

# General Session I

## "Bovine Diseases"

Moderator — Kelly Lechtenberg

### Bovine Mycoplasmosis: Current Perspectives

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#### Introduction

The bovine mycoplasmas interact in varying degrees with their host. This interaction can range from purely commensal in nature to one with devastating consequences for the host. In some disease processes the role of mycoplasma is well documented and straightforward. However, because of the commensal nature that can exist with many of the bovine mycoplasma species, even species considered to be highly pathogenic, it is not always clear what role, