT may cause subclinical inflammation.⁵⁵ Despite these findings, isolation of *M bovis* as the predominant pathogen in numerous outbreaks of respiratory disease and experimental confirmation of its ability to cause pneumonia in calves verify its role as an important respiratory pathogen.

Field cases of respiratory disease caused by *M bovis* are sometimes accompanied by arthritis, and *M bovis* has been isolated in pure culture from affected joints, as well as from the lungs of calves with concurrent respiratory disease.^{56–61} Consistent with the observations of natural disease, arthritis has been induced by inoculation of *M bovis* into joints or lungs, or intravenously.^{33,48,56,62,63} Variation among clinical isolates of *M bovis* in their ability to cause arthritis in an experimental infection model has been reported.⁵⁸

In addition to causing disease of the LRT and arthritis, *M bovis* is the predominant pathogen isolated from the middle ear of young calves with otitis media.^{64–68} However, other bacteria, including *Mycoplasma bovirhinis*, *Mycoplasma alkalescens*, *Mycoplasma arginini*, *Pasteurella multocida*, *Mannheimia hemolytica*, *Histophilus somni*, and *Arcanobacterium pyogenes* are isolated sporadically, and some have been associated with outbreaks of otitis media, especially in feedlot cattle.^{10,64,68–70} However, in the past 15 years, outbreaks of otitis media in groups of North American dairy calves have been largely attributable to *M bovis* infection.^{64,65,68} In an experimental infection study, we inoculated immunocompetent calves at 7 to 10 days of age by feeding milk replacer containing a field strain of *M bovis*.³⁷ Inoculated calves were consistently colonized in the URT and the eustachian (auditory) tubes, and 37% of calves developed otitis media by 2 weeks postinoculation. No pathogens other than *M bovis* were isolated from the inoculated calves. Therefore, *M bovis* has been implicated as a primary pathogen of the middle ear in both natural and experimental infections.

Mycoplasmas other than Mycoplasma bovis

Disease in young calves is occasionally attributed to mycoplasmas other than *M bovis*, including *Mycoplasma dispar*, *Mycoplasma californicum*, *Mycoplasma canis*, *Mycoplasma alkalescens*, *Mycoplasma arginini*, *Mycoplasma bovirhinis*, *Mycoplasma bovisgenitalium*, and *Mycoplasma bovoculi*, and a variety of other species have been isolated from the middle ear or LRT of diseased calves.^{68,71–76} A number of these species are often found as part of the microbial flora of the URT in healthy calves and in most reports they have been isolated in mixed infections with other known pathogens.^{77,78} Although specific episodes of disease are occasionally associated with one or more of these species, for the most part, their role in calfhood disease remains

poorly defined. *M bovirhinis* is a particularly common inhabitant of the URT in intensively managed cattle and has been isolated from pneumonic lungs^{71–73} and from the tympanic bulla of calves with otitis media;⁶⁸ however, it is believed to be an opportunistic invader and to play a minimal role in disease. In Australia, *Mycoplasma* species bovine group 7 has been isolated from cases of respiratory disease and arthritis in dairy calves, along with mastitis and abortion in cows.^{79,80} Outbreaks of disease associated with this mycoplasma have been reported⁸⁰ but there are few data available to determine its overall importance to calf disease in that country.

M dispar is occasionally isolated from the respiratory tract of diseased cattle, typically in mixed infections with other pathogens such as *Mannheimia hemolytica*.^{71–75} *M dispar* causes disruption of normal ciliary function in tracheal epithelium, suggesting that it could play a role in predisposing the LRT to infection with primary lung pathogens.^{54,81,82} Experimental infection of calves with *M dispar* results in colonization of the LRT and occasionally causes pneumonia.^{74,81} A rise in serologic titers to *M dispar* has been associated with increased risk of pneumonia⁸³ and with reduced weight gain⁵⁰ in feedlot cattle, supporting a role for *M dispar* in some cases of respiratory disease in feedlot cattle. There are few data on the importance of *M dispar* infections in young calves. In one case-control study, a rise in serologic titers to *M dispar* was associated with treatment for pneumonia during the first 3 months of life,⁵⁴ but there are few recent reports of its isolation from dairy calves in North America.

Prevalence

M bovis appears to be widespread within the North American dairy cattle population.^{2,4,51,84,85} In the National Animal Health Monitoring System (NAHMS) Dairy 2002 study, 7.9% of 871 dairies tested positive for mycoplasmas upon culture of a single bulk tank milk sample; *M bovis* was identified in 86% of the positive herds. States in the Western region had a greater percentage of operations with positive *Mycoplasma* culture (9.4%) than states in the Midwest (2.2%), Northeast (2.8%), and Southeast (6.6%) regions. These values are likely an underestimate of true prevalence, as subclinically infected cows shed mycoplasmas intermittently in milk,^{86,87} and milk from cows with clinical mastitis is usually withheld from the bulk tank. In a study of 463 dairy operations in the Northwestern United States (US), 20% (93) of herds had at least one *Mycoplasma*-positive bulk tank milk sample between 1998 and 2000.⁴

Because of the multifactorial nature of calfhood respiratory disease, it is very difficult to estimate the contribution of a single pathogen such as *M bovis*. This is further hampered by a lack of epidemiologic data on *M bovis* infections of calves in North America. In Europe it has been estimated that *M bovis* is responsible for 25% to 35% of calfhood respiratory disease.⁸⁸ Although specific data for MbAD in dairy calves in North America have not been published, undifferentiated respiratory disease is the second most important cause of morbidity and mortality in US dairy heifers.^{54,89–91} It is clear from the reports on outbreaks of *M bovis*-associated respiratory disease in North American dairy calves that *M bovis* can be a significant contributor to overall rates of disease and mortality in affected herds.^{43,60,65,92} For example, in a 1996 prospective study of five New York dairies, 40 cases of pneumonia occurred in 78 calves that were prospectively followed for the first 3 months of life; and 22 (55%) of these cases were attributed to *M bovis* infection.⁹²

Economic Losses

There are limited data available on the economic impact of MbAD. Losses to the US beef industry as a result of reduced weight gain and carcass value because of MbAD have been estimated at \$32 million per year, and in the United Kingdom, it is estimated

that *M bovis* contributes to at least a quarter of the economic loss due to bovine respiratory disease.⁹³ However, the cost of *MbAD* in dairy heifers has not been reported. In addition, there is scant recent information available on the cost of undifferentiated respiratory disease in dairy heifers in North America. In a 1990 study of Michigan dairy herds, the cost of respiratory disease in calves was estimated at \$14.71 per calf year.⁹⁴ Esslemont and Kossaibati⁹⁵ estimated that the average cost of respiratory disease in dairy heifers in the UK was \$61 per calf in the herd, based on 30% morbidity and 5% mortality rates. Economic costs associated with calf respiratory disease include treatment costs, labor costs, veterinary services, increased mortality, increased premature culling, reduced weight gain, reduced fertility, increased age at first calving, and possibly reduced milk production.^{96–101} Without pathogen-specific data being available, it is reasonable to assume that *MbAD* incurs many of the same costs.

M bovis-associated disease tends to be debilitating and unresponsive to therapy.^{42,44,47,102,103} Tschopp and colleagues⁷ give an example of an outbreak of *MbAD* in which 54% of 415 calves introduced into an *M bovis*-endemic facility seroconverted to *M bovis*. Calves that seroconverted within 7 weeks of arrival experienced an 8% reduction in weight gain and required twice as many antibiotics as did seronegative calves. The proportion of clinical episodes of respiratory disease attributable to *M bovis* in these calves was 50.3%. In another reported outbreak of severe *MbAD*, 70% of the calves in one dairy herd required treatment for respiratory disease or otitis media before 3 months of age.⁴³ On the individual farm affected with *M bovis*-associated calf disease, losses resulting from treatment costs, death, and culling can be substantial, and economically devastating outbreaks with very high morbidity rates and death losses of up to 30% have been observed.^{7,42,44,45,57,60,64,65,104}

Animal Welfare

In addition to any economic consequences, *M bovis* must be considered important from a calf welfare perspective. *MbAD* is often chronic, responds poorly to antibiotic therapy, often affects a substantial proportion of calves in a herd, may cause permanent health issues for affected calves, and available vaccines appear to be, at best, of limited efficacy.^{10,42,44,53,88,102,103} Taken together, these characteristics result in affected calves that may be subject to long periods of illness for which the producer or veterinarian can provide only limited relief.

EPIDEMIOLOGY OF MYCOPLASMA INFECTIONS IN CALVES

Colonization and Shedding

M bovis is a frequent colonizer of the URT of healthy or diseased calves. In diseased herds, nasal prevalences of up to 100% of calves have been reported.^{31,37,43,52,105} Within-herd prevalence is generally higher in herds with a history of *MbAD* than in herds without such a history. For example, Bennett and Jasper³¹ reported a nasal prevalence of 34% in dairy calves younger than 8 months of age in herds with *MbAD*, compared with 6% in nondiseased herds. Cattle can remain infected for long periods of time and may shed *M bovis* intermittently for many months and even years, acting as reservoirs of infection in the herd.^{3,31} Chronic colonization of tonsils, with or without nasal shedding, has been described for mycoplasmal respiratory pathogens in other hosts,^{106,107} and data from our experimental infection studies suggest that the tonsils are the primary site of URT colonization for *M bovis*.³⁷ In those studies, we infected calves by feeding milk replacer containing a field isolate of *M bovis*. All inoculated calves became heavily colonized at both palatine and pharyngeal

tonsil sites by 2 weeks postinoculation, without significant nasal shedding of *M bovis* detected in most calves.

The significance of colonization of the URT with *M bovis* as a risk factor for the development of clinical disease in the individual animal is unknown. At the herd level, a high prevalence of nasal colonization is associated with increased rates of MbAD and with isolation of *M bovis* from the LRT.^{31,43,52,108} However, isolation of *M bovis* from nasal swabs in individual calves is generally poorly correlated with both clinical disease and the presence of *M bovis* in the LRT,^{31,108,109} although a positive correlation between *M bovis* isolation from nasal swabs and clinical disease was reported in one study of backgrounding and stocker cattle.¹¹⁰

Little is known about the typical age of onset and duration of nasal shedding of *M bovis* in endemically infected herds. Bennett and Jasper³¹ reported that in calves younger than 1 week of age, nasal prevalence was 38% in herds with MbAD and 7.5% in nondiseased herds. Prevalence in the diseased herds peaked at 48% between 1 and 4 months of age. *M bovis* was still detected in nasal swabs from some calves at 8 months of age and from pre-partum heifers, although whether these represented new or chronic infections was not determined. Other investigators reported that almost 50% of calves in a herd with severe *M bovis* and *P multocida* pneumonia were shedding *M bovis* at 5 days of age and over 90% were shedding *M bovis* by 4 weeks. The onset of clinical disease in this herd peaked between 10 and 15 days of age.⁴⁵ Approximately 10% of the calves died as a result of severe pneumonia, and surviving calves had poor weight gain. In a Florida dairy experiencing an outbreak of MbAD, *M bovis* was isolated before 14 days of age from nasal swabs of all of 50 calves sampled, and 70% of these calves required treatment for respiratory disease or otitis media.⁴³ In another study of 85 calves in a Florida dairy with a history of MbAD, *M bovis* was isolated from weekly nasal swabs of every calf at least once before 90 days of age. Most calves were shedding *M bovis* by 3 weeks of age and remained shedders for several weeks.³⁷ It is apparent from these studies that calves in infected herds are often colonized when they are very young (even at less than 1 week of age), and that the highest rates of nasal shedding occur in the first 2 months of life. In addition, Bennett and Jasper³¹ found that *M bovis* may be shed in nasal secretions of calves in herds with no history of MbAD.

Although the URT is the most common site of infection, *M bovis* may similarly colonize and be shed from other body systems without causing clinical disease. In cows, subclinical *M bovis* mastitis is common, and infected cows may intermittently shed the bacteria in milk for months to years.^{3,86} *M bovis* has also been isolated from the conjunctiva,¹¹¹ semen, and vaginal secretions³ of cattle without clinical disease. Although both respiratory tract and mammary gland shedding have been implicated as reservoirs of infection within a herd,³ colonization at other sites does not seem to play a major role in the epidemiology of *M bovis*. Long-term epidemiologic studies would be helpful to determine the impact of *M bovis* colonization or MbAD in young calves on the risk of URT or mammary gland infection with *M bovis* as adults.

Transmission and Risk Factors

M bovis is thought to be introduced into *M bovis*-free herds by clinically healthy cattle that are carrying this microorganism.^{5,7,86} Spread to uninfected animals may occur at the time of introduction into the herd or may be delayed until shedding occurs.¹¹ Little is published on the epidemiology of *M bovis* within young calf populations, but there are several potential routes of initial exposure. Calves could become infected from their dams or from other adult cows in the maternity area that are shedding *M bovis* in colostrum, vaginal, or respiratory secretions.³ The isolation of *M bovis* from vaginal

secretions of cows at calving⁴³ and congenital infection of calves^{57,112} have been reported, although both events appear to occur infrequently and probably do not play a major role in transmission.

One of the major means of transmission to young calves is thought to be ingestion of milk from cows shedding *M bovis* from the mammary gland (Fig. 1).^{43,60,65} Colonization of the URT by *M bovis* occurs more frequently in calves fed infected milk than in those fed uninfected milk,³¹ and clinical disease has been documented following feeding of *M bovis*-contaminated waste milk or in calves nursing cows with *M bovis* mastitis.^{43,56,60,65} Because milk in modern husbandry systems is typically batched for feeding to calves, a single cow shedding *M bovis* can potentially expose a large number of calves to infection, and calves may be repeatedly exposed over the milk-feeding period. In a field study to determine the method of transmission of *M bovis* in one Florida dairy herd, 100% of 50 calves exposed to *M bovis*-contaminated waste milk became colonized in the URT by 14 days of age.⁴³ Culture of nasal and vaginal swabs of cows at calving was only positive for *M bovis* in one instance each. This led the authors to conclude that the main method of spread of *M bovis* from dam to calf was through contaminated waste milk. This hypothesis has been supported by other investigators.^{60,65} Additionally, in an experimental infection study using young calves, feeding of milk replacer containing a clinically relevant dose of *M bovis* consistently resulted in colonization of the URT and mild clinical disease by 14 days postinoculation.³⁷ However, feeding of unpasteurized waste milk is clearly not the only important factor in the epidemiology of *M bovis* in calves, because clinical disease can occur in herds that feed only milk replacer or in herds that effectively pasteurize milk before feeding.⁶⁸ The importance of colostrum as a source of *M bovis* infection in calves is unknown, although in one study, investigators did not isolate *M bovis* from 50 colostrum samples collected during an outbreak of *MbAD*.⁴³

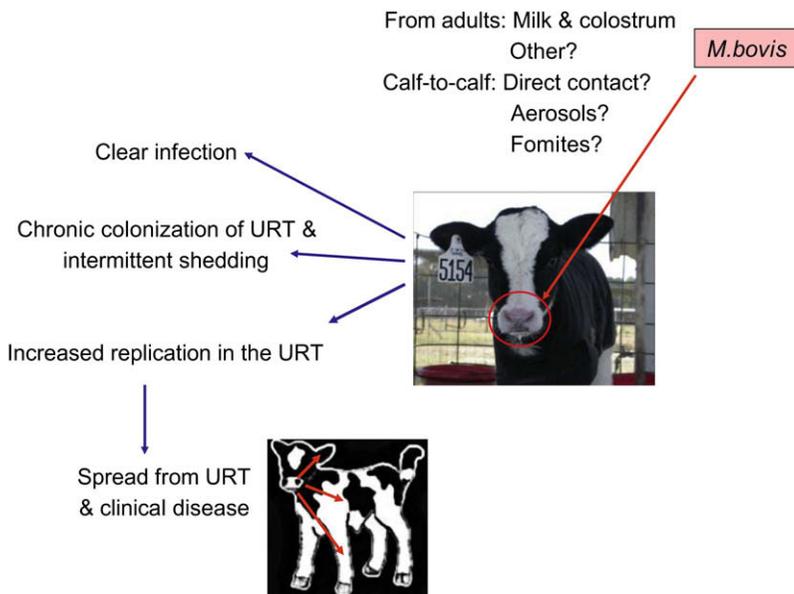


Fig. 1. Proposed transmission and infection dynamics of *M bovis* infections in young calves. URT, upper respiratory tract.

Whatever the mechanism (infected milk, colostrum, respiratory or vaginal secretions, or congenital infection) by which calves become infected, they may then shed *M bovis* in respiratory secretions (see Fig. 1). Once established on farms, *M bovis* becomes extremely difficult to eradicate, suggesting that continual transmission from older animals to incoming calves occurs.³¹ Transmission is likely to be a result of direct or indirect contact of uninfected calves with calves that are shedding *M bovis* in respiratory secretions.^{7,31,88}

In general, for bacterial pathogens involved in multifactorial diseases, the risk of infection and of developing clinical disease depends on a large number of pathogen, host, and environmental factors. With the exception of exposure to *M bovis*-contaminated milk (discussed previously), few specific risk factors for the transmission of *M bovis* or for outbreaks of clinical disease have been identified. Mixing of calves from different sources and the presence of at least one seropositive animal in new purchases increased the risk of MbAD on a ranch that raised dairy bull calves.⁷ This result is in agreement with epidemiologic studies of *M bovis* mastitis, where one of the few consistently identified herd-level risk factors has been a history of purchasing cattle.² Herd size is the only other commonly identified risk factor for mycoplasmal mastitis.^{4,113} Herd size was identified as a risk factor for an *M bovis*-positive bulk tank in the NAHMS Dairy 2002 study, with 21.7% of herds of 500 head or more having positive samples, compared with 3.9% and 2.1% of medium (100 to 400 head) and small (<100 head) herds, respectively.⁸⁴ Larger herd size was associated with increased rates of undifferentiated respiratory disease in calves in the 1991 to 1992 National Dairy Heifer Evaluation Project,⁹⁰ but the effect of herd size on MbAD in young calves has not been reported.

Despite the lack of published studies, other potential risk factors for *M bovis* infection in young calves can be identified from the limited research on *M bovis* epidemiology in calves, from studies of *M bovis* mastitis, and by extrapolating from what is known about risk factors for other respiratory pathogens in calves. For example, calves with MbAD shed huge numbers of bacteria³¹ and are therefore likely to be the greatest contributors to the load of *Mycoplasma* within a calf-rearing facility, and the most important factor in calf-to-calf spread of disease. For undifferentiated respiratory disease, high bacterial counts in the air of calf pens are associated with increased disease prevalence.¹¹⁴ Large numbers of *M bovis* can be isolated from the air in barns housing calves with MbAD.¹¹⁵ Therefore, factors that influence airborne bacteria counts in calf pens, such as pen design, barn ventilation, and stocking density¹¹⁴ may affect transmission rates. Independent of effects on bacterial load, poor air quality compromises respiratory defenses, which may increase the risk of respiratory disease.⁹⁹ However, this has not been specifically evaluated with respect to *M bovis* infections.

Mechanical transmission via fomites has been implicated in udder-to-udder spread of *M bovis* mastitis. Milking of uninfected and infected cows at the same time increases the risk for new cases, and milking equipment, teat dip, hands, sponges, washcloths, and poor hygiene during intramammary infusion of antibiotics have been implicated in the spread of *M bovis*.^{5,115-117} It is plausible that similar mechanical means of transfer could occur in calf facilities. Despite being enveloped by only a thin cell membrane, some mycoplasmas survive well in the environment. *Mycoplasma bovis* was reported to survive at 4°C for nearly 2 months in sponges and milk, over 2 weeks on wood and in water, and 20 days in straw, although higher environmental temperatures dropped survival considerably.³ In general, survival is best under cool, humid conditions.³ In surveys of Florida dairy farms, *M bovis* was commonly isolated from cooling ponds and from dirt lots with recently calved cows on farms that had

a history of *M bovis*-positive bulk tank milk culture.¹¹⁸ These studies demonstrate that *M bovis* can survive well in the dairy environment, and that mechanical transmission via fomites could theoretically occur among calves. However, further studies are required to examine the role of fomites in the epidemiology of *M bovis* infection in calf-rearing facilities.

In a study of the effect of temperature and humidity on nasal shedding of mycoplasmas in calves, an abrupt change from warm (17°C) to cold (5°C) conditions was associated with increased rates of nasal shedding of *M bovis*. In addition, calves that were permanently housed at 5°C had higher rates of nasal shedding of *M bovis* than calves housed at 16°C.¹¹⁹ Other investigators subjected healthy calves to extreme environmental temperatures (5°C or 35°C) for 4 hours; calves were housed at 18°C to 20°C before and after the exposure. Calves exposed to environmental extremes experienced significantly higher rates of respiratory disease over the following 3 weeks than did unexposed control calves. *Mycoplasma* spp were identified as the cause of respiratory disease in calves that were exposed to cold temperatures (5°C), whereas no mycoplasmas were isolated from the lungs of calves exposed to warmer temperatures (35°C) or in control calves.¹²⁰ Together, these findings suggest that mycoplasmal nasal shedding and, perhaps, clinical disease are favored by low environmental temperatures. However, epidemiologic studies to evaluate the association between temperature and MbAD have not been published.

Season may have some effect on *M bovis* infections in calves. Lamm and colleagues⁶⁸ reported that there was a seasonal distribution of cases of mycoplasmal otitis media in calves submitted for necropsy to a Californian diagnostic laboratory, with the highest proportion of cases submitted in the spring and the lowest in the summer months. Seasonal effects have been observed in some studies of mycoplasmal mastitis, with the incidence generally being higher in the cooler months of the year,^{2,121} but not in others.^{4,85} There are several possible explanations for increased rates of MbAD in winter or early spring compared with other times of year. Survival of mycoplasmas in the environment is best in cool, humid conditions³ and the risk of indirect transmission between animals may be greatest when these conditions predominate. Second, a seasonal distribution could reflect an association of *M bovis* infection with exposure to cold environmental temperatures, as discussed above.^{119,120} Last, air quality in enclosed cattle facilities may be worse in winter than at other times of the year, predisposing animals to increased rates of respiratory disease.^{99,114} Further epidemiologic studies are required to definitively determine if there is a seasonal distribution of MbAD in calves.

The immune status of the calf is important in determining susceptibility to respiratory infections. Numerous investigators have found a strong association between failure of passive transfer of maternal immunoglobulins and increased risk and severity of respiratory disease in young calves.^{51,91,122-124} However, whether maternal antibodies have any protective effects against *M bovis* infection is not clear. In one study, there was no significant association between *M bovis*-specific serum antibody titers in the first 2 weeks of life and occurrence of pneumonia in 325 colostrum-fed dairy calves.⁵¹ Likewise, Brown and colleagues⁴³ did not find an association between *M bovis*-specific serum antibody concentrations at 7 days of age and occurrence of MbAD in 50 Holstein calves. Nonspecific respiratory defenses are important in protection from mycoplasmal respiratory infections in other hosts,³⁸ and it is logical that they would also be important in *M bovis* infections. The nonspecific respiratory defenses of calves can be compromised by a variety of factors including infection with viral pathogens, sudden changes in environmental temperature, heat or cold stress, overcrowding, transportation, poor air quality, and inadequate nutrition.^{99,125} However,

further studies are required to define the role of factors affecting the nonspecific respiratory defenses of calves as well as the role of passive immunity in *M bovis*-associated calf disease. Induction of specific immunity to *M bovis* will be discussed under vaccination later in this article.

Colonization of the URT of calves with *M bovis* often occurs within the first few weeks of life,^{31,37,43} with the peak incidence of clinical disease at around a month of age. During this period, the immune system of the young calf is undergoing the rapid changes associated with maturation.^{126,127} Therefore, age-specific features of the immune system are likely to be important in determining the susceptibility or resistance of the young dairy calf to *MbAD*. For example, the tendency toward an IgG₁-dominated humoral response in young calves may not be optimal for clearance of *M bovis*, given that IgG₂ is a superior opsonin for macrophage- and neutrophil-mediated killing of *M bovis*.¹²⁸ Additionally, the presence of age-specific immune responses means that vaccine strategies targeting young calves need to be tailored specifically to this age group. Readers are referred to the article in this issue on neonatal vaccination for more information on the challenges to successful vaccination of calves in this age group.

Genetic background is thought to play an important role in the susceptibility of cattle to infectious disease.^{129,130} Genetic background is also important in determining susceptibility or resistance to mycoplasmal respiratory infections of nonbovine species. In many cases, genetic susceptibility to mycoplasmal respiratory disease appears to be a result of increased immunoreactivity of the host when compared with resistant animals.¹³¹ Additionally, innate responses such as alveolar macrophage clearance of mycoplasmas from the lung early in the infection process are influenced by genetic background, at least in rodents.¹³² Interestingly, male mice are more susceptible than females to mycoplasmal infection, suggesting that hormonal regulation may also be important in disease susceptibility.¹³³ Genetic susceptibility to mycoplasmal infections is not limited to rodents. In pigs that were bred for high or low cellular and humoral immune responses, high responders that were experimentally infected with *M hyorhinis* had more severe arthritis than did pigs bred for low immune response.¹³⁴ Given these findings coupled with the fact that immune responsiveness in cattle has a substantial genetic influence, it would not be surprising if genetic background is associated with susceptibility to *MbAD* in cattle. However, to date no studies have addressed the role of genetics in susceptibility of cattle to mycoplasmal infections.

Bovine respiratory disease frequently involves a number of viral and bacterial pathogens,^{99,125} and *M bovis*-associated respiratory disease is no exception.^{9,10,44,49,54,135–138} In fact, *M bovis* infection may predispose the respiratory tract to invasion by other bacterial pathogens.^{10,49,54,139} Similarly, other pathogens may enhance *M bovis* infection. Viral infections can damage the respiratory mucosa, reduce ciliary activity, and impair secretory and cellular immune defenses in the respiratory tract.^{99,140} Any or all of these changes could increase susceptibility to mycoplasmal infection. Studies in feedlot calves with chronic, antibiotic-resistant pneumonia suggest that there may be synergism between bovine viral diarrhea virus (BVDV) and *M bovis*.¹⁴¹ Experimental infection studies have confirmed that *M bovis* plays a synergistic role with other respiratory pathogens,^{33,142,143} especially *P multocida* and *M hemolytica*. In cases of *M bovis*-associated arthritis, mixed infections in affected joints are uncommon, although calves with arthritis often have concurrent respiratory disease from which multiple pathogens may be isolated.^{6,60,68}

Mixed infections can occur in *M bovis*-associated otitis media, although their significance is unknown.^{64,68} In other host species, viral infections of the URT are important

risk factors for increased incidence, severity, and chronicity of bacterial otitis media. One mechanism by which viral infections can potentiate bacterial otitis media is by perturbing the ciliary clearance mechanisms of the eustachian tubes.^{144,145} Specific viral etiologies have not been identified in the lungs of preweaned calves with *M bovis*-associated otitis media.^{65,66,68,146} However, attempts to isolate viruses from lesions in the tympanic bullae have been reported only once,⁶⁶ and no attempts to isolate viruses from the nasopharynx or eustachian tubes of affected calves have been reported.

Susceptibility to *M bovis*-induced otitis media appears to be age related, with the peak incidence of clinical disease at 2 to 6 weeks of age.^{64,65} *M bovis*-associated otitis media is uncommon in other age groups. In one recent study of feedlot cattle, *M bovis* was frequently isolated from the tympanic bullae of animals with no clinical or gross lesions of otitis media,¹⁰ suggesting it is the expression of clinical disease rather than dissemination to the middle ear that is age related. Age-related susceptibility to otitis media is also observed in *M hyorhinae* infections of piglets, although age-specific factors contributing to susceptibility in this species have not been determined.^{36,147} In other species, the age at which colonization of the nasopharynx or tonsils first occurs also affects the risk of developing otitis media. For example, infants who are first colonized in the nasopharynx with *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis* before 3 months of age have increased risk and severity of otitis media compared with infants who are first colonized after 3 months of age.¹⁴⁸ Colonization of the nasopharynx with bacterial pathogens within the first week of life is associated with extremely high rates of otitis media in infants. Interestingly, whereas complete eradication of *H influenzae* from the nasopharynx was highly effective at preventing otitis media, reduction of the bacterial load in the nasopharynx to below a critical threshold level appeared similarly effective.¹⁴⁹ These findings suggest that the ability to delay colonization by only a few weeks might have a dramatic impact on susceptibility to *M bovis*-associated otitis media in calves.

In summary, young calves can be infected at a very early age by ingestion of milk from cows infected with *M bovis*. They are also likely infected by direct or indirect transmission from other calves shedding *M bovis* in nasal secretions. However, other than the feeding of infected milk, few specific risk factors have been identified, and factors associated with dissemination from the URT to the LRT and clinical disease expression are poorly understood. Clearly, new epidemiologic studies would be helpful to establish risk factors and to provide guidance for calf producers to reduce MbAD.

Molecular Epidemiology

M bovis is well equipped to generate genetically diverse populations, and has been observed to undergo DNA recombination and rearrangement events at high frequency.^{21,24,28} The *M bovis* genome contains a large number of insertion sequences that are also likely to lead to heterogeneous populations.¹⁵⁰ There have been several molecular epidemiologic studies of *M bovis* using a variety of DNA fingerprinting techniques including randomly amplified polymorphic DNA analysis, amplified fragment length polymorphism analysis, restriction fragment length polymorphism analysis, pulsed-field gel electrophoresis (PFGE) analysis, and insertion sequence profile analysis.^{27,150-154} Considerable genomic heterogeneity among field isolates of *M bovis* has been reported, especially when isolates were collected from diverse geographic regions and over a period of several years.^{27,150,151} The significance of particular DNA fingerprint types in *M bovis* infections are currently unknown, and correlations between particular DNA fingerprint types and geographic location, year of isolation,

and type or severity of pathology have not been reported.^{27,150–152} However, the vast ability of *M bovis* to create genetically diverse populations as well as the frequent movement of cattle among herds in modern management systems may make it difficult to identify any such associations.

Comparison of PFGE patterns for isolates of *M bovis* or *Mycoplasma californicum* obtained at necropsy from multiple body sites in seven cows with mycoplasmal mastitis was reported.¹⁵⁴ Within each cow, the same PFGE pattern was found in 100% of isolates from sites in the mammary system (milk, mammary parenchyma, and supra-mammary lymph nodes). Forty-one percent of isolates obtained from the respiratory system and 90% of isolates obtained from other body systems had PFGE patterns identical to that of the mammary isolates. These findings indicate that the same strain of *M bovis* often colonizes multiple body sites, but also that multiple strains may be present within an animal. Isolates of *M bovis* from multiple sites of pathology within the same animal, or from multiple animals in the same disease outbreak, are typically closely related or identical by DNA typing methods, especially when the herd is closed.^{152,153,155} In contrast, endemically infected open herds (including dairy calf ranches), harbor numerous genetically diverse strains of *M bovis*. This has been attributed to introduction of animals from multiple sources over time.¹⁵³ Further molecular epidemiologic studies will hopefully enhance the current understanding of the transmission dynamics of *M bovis*.

CLINICAL DISEASE IN CALVES

Clinical disease associated with *M bovis* infection of young calves typically presents as pneumonia, otitis media, or arthritis, or any combination of these.^{43,45,56,60,65,67,68} *M bovis* has also been associated with a variety of other less common clinical manifestations in calves, including tenosynovitis, decubital abscesses, and meningitis.^{57,59,104} The age of onset of clinical disease in affected calves is typically between 2 and 6 weeks,^{43–45,65} but has been reported as early as 4 days of age.⁵⁷ Clinical disease caused by *M bovis* tends to be chronic, debilitating, and unresponsive to therapy.^{10,42,44,53,102,103,141} Chronic endemic disease as well as epizootics can occur.¹³⁶

M bovis-associated respiratory disease has a similar clinical presentation to other types of calf pneumonia. Fever, loss of appetite, nasal discharge, coughing, and both increased respiratory rate and effort are typically reported, and concurrent cases of otitis media and arthritis may occur.^{43,45,59,65,67,68} As for undifferentiated calf pneumonia, auscultation reveals abnormal breath sounds including increased bronchial sounds, crackles, wheezes, and areas of cranioventral consolidation in severe cases.⁹⁹ Both acute and chronic disease can occur, and mixed infections are common.^{9,42,44,49,54,81,137,138} Calves with chronic pneumonia often develop extreme dyspnea and emaciation.⁹⁹

Otitis media has been an increasingly recognized form of *MbAD* in North American dairy calves over the past 15 years.^{64,65,68} The clinical signs of otitis media observed include loss of appetite, fever, listlessness, ear pain evidenced by head shaking and scratching or rubbing ears, epiphora, ear droop, and signs of facial nerve paralysis (**Fig. 2A–C**).^{43,65–67,146} One or both tympanic bullae can be affected. In some cases, purulent discharge from the ear canal is observed following rupture of the tympanic membrane.^{65,67} In addition, calves with *M bovis*-induced otitis media often have concurrent pneumonia.^{64–66,68}

Otitis interna is a common sequela to otitis media in calves, and affected animals exhibit varying degrees of vestibulocochlear dysfunction including head tilt, horizontal

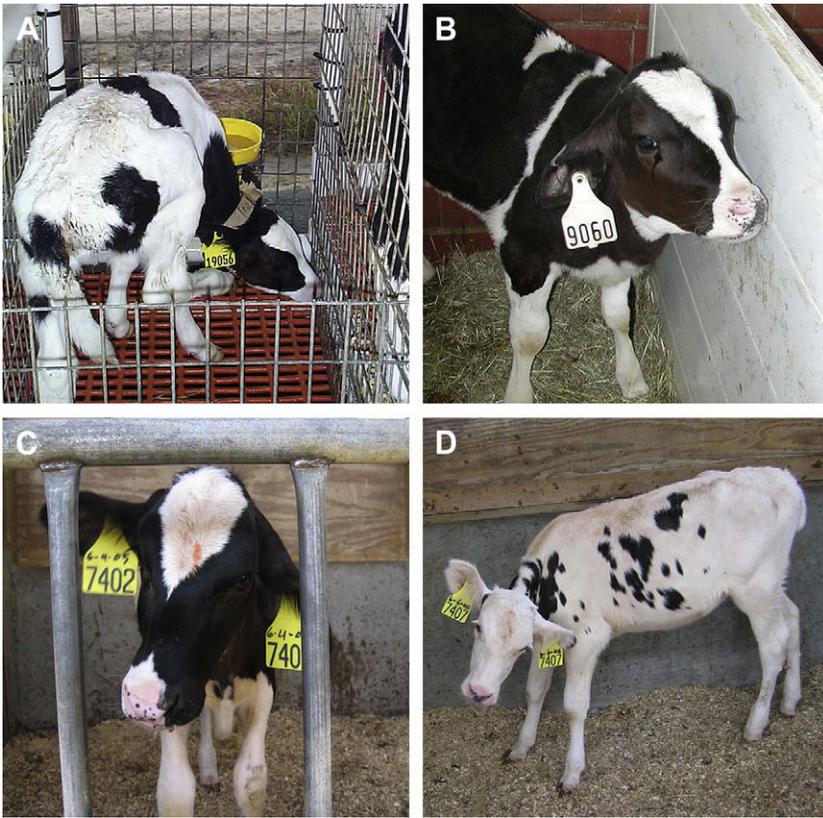


Fig. 2. Examples of the clinical manifestations of *Mycoplasma bovis*-associated otitis in calves. Ear scratching is frequently one of the earliest signs of otitis media (A), followed by unilateral or bilateral ear droop and epiphora (B and C). Head tilt is indicative of otitis interna and more advanced disease (D).

nystagmus, staggering, circling, falling, and/or lateral recumbency (Fig 2D).^{64,66,68,146} Meningitis can occur as a complication of otitis interna.^{57,67,68,146} Spontaneous regurgitation, loss of pharyngeal tone, and dysphagia have also been reported in calves with *M bovis*-associated otitis media-interna, indicative of glossopharyngeal nerve dysfunction with or without vagal nerve dysfunction.¹⁴⁶ Whether these nerves are affected by inflammation associated with meningitis or with inflammation at the sites where the nerves pass over the tympanic bullae is unknown. As is observed with *M bovis*-associated respiratory disease, calves with chronic otitis media-interna may become emaciated.^{65,146}

Clinical cases of *M bovis*-induced arthritis in preweaned calves tend to be sporadic and are typically accompanied by respiratory disease within the herd and often within the same animal.^{155,156} Clinical signs are typical of septic arthritis. Affected joints are painful and swollen, and calves exhibit varying degrees of lameness and may be febrile in the acute phase of disease.^{57,61,157} Large rotator joints such as the shoulder, elbow, carpus, hip, stifle, and hock are most frequently involved.^{10,57,59,157,158} One or multiple joints can be affected, and cattle with *M bovis* arthritis are frequently culled because of poor response to therapy.^{59,61,159} Arthritis appears to be a less frequent

clinical manifestation of *M bovis* infections in preweaned calves than in feedlot cattle. However, outbreaks of disease in young calves where arthritis was the predominant clinical presentation have been reported.^{57,60}

M bovis also may cause a variety of less common clinical syndromes in calves, with or without concurrent respiratory disease. In addition to its occurrence as a sequela of otitis media,^{66,67} meningitis has occurred as a consequence of mycoplasmaemia in very young calves. For example, in one case report, 3- to 21-day-old calves developed polyarthritis and meningitis with high mortality rates. *M bovis* was the only pathogen isolated from joints and meninges of affected calves.⁵⁷ In very young calves, arthritis caused by *M bovis* must be distinguished from septic arthritis secondary to navel infections or other causes of bacterial sepsis.

M bovis infections can occur in or around tendons and synovial structures, and tenosynovitis and bursitis are commonly reported in feedlot calves with concurrent chronic *M bovis* arthritis.^{59,157,158} In addition, intra-articular inoculation of *M bovis* in calves resulted in arthritis plus tenosynovitis.^{56,63} In an unusual presentation of *M bovis* infection, an outbreak of subcutaneous decubital abscesses over carpal and stifle joints and over the brisket was reported in 50 calves fed unpasteurized waste milk on a California calf ranch.¹⁰⁴ *M bovis* was the only pathogen isolated from abscesses, which occurred at the sites of pressure sores. Whether the bacteria gained entry through skin abrasions or via hematogenous spread is unknown, but the authors hypothesized that *M bovis* in nasal secretions may have contaminated pressure sores when calves licked these areas. There was no evidence of joint involvement in affected calves, but at least one calf had concurrent *M bovis*-associated respiratory disease.

M bovis can be isolated from the conjunctiva of cattle in infected herds,¹¹¹ although *M bovis*-associated ocular disease is considered uncommon. However, there are several reports of outbreaks of keratoconjunctivitis involving *M bovis* alone, or in mixed infections with *Mycoplasma bovoculi*.¹⁶⁰⁻¹⁶³ An outbreak of severe keratoconjunctivitis, from which *M bovis* was the only consistently isolated pathogen, was reported in a group of 20 calves. Clinical signs included mucopurulent ocular discharge, severe eyelid and conjunctival swelling, and corneal edema and ulceration. Most clinical signs resolved within 2 weeks but some animals had residual corneal scarring.¹⁶² In a recent report, an outbreak of *M bovis*-associated keratoconjunctivitis in beef calves in Italy was followed by cases of pneumonia and arthritis.¹⁶⁰

In summary, *M bovis* infections primarily result in pneumonia, otitis media, and, to a lesser extent, arthritis in young calves, but other more unusual clinical presentations affecting a wide variety of body systems can occur.

PATHOLOGY

The macroscopic and microscopic lesions of the respiratory tract in experimental *M bovis* infection vary considerably among studies, probably reflecting differences in the route of inoculation, the dose and strain of *M bovis*, the age and health status of the host, and the duration of infection. Gross lesions have consisted of cranioventral lung consolidation, sometimes accompanied by multiple necrotic foci.^{33,48,136,143} Histologically, experimental lung infections with *M bovis* are characterized by peribronchiolar lymphoid hyperplasia or cuffing, often accompanied by acute or subacute suppurative bronchiolitis, thickening of alveolar septa as a result of cellular infiltration, atelectasis, and, in some cases, foci of coagulative necrosis.^{33,48,125,136,143}

Lesions described for the lungs of cattle with natural *M bovis* infections are similar to those described for experimental disease, although typically of much greater severity. There are relatively few studies describing the naturally occurring pathology in young

calves, so most information comes from feedlot cattle. The pathology associated with *M bovis* pneumonia in feedlot cattle has recently been reviewed.^{10,12} Grossly, affected lung lobes are a deep red color and have varying degrees of consolidation, often accompanied in subacute to chronic cases by multifocal necrotizing lesions.^{8,10,42,59,102,158,164} Similar lesions are observed in 6-month-old veal calves with *M bovis*-associated pneumonia.¹⁶⁵ Lesions usually have a cranioventral distribution, but can involve whole lung lobes and the cranial portions of the caudal lobes. Necrotic lesions can vary from 1 to 2 mm to several centimeters in diameter and contain yellow caseous material (**Fig. 3**). They are distinct from typical lung abscesses in that they are not usually surrounded by a well-defined fibrous capsule.^{158,164} Diffuse fibrinous or chronic fibrosing pleuritis are sometimes observed, and interlobular septae may contain edema fluid or linear yellow necrotic lesions.^{10,46,136,157,158} Occasionally, chronic cases of *M bovis* pneumonia contain areas of lung sequestration.¹⁰ Fibrinosuppurative tracheitis has been reported in calves with mycoplasmal lung infections.⁷⁶ Experimental and natural *M bovis*-associated respiratory disease is typically accompanied by hyperplasia of the lymphoid tissues in both the URT and LRT.^{10,33} Foci of caseous necrosis in bronchial and mediastinal lymph nodes of affected calves have been observed.¹⁰

Histologically, lung lesions in naturally occurring *M bovis* infections are characterized by a subacute to chronic suppurative bronchopneumonia that is frequently necrotizing.^{8,10,102,136,158,164,165} Mixed infections are common and often complicate characterization of lesions.^{8,10,59,102,158,164} Bronchioles are filled with purulent exudate that contains abundant *M bovis* antigen on immunohistochemical staining, accompanied by varying degrees of peribronchiolar lympho-histiocytic cuffing, thickening of alveolar septa as a result of cellular infiltration, and atelectasis.

Two distinct types of necrotic lesions have been reported in *M bovis* pneumonia, the most common being multifocal pyogranulomatous inflammation with centers of caseous necrosis.^{10,102,136,158,164,165} These well-delineated necrotic foci have centers of amorphous eosinophilic material in which degenerative neutrophils are sometimes visible, especially at the periphery, and are surrounded by a band of lymphocytes, plasma cells, macrophages, and fibroblasts. In many cases, it appears that foci of caseous necrosis are centered on obliterated bronchioles. Edema fluid, fibrin, and variable numbers of neutrophils and macrophages are often present in adjacent pulmonary parenchyma. The second, and less common, type of necrotic lesion described is fibrinopurulent broncho- or bronchointerstitial pneumonia accompanied by multifocal irregular areas of coagulative necrosis, surrounded by a dense zone of necrotic cells, especially neutrophils.^{8,46,66,164,165} These types of lesions were reported to be the most common type observed in 6-month-old veal calves with mycoplasmal pneumonia.¹⁶⁵ Edema, fibrin deposition, and vascular and lymphatic



Fig. 3. *Mycoplasma bovis* pneumonia. Lungs may contain numerous foci of caseous necrosis.

thromboses in the interlobular septa may accompany these types of lesions.^{136,164} Large amounts of *M bovis* antigen have been demonstrated in both caseous and coagulative necrosis by immunohistochemical staining, especially at the periphery of lesions.^{10,66,136,158,164} Whether the two distinct types of necrosis are a result of temporal events, co-infection with other pathogens, variation among strains of *M bovis*, or variation in the host response is unknown.

Lesions in the joints and tendon sheaths of calves after experimental inoculation of *M bovis* are characterized as necrotizing fibrinosuppurative arthritis or tenosynovitis.^{32,33,63} Similar lesions have been reported in naturally occurring *M bovis* arthritis.^{10,59,76,158} Gross lesions vary from minimal to severe, but chronically affected joints usually contain nonodorous, turbid, yellow, and fibrinous to caseous exudate accompanied by thickening of the joint capsule. Histologically, affected joints usually have severe erosion of articular cartilage, hyperplasia and caseous necrosis of synoviae, and thrombosis of subsynovial vessels.^{10,63} Adjacent soft tissues, including ligaments and tendons, are frequently involved.^{10,69,158} Large amounts of *M bovis* antigen in the periphery of necrotic lesions and within joint exudates have been demonstrated by immunohistochemical staining of the joints in cattle with natural and experimental *M bovis* arthritis.^{10,33,59,158}

In calves with *M bovis*-associated otitis media, affected tympanic bullae are filled with fibrinosuppurative to caseous exudate.^{65,66,68} Histologically, extensive fibrinosuppurative exudates fill the tympanic bullae and normal architecture may be obliterated.^{65,66,68} The tympanic mucosa may have areas of ulceration and/or squamous metaplasia and is markedly thickened as a result of infiltrates of macrophages, neutrophils, and plasma cells, and proliferation of fibrous tissue. There is usually extensive osteolysis and/or remodeling of adjacent bone.^{65,68,146} Lesions are accompanied by fibrinosuppurative eustachitis.⁶⁸ Large quantities of *M bovis* antigen have been observed within necrotic exudates, particularly at the margins of necrotic lesions within the tympanic bullae. This is similar to findings in calves with *M bovis* pneumonia.⁶⁶ In chronic cases, lesions frequently extend into the inner ear and include petrous temporal bone osteomyelitis.^{66,68} Meningitis as a consequence of otitis interna is usually localized to the regions adjacent to the affected petrous temporal bone and characterized as fibrinous to fibrinosuppurative and sometimes necrotizing.^{68,166} In addition, diffuse fibrinous meningitis was described in neonatal calves with *M bovis* meningitis, which likely originated from mycoplasmaemia.⁵⁷

M bovis-associated lesions have occasionally been identified in other body systems in both experimentally and naturally infected calves.^{33,66,102,166} Ayling and colleagues¹⁶⁶ described a 10-month-old calf with a history of respiratory disease that had lesions of endocarditis and encephalitis from which *M bovis* was the only pathogen isolated. In another report, intratracheal inoculation of *M bovis* resulted in arthritis in one calf, and mycoplasma were isolated from the blood during the first week post-inoculation.³³ At necropsy, investigators observed perivascular mononuclear cell infiltration in portal areas of the liver, and immunohistochemical staining revealed *M bovis* in association with these lesions. Other investigators identified *M bovis* antigen within foci of mononuclear cell infiltrates in the liver and kidneys of two calves with chronic *M bovis* pneumonia.¹⁰²

DIAGNOSIS

The occurrence of *M bovis* is generally underestimated for several reasons. *Mycoplasma* culture requires special equipment and expertise.¹⁶⁷ Although the role of mycoplasmas in disease of cattle has received increased recognition in the past

decade, many laboratories will not routinely monitor for this organism unless *Mycoplasma* culture is specifically requested. In respiratory disease, multiple pathogens are often present. Because other bacteria such as *M hemolytica* and *P multocida* are easier to culture, the presence of *M bovis* may be missed.^{10,99} Recent studies suggest that *MbAD* is underdiagnosed, perhaps because veterinarians and pathologists fail to recognize the infection during routine physical, gross, and microscopic examination.^{10,88} *M bovis* is sometimes associated with a variety of unusual clinical presentations in which its involvement is not widely recognized, and so appropriate diagnostic tests may not be requested.

A history of respiratory disease that is poorly responsive to antibiotic therapy is suggestive of *M bovis* involvement, especially when accompanied by cases of arthritis and/or otitis media. Although the associated lung pathology can be variable, multiple nodular lesions of caseous necrosis are strongly suggestive of *M bovis* infections.^{10,102} However, as there are no pathognomonic clinical or pathologic signs for *MbAD*, a definitive diagnosis is based on isolation of *M bovis* from the affected site, and/or by demonstration of its presence in affected tissues by polymerase chain reaction (PCR), capture enzyme-linked immunosorbent assay (ELISA), or immunohistochemistry (IHC).

The culture of bovine mycoplasmas requires the use of nutritionally complex media as well as a moist carbon dioxide-enriched atmosphere.^{86,167–169} Growth of *M bovis* in appropriate media is typically apparent by 48 hours, but may take up to 10 days.^{86,88,168} Mycoplasmal colonies on solid media are identified by their characteristic morphology (Fig. 4); growth in broth is indicated by turbidity, film formation, and by subculture onto solid media.¹⁶⁸ A number of pathogenic and nonpathogenic bovine mycoplasmas or other mollicutes (especially acholeplasmas) may be isolated from the URT or from sites of pathology, either alone or in mixed infections.^{10,88,88} Many of these cannot be differentiated morphologically from *M bovis*, so speciation by immunologic methods (direct or indirect immunofluorescence or immunoperoxidase testing) or by PCR is necessary.^{47,86,170}

In live calves with clinical signs of respiratory disease, mycoplasmal culture of transtracheal wash or broncho-alveolar lavage (BAL) fluids are suitable for the diagnosis of *M bovis* infections.^{108,109,171} Comparisons of paired culture results from nasopharyngeal swabs and BAL samples in cattle with respiratory disease indicate that, in individual animals, isolation of *M bovis* from the URT is not well correlated with its presence in the LRT or with clinical disease.^{108,109} For example, in one study,¹⁰⁹ nasal swabs had a sensitivity of only 21% for predicting *M bovis*-associated lung disease.



Fig. 4. Colonies of *Mycoplasma bovis* on solid media have the “fried egg” morphology that is typical of many mycoplasma species when viewed under a stereomicroscope ($\times 40$ magnification).

Nasopharyngeal swabs can be used at the group level to indicate the presence of *M bovis* within a calf facility,³¹ although the sensitivity of this test has not been determined. In calves with arthritis or tenosynovitis, affected joints and tendon sheaths can be aspirated for culture.⁶¹ Because of difficulties with access to the site of infection, samples are not usually collected from the tympanic bulla in live calves with otitis media. Meningitis secondary to otitis media-interna may be localized. In one report, cerebrospinal fluid samples collected for diagnostic purposes were more useful when collected from the atlanto-occipital rather than the lumbo-sacral space.¹⁴⁶

Mycoplasma culture of necropsy specimens can be performed directly from homogenates of fresh tissues, aspirates, swabs collected from lesion sites, and lavage samples.^{47,109,168} As for other infectious diseases, calves that are selected for necropsy to diagnose a herd problem should be representative of the cases seen in that herd. Culture of BAL samples collected at necropsy may be preferable to culture of lung tissue when tissues cannot be processed immediately. Mycoplasmas remain viable in BAL fluids for months at -20°C or -70°C , for a few days at 4°C and for several hours at room temperature, whereas isolation rates from lung tissue decrease markedly over a few hours after collection because of release of mycoplasmal inhibitors from disrupted tissue.^{169,172,173} Complete agreement between mycoplasmal cultures of paired BAL fluids collected at necropsy and corresponding lung tissue cultured immediately after collection from cattle euthanized for respiratory disease has been reported.¹⁰⁹ In the authors' experience, when there will be more than a few hours' delay until processing of tissue samples submitted to the diagnostic laboratory, it is advisable to also submit swabs of that tissue for *Mycoplasma* culture.

Sample handling and transport are particularly important to ensure the survival of *M bovis*. Swabs should be collected into transport media such as Ames (without charcoal) or Stuart's.¹⁷⁴ Swabs, lavage fluids, aspirates, milk, and colostrum samples should be refrigerated, and tissue samples should be collected as soon as possible after death and placed in sealed plastic bags on ice.¹⁷⁴ Samples should be transported to the laboratory within 24 hours.^{174,175} If samples such as milk are stored frozen, they should still be submitted within 7 to 10 days of collection, as longer storage significantly decreases the isolation of *M bovis*.¹⁷⁵ Detection of mycoplasmas in clinical samples can potentially be improved by using enrichment techniques and large inoculum sizes.⁸⁷ Limitations of mycoplasma culture include the requirement for specialized equipment and expertise, the need to speciate any mycoplasmas that are isolated, the length of time before results are obtained, the overgrowth of slower growing species by other more rapidly growing mycoplasmas or other bacteria and fungi, the need to process samples rapidly after collection to maximize sensitivity, and the occurrence of false negative cultures because of the presence of antibiotics or other inhibitors in clinical samples.

In part to address frustrations with conventional culture techniques, a variety of PCR systems have been developed for the diagnosis of *M bovis* infections.¹⁷⁶⁻¹⁸⁰ Three PCR systems have been widely adopted for clinical diagnostics, including (1) amplification of the 16S*rRNA* gene with species- or class-specific primers followed by digestion with various restriction enzymes to permit differentiation of several species of mollicutes within a single assay,^{176,177} (2) amplification of the 16S*rRNA* gene with species-specific primers,^{177,181} and (3) amplification of the housekeeping gene *uvrC* with species-specific primers.^{177,179,182} PCR can be used for the speciation of mycoplasmas that have already been isolated by routine culture methods,^{176,179,182} as well as for the direct detection of *M bovis* in clinical samples.^{178,180,181,183} However, PCR performed directly from clinical samples can have variable sensitivity, and some authors report that samples containing fewer than 10^2 colony-forming units/mL were often detected as

negative by PCR.¹⁸³ This detection level would be no better than standard culture procedures. Sensitivity has been improved by antigen capture before PCR using an *M bovis*-specific monoclonal antibody.¹⁸³ A nested PCR was slightly more sensitive than culture of fresh milk samples, but was much more sensitive than culture (100% compared with 27%) for detection of *M bovis* in milk after 2 years of frozen storage.¹⁸⁰

Because of the very close genotypic relationship between *M bovis* and *Mycoplasma agalactiae* there has been considerable work invested in developing assays that accurately differentiate these two species.^{177,179,181,182,184} However, because *M agalactiae* is a pathogen of small ruminants that is presumed to be absent from North America and is rarely isolated outside of its typical hosts, differentiation from *M bovis* is less of a concern on this continent than in regions where both pathogens exist.

A sandwich ELISA has been developed to capture *M bovis* antigen from culture medium or clinical samples, and is commercially available in Europe (Bio-X Diagnostics, Belgium).¹⁸⁵ The ELISA has a similar sensitivity to conventional culture when performed directly from clinical samples, but sensitivity is improved when samples are incubated in broth culture medium for a brief period before antigen capture.

Immunohistochemical demonstration of *M bovis* antigen within tissues is a sensitive and specific means of determining the involvement of *M bovis* in observed pathology.^{6,8,10,66,136,158,164,165,186} Advantages of IHC are that it performs well using formalin-fixed, paraffin-embedded tissues, and can be performed retrospectively, especially when other findings suggest a *M bovis* infection but culture is negative. An additional advantage of IHC is that it reveals the location of *M bovis* within lesions. In one recent retrospective study,¹⁰ 98% and 100% of cases of caseonecrotic bronchopneumonia from feedlot cattle submitted to a diagnostic laboratory were positive for *M bovis* by culture and IHC, respectively. In cases of fibrinosuppurative pneumonia where *M hemolytica* was isolated, *M bovis* was also isolated in 82% of cases, and was demonstrated by IHC in lesions of mixed infections (with *M hemolytica*) in 85% of cases.¹⁰ The involvement of *M bovis* in lesions at a variety of other body sites has also been verified by IHC.^{10,33,57,59,66,104,158} An indirect fluorescent antibody test using polyclonal antisera has been described for the detection of *M bovis* in fresh, frozen lung tissue.⁷²

A variety of methods for the detection of *M bovis*-specific antibodies in serum and other body fluids have been described.^{83,187-189} An indirect hemagglutination test (IHA) has been successfully used to demonstrate the presence of *M bovis*-specific antibody in serum, colostrum whey, and joint fluid.^{10,83,187} However, the most widely applied method to detect *M bovis*-specific antibodies is an indirect ELISA.^{88,190} Most studies have used whole cell or membrane protein antigens derived from various reference or field strains of *M bovis*. Laboratory-grown strains of *M bovis* vary over time in their variable surface protein (Vsp) expression profiles, and it has been proposed that this may effect the reliability of immunologic assays¹⁹¹; however studies addressing whether this issue is of practical concern in diagnostic ELISA have not been published. Le Grand and colleagues¹⁹⁰ developed an indirect ELISA using membrane proteins derived from a phenotypic clonal variant of the *M bovis* type strain PG45 with a high level of expression of Vsp A. The assay performed well in experimentally and naturally infected cattle populations, although whether or not the antigen was superior to traditional antigens was not determined. A variety of ELISA tests for serologic detection of *M bovis* antibodies are now commercially available in Canada and Europe. For example, Biovet in Canada, Bio-X Diagnostics in Belgium, and Bommelli in Switzerland currently manufacture ELISA kits that detect *M bovis* antibodies.

M bovis-specific serum immunoglobulin is detectable as early as 6 (IgM) to 10 days (IgG) after experimental inoculation of *M bovis* into the respiratory tract.^{188,190} Specific

serum immunoglobulin concentrations remain elevated for months to years after *M bovis* infection, so a high titer does not necessarily indicate very recent exposure.^{88,192} Maternal antibody can also result in high antibody levels in young calves, although with a half-life of 12 to 16 days, this typically wanes by a few months of age.⁷ Virtala and colleagues¹⁷¹ reported that of 75 pneumonic dairy calves younger than 3 months of age in which *M bovis* was isolated from tracheal wash samples, only 57% had a fourfold or greater increase in *M bovis* serum antibody titers by IHA.¹⁷¹ The authors concluded that paired serum samples were not a good predictor of *M bovis*-associated respiratory disease, possibly because of the presence of maternal antibody titers. Other investigators also failed to find a correlation between serum antibody titers to *M bovis* and *M bovis*-associated respiratory disease in naturally infected individual animals.^{83,193} However, calves with severe chronic respiratory disease caused by *M bovis* generally have high serum IgG titers.¹⁶⁵ On a group level, seroconversion has been predictive of *M bovis*-associated respiratory disease.^{8,50} Therefore, serology is of limited diagnostic value in individual animals and is really most useful in epidemiologic surveillance.^{83,190} Serology has also been effective as a biosecurity tool to screen new groups of cattle before introduction into a herd, but this would be applicable only to animals more than a few months of age, after maternal antibodies have waned.⁸⁸

TREATMENT

The fact that *Mycoplasma* species lack a cell wall has important implications for treatment, as it means the beta-lactam antibiotics are ineffective.¹⁹⁴ *Mycoplasma* species are also naturally resistant to sulfonamides. Currently, only one product containing the triamidine antibiotic tulathromycin (Draxxin; Pfizer, Inc.) is approved for treatment of *MbAD* in dairy calves in the United States. Other antimicrobials that have a theoretic basis for efficacy against *M bovis*, and that are approved in the United States for treatment of respiratory disease in dairy heifers younger than 20 months of age, include enrofloxacin, florfenicol, oxytetracycline, spectinomycin, tilmicosin, and tylosin. Recent evidence suggests that antimicrobial resistance to antibiotics traditionally used for treatment of *Mycoplasma* infections is increasing in field isolates of *M bovis* in North America^{195,196} and Europe.^{197,198} Isolates from both continents show widespread resistance to tetracyclines and tilmicosin, and European isolates show increasing resistance to spectinomycin. Although in vitro antibiotic susceptibility profiles of *M bovis* may be useful in making broad generalizations about antibiotic resistance, data have not been published on the relevance of these profiles to clinical efficacy on an individual or a herd level. The antibiotic susceptibility profiles of paired *M bovis* isolates obtained from nasal swabs and BAL samples in calves with respiratory disease were found to differ considerably within animals, suggesting that if susceptibility profiles are used, they need to be based on isolates obtained from the site of infection.¹⁰⁹ Methodology for and application of antimicrobial sensitivity testing of *M bovis* has been recently reviewed.¹²

In spite of the limited choice of potentially effective antibiotics available, antibiotics are widely used to treat *MbAD*. However, treatment is frequently unrewarding, with affected cattle requiring a long duration of treatment or failing to respond to therapy.^{3,49,53,57,59,65,67,103,146,159,199} Calves with chronic and/or multisystemic disease are reported to have a particularly poor response to treatment.^{57,59,103,159,199} There are few controlled clinical trials evaluating the efficacy of various antibiotics available for treatment of *MbAD*, and the few efficacy studies published must be interpreted with caution because most use experimentally infected calves and treatment is often

started early in the disease course.^{49,200,201} In an industry-sponsored study, tulathromycin was an effective treatment for respiratory disease in dairy calves that had been experimentally infected with *M bovis*, when treatment was initiated at 3 or 7 days after inoculation.²⁰¹ Likewise, tilmicosin administered 6 hours before inoculation or at the onset of clinical disease was effective in reducing lung colonization by *M bovis* in calves that had been experimentally infected with *M hemolytica* and *M bovis*.²⁰⁰ However, treatment with spectinomycin did not alter the clinical course of disease in calves with *M bovis* and *P multocida* pneumonia when treatment was started 6 days after inoculation, although the numbers of *M bovis* in the lung were reduced in treated calves.⁴⁹

Scant information is available regarding treatment of *MbAD* in field situations, and most studies have come from Europe. Marbofloxacin, a fluoroquinolone antibiotic, was an effective treatment for naturally occurring *M bovis*-associated respiratory disease,²⁰² but this antibiotic cannot be used in cattle in the United States. Enrofloxacin, another fluorquinolone, recently has been approved for the treatment of respiratory disease in dairy heifers under 20 months of age in the US; however extra-label use (eg, for treatment of otitis media) is prohibited. Available therapies that have resulted in clinical improvement in calves with *M bovis*-associated respiratory disease in field trials include oxytetracycline, tilmicosin, or a combination of lincomycin and spectinomycin.^{92,203} However, given the recent evidence that resistance against these drugs is increasing, these antibiotics may no longer be appropriate choices. Without other data to guide choice of an antibiotic, selection of a specific treatment regimen from the list of potentially effective antibiotics based on past performance in the affected herd is frequently recommended.^{103,157}

In addition to antibiotics, short-term use of anti-inflammatory drugs can be beneficial in the treatment of bovine respiratory disease.²⁰⁴ Although these therapeutic agents have not been specifically evaluated for the treatment of *MbAD*, there is a logical basis for their use, as the inflammatory response may contribute significantly to the pathology of *M bovis* infections.^{39,47} Nonspecific supportive therapy including oral or intravenous fluids and nutritional support may be indicated in specific animals.¹⁴⁶

Irrigation of the middle ear after the tympanic membrane has ruptured has been recommended for treatment of undifferentiated otitis media in calves.³⁴ Puncture of the tympanic membrane (myringotomy) followed by insertion of tympanostomy tubes is commonly used in the treatment of children with chronic or recurrent otitis media,^{205,206} and some veterinarians have promoted blind myringotomy using a sharp object such as a knitting needle in the treatment of otitis media in calves.²⁰⁷ To the best of the authors' knowledge, studies on the risks and efficacy of this procedure in clinical cases have not been published. The potential benefit of myringotomy is the relief of pain and pressure caused by the buildup of exudate in the middle ear, as well as access to the middle ear for irrigation. Whether the procedure might provide relief for calves that have the thick, caseous exudate characteristic of chronic *M bovis* otitis media is not clear. In a recent study using calf cadavers, investigators reported that blind insertion of a 3.5-mm diameter straight knitting needle approximately 3 cm into the ear canal to perforate the ear drum was anatomically feasible.²⁰⁸

Surgical treatment of *M bovis*-associated otitis media/interna has been described.¹⁴⁶ A bilateral tympanic bulla osteotomy was performed on a 4-week-old calf with severe, chronic *M bovis*-associated otitis media-interna that had failed to respond to antibiotic treatment. Postsurgically, the tympanic bullae were lavaged daily with warm saline for 3 days, and antibiotics were continued for 16 days. Surgery coincided with a dramatic improvement in clinical signs and the calf was reported to be clinically normal at 1 year of age. Because of the cost and complexity associated with

this procedure, as well as the requirement for general anesthesia, its application is probably limited to refractory cases of otitis media in valuable calves without concurrent respiratory disease.

To summarize, antibiotic treatment of *MbAD* is often unrewarding, especially in calves with chronic or multisystemic infections. Improved efficacies are reported in experimental infection studies when treatment is initiated early in the disease course, suggesting that early intervention or, perhaps, metaphylactic therapy in high-risk calves (discussed later in this article) may be more rewarding. For example, when dealing with otitis media it is likely that initiation of treatment at the earliest onset of clinical signs (such as ear scratching or head shaking accompanied by fever) will be more successful than waiting until disease is more advanced and an obvious ear droop is present. Extended duration of antimicrobial therapy is frequently recommended for *MbAD*. Given the lack of data on the efficacy of antimicrobials or other treatment options (such as middle ear lavage) for *MbAD* that are specific to North American calves, together with concerns regarding antimicrobial use in today's livestock industries, controlled clinical trials to evaluate current therapeutic and metaphylactic antibiotic regimens are needed.

CONTROL AND PREVENTION

Biosecurity

Results of epidemiologic studies of mycoplasmal mastitis suggest that the best way to prevent *M bovis* infection is to maintain a closed herd or to screen and quarantine purchased animals.^{2,117,209} Results of such studies also suggest that *M bovis*-associated mastitis can be effectively eliminated from dairy herds through aggressive surveillance and culling of infected cows.^{4,210} In feedlot cattle, where these types of biosecurity measures are not practical, recommendations for the control and prevention of *M bovis*-associated respiratory disease and arthritis focus on limiting stress, vaccinating to reduce the incidence of other respiratory pathogens, and segregating affected groups of calves from new arrivals to reduce exposure of high-risk animals to *M bovis*.^{157,159} Dairies that are expanding and calf ranches that rear animals from multiple sources obviously cannot maintain closed herds, and calf ranches are not usually able to screen new calves before introduction into the facility. However, calf ranches do have the ability to be selective in purchasing calves, and animals could be screened on arrival to determine if a particular supplier is consistently providing *M bovis*-infected calves. Prevention of *MbAD* is hampered in dairy calf operations by the extremely limited understanding of its epidemiology and risk factors.

Management of Calves in Mycoplasma bovis-Infected Herds

Current recommendations for prevention of *MbAD* in calf-rearing facilities are based on reducing exposure to *M bovis* (**Box 1**). Potential sources of exposure that could be controlled include unpasteurized bulk tank or waste milk, colostrum, and indirect or direct contact with respiratory aerosols from infected calves. Exposure to *M bovis* in milk could be limited by culling infected cows or avoiding the feeding of milk from infected cows in herds where the *M bovis* status of the lactating cows is monitored. A more widely applicable method of reducing exposure to *M bovis* is by pasteurizing milk before feeding, or by feeding milk replacer.^{60,65,211} On-farm batch pasteurization of waste milk to 65°C for 1 hour or 70°C for 3 minutes,⁶⁰ or the use of a high-temperature short-time pasteurizer²¹¹ will inactivate *Mycoplasma* species. Frequent monitoring by culture of pasteurized milk samples to ensure that pasteurization has been effective is important in any on-farm pasteurization program.²¹² Pasteurization of

Box 1**Proposed strategies for the control of *MbAD* in pre-weaned calves***Reduce the level of exposure to Mycoplasma bovis*

1. Reduce exposure in milk
 - a. Pasteurize whole milk
 - b. Feed milk replacer
2. Reduce potential exposure in colostrum
 - a. Avoid pooling
 - b. Consider pasteurization
3. Reduce potential airborne exposure
 - a. Provide adequate ventilation in calf housing
 - b. Consider the impact of pen design on air quality
 - c. Consider ways to reduce stocking density
4. Reduce exposure to sick calves
 - a. Considering segregation of calves with clinical *MbAD*
 - b. Promptly treat clinical cases
5. Prevent fomite transmission
 - a. Sanitize pens, hutches, buckets, and other equipment between uses
 - b. Wear gloves when handling sick calves and change them between calves. Wear gloves when assisting calves to drink.
 - c. Handle the youngest calves first
6. Consider "all-in, all-out" practices, or segregate older and younger calves at the earliest possible opportunity
7. Where *M bovis* is not already present, use biosecurity practices appropriate to the particular operation and monitor for *MbAD*

Maximize calf defenses against Mycoplasma bovis

1. Use nonspecific measures to maximize respiratory and immune system health
 - a. Provide good air quality
 - b. Control other pathogens, and in particular address any deficiencies in the vaccination and monitoring programs for respiratory viruses and BVDV
 - c. Provide good nutrition
 - d. Address any colostrum management issues
 - e. Minimize other sources of stress such as transport, heat and cold stress, and overcrowding
2. Consider metaphylactic antimicrobial use when high morbidity and mortality caused by *MbAD* are being sustained
3. There are insufficient data on the efficacy of currently available vaccines to recommend their use in neonatal calves at this time

colostrum is also possible. Some recent studies have reported that on-farm batch pasteurization at 60°C for 30 minutes eliminated viable *M bovis* while immunoglobulin concentration and colostrum consistency were not significantly affected.^{213,214} Pasteurization methods that use higher temperatures have resulted in reduced colostrum quality and unacceptable feeding characteristics.^{211,213,214} If colostrum is not pasteurized, it has been recommended that it should not be pooled to minimize potential exposure of calves to *M bovis*.⁴⁷

Large numbers of *M bovis* are shed in the respiratory secretions of calves with clinical *MbAD*.^{3,31} It has therefore been recommended to segregate affected and healthy calves, although this is frequently impractical.¹⁵⁷ Other recommendations that have been made include taking appropriate precautions to prevent potential transfer of *M bovis* between calves by personnel or equipment.⁸⁸ Nipples, bottles, tube feeders, and buckets should be adequately sanitized, and pens disinfected between calves. As discussed earlier, *M bovis* survives surprisingly well in the environment, but it is highly susceptible to heat and to most commonly used chlorine-, chlorhexidine-, acid-, or iodine-based disinfectants.²¹⁵ Personnel should wear gloves when feeding calves (especially when feeding newborns or assisting sick calves to nurse), and change them between animals. Wherever possible, consideration should be given to “all-in, all-out” practices to prevent older animals from infecting younger ones,⁸⁸ or to segregation of older and younger animals within the facility if “all-in, all-out” is not practical.

Management practices that help control other respiratory diseases by maximizing the ability of the calf’s respiratory system to resist and control infection have been recommended for *M bovis*, although none of these have been specifically evaluated with respect to this pathogen.^{47,99,157} Such measures include providing proper nutrition and adequate ventilation at the pen level, and reducing environmental stressors such as overcrowding and heat and cold stress. Because viral respiratory pathogens, especially BVDV, may predispose to *M bovis* infection,^{99,141} herd vaccination protocols for infectious bovine rhinotracheitis virus (IBR), parainfluenza type 3 virus (PI₃), BVDV, and bovine respiratory syncytial virus (BRSV), as well as the herd BVDV monitoring program, should be evaluated to ensure that they are appropriate. Although the role of passive transfer of *M bovis*-specific antibodies in protection of calves from *MbAD* is unclear, a sound colostrum feeding program can reduce the risk of infection with other respiratory pathogens,⁹⁹ and may therefore decrease the risk of secondary *M bovis* infections. Readers are referred to the article on respiratory disease in this issue for more discussion of respiratory disease control practices.

The prophylactic or metaphylactic use of antibiotics is generally undesirable but may be justified when high levels of morbidity and mortality are occurring. Strategic antibiotic treatment of calves that are deemed to be at high risk for respiratory disease upon arrival at feedlots has clearly been demonstrated to reduce the incidence and severity of respiratory disease.^{216,217} In addition, feeding metaphylactic levels of antibiotics in milk replacer to dairy calves on calf ranches reduces disease incidence and delays the onset of clinical disease during the preweaning period.²¹⁸ For *MbAD*, the response to treatment when antibiotics are given before, or early in the course of experimentally induced disease, is often better than the response rates reported in field cases. This suggests that metaphylactic treatment might be more successful than initiating treatment after disease is clinically apparent. In one European study, investigators found that valnemulin added to the milk from 4 days of age for 3 weeks was effective in limiting *MbAD* in calves.⁴⁵ Animals in the treated group had fewer clinical signs and reduced clinical scores; however, disease was not eliminated and calves still required a considerable number of individual treatments. Nagatomo and colleagues²¹⁹ treated calves that were at high risk of *MbAD* with chloramphenicol.

Untreated calves had high mortality rates (up to 41%), while the onset of clinical disease was delayed in treated calves and all treated calves survived. Prophylaxis or metaphylaxis with antibiotics that are approved for use in US cattle has not been evaluated with respect to *MbAD* in young calves.

Vaccination

From studies of the immune response to respiratory mycoplasmal infections in other species, it can be concluded that adaptive responses in place at the time of mycoplasmal exposure contribute to the control or prevention of new mycoplasmal infections.³⁸ It is therefore not surprising that under some circumstances, vaccination can at least partly protect calves from *MbAD*. For example, in an experimental study, 1- to 5-month-old beef calves were vaccinated subcutaneously with live *M bovis*, intraperitoneally with live *M bovis*, or subcutaneously with a formalin-inactivated bacterin.⁶² Two boosters were given at 10-day intervals and animals were challenged by intravenous inoculation of *M bovis*. Clinical arthritis was seen in 100% of nonvaccinated as compared with only 13% of vaccinated calves, and lesion severity was decreased in vaccinated calves that did develop arthritis.

In another study of an apparently efficacious vaccine in young calves, Nicholas and colleagues²²⁰ vaccinated 3-week-old dairy calves with a single dose of saponin-inactivated bacterin. Calves received an aerosol challenge with live *M bovis* 3 weeks after vaccination. Vaccinated calves had fewer numbers of *M bovis* at colonized sites, fewer numbers of body sites colonized by *M bovis*, and reduced severity and incidence of clinical disease and lesions compared with control calves. There was also a significant decrease in body weight gain in control calves compared with vaccinates. Additionally, no vaccinated calves as compared with two of seven control calves developed arthritis. Vaccinated calves produced a strong IgG response before challenge, but IgG subtypes were not reported. No adverse events associated with vaccination were noted.

In another report of an apparently efficacious vaccine, a killed vaccine against four bovine respiratory pathogens (BRSV, PI₃, *M bovis*, and *M dispar*) was evaluated for protection against naturally occurring respiratory disease in beef calves.^{135,221} Calves were vaccinated subcutaneously and received two boosters at 3-week intervals. In one study, three groups of beef calves aged 12, 7, and 3 weeks at the time of first vaccination were used, and calves were followed for 6 months.²²¹ Respiratory disease occurred in a significantly higher ($P < .05$) proportion of the control calves (27%) than vaccinated calves (16.3%). In a second study using the same vaccination protocol, *M bovis* and BRSV were implicated in outbreaks of respiratory disease during the trial period.¹³⁵ Morbidity as a result of respiratory disease was significantly reduced in vaccinated calves (25%) compared with controls (32%), and mortality in the vaccinated group was similarly reduced (2% and 9% for vaccinates and controls, respectively). No adverse effects of vaccination were observed.

In a report of *M bovis* vaccination of feedlot cattle, a bacterin consisting of autogenous formalin-inactivated strains of *M bovis* and *M hemolytica* was used in 3000 cattle at arrival.²²² The feedlot had a history of *MbAD*. The vaccine was reported to be efficacious for the prevention of respiratory disease in newly introduced cattle, but, unfortunately, comparisons were made to a historical control group. No adverse effects of vaccination were noted.

Despite the promise shown in some of the studies discussed previously, other vaccine trials have been less successful. Rosenbusch vaccinated 2-month-old dairy calves with a formalin-inactivated bacterin prepared from two strains of *M bovis*.²²³ Calves received a single booster at 3 weeks postvaccination, and were challenged

by transthoracic inoculation of *M bovis*. Vaccination exacerbated disease, with four of five vaccinated calves as compared with only one of five control calves developing severe respiratory disease. A similar exacerbation of disease was seen in calves vaccinated with partially purified membrane proteins from *M bovis*, as clinical disease and pathology following aerosol challenge were more severe in vaccinated calves than in controls.²²⁴ In a field trial using 330 neonatal dairy calves in two north-central Florida herds with endemic *M bovis* disease, calves were vaccinated at 3, 14, and 35 days of age using a commercially available *M bovis* bacterin.³⁷ The vaccine was not efficacious in reducing morbidity or mortality caused by respiratory disease, otitis media, or arthritis in these herds. The response to vaccination was herd-dependent, with a higher rate of otitis media associated with vaccination in one herd. Most calves in the study became colonized with *M bovis* in the first 2 weeks of life, and most clinical disease occurred between 3 and 6 weeks of age. A humoral immune response to vaccination was not detected until approximately 2 weeks after the third vaccine booster at approximately 7 weeks of age. The early age at which calves can become infected with *M bovis* in endemically infected facilities and represents perhaps the biggest challenge to the development of an effective *M bovis* vaccine for use in young calves. Other challenges to vaccination of neonatal calves are discussed in an article on neonatal vaccination in this issue.

Even where *M bovis* vaccines have been associated with clinical benefits, they often fail to induce an immune response that clears infection.^{62,220} For example, intramuscular injection with formalin-killed *M bovis* with adjuvant followed after 14 days by intratracheal inoculation with killed organisms resulted in reduced *M bovis* in the lungs compared with control calves after intratracheal challenge, however significant numbers of mycoplasmas were still present in vaccinated calves.²²⁵ Induction of protective immune responses against *M bovis* by vaccination is also complex. For example, in the aforementioned study, a vaccination protocol of three subcutaneous injections also induced protective responses, but two intramuscular or two intratracheal inoculations did not.²²⁵ In these studies, the number of *M bovis* bacteria isolated from the lungs of calves was negatively correlated with IgG concentrations in BAL fluid, and different vaccination regimens were more or less effective at inducing an IgG response in the respiratory tract.

Despite very limited data on the field efficacy of *M bovis* vaccines, a number of bacterin-based vaccines for *M bovis* are licensed for marketing in the United States. To the best of the authors' knowledge, one vaccine is currently licensed for reducing the duration and severity of mycoplasmal mastitis in adult dairy cattle (Mycomune; Biomune, Lenexa, Kansas), and at least three vaccines are licensed for prevention of *M bovis*-associated respiratory disease in cattle. One product (Myco-B Bac; Texas Vet. Labs, Inc., San Angelo, Texas), is aimed at stocker and feeder cattle. Another product (Pulmo-Guard MbP; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, Missouri) is licensed for vaccination of cattle older than 45 days of age and is primarily marketed to the beef industry. A third vaccine (Mycomune R; Biomune, Lenexa, Kansas) recently has been approved for use in calves 3 weeks of age or older as an aid in the prevention of *M bovis*-associated respiratory disease. In addition to these vaccines, a number of US companies are licensed to produce custom autogenous bacterins using strains of *M bovis* isolated from the target herds.

In conclusion, vaccination against *M bovis*, at least in older calves, is possible. However the vaccines reported to date do not prevent colonization of the URT with *M bovis* and vaccination can also induce harmful effects. The very young age at which calves often become infected with *M bovis* is perhaps the greatest challenge to the development of a successful vaccine. A better understanding of the immunology of the

neonatal calf, especially with respect to ability to respond to different antigens, the types of responses that are produced, and modulation of these responses by mucosal and systemic adjuvants may improve our ability to produce efficacious *M bovis* vaccines, if indeed vaccination of the very young calf against *M bovis* is possible. Ongoing research is continually leading to a better understanding of *M bovis* antigens (for example, see Perez-Casal and Prysliak²²⁶) and this may lead to the development of more targeted vaccine approaches. In addition to research into new vaccination strategies, critical evaluation of currently marketed *M bovis* vaccines and autogenous bacterins in well-designed, independent efficacy studies that include a valid control group, blinding, adequate power, and relevant clinical outcomes, and that are conducted in an appropriate age group are clearly required. The paucity of such studies is a major gap in understanding the potential of currently available vaccines as a management strategy to control *M bovis* infections in young calves.

SUMMARY

M bovis has emerged as an important pathogen of young intensively reared calves in North America. A variety of clinical diseases are associated with *M bovis* infections of calves, including respiratory disease, otitis media, arthritis, and some other less common presentations. Clinical disease associated with *M bovis* is often chronic, debilitating, and poorly responsive to antimicrobial therapy. Current control measures are centered on reducing exposure to *M bovis* through contaminated milk or other sources, as well as nonspecific control measures to maximize the respiratory defenses of the calf; however, these management strategies often fail to control clinical mycoplasmal disease. The development of improved preventive, control, and treatment strategies for *Mycoplasma*-associated disease in young calves is hampered by a lack of understanding of the epidemiology of *M bovis* infections in young calves and of the host-pathogen interactions involved in the establishment of infection and development of clinical disease.

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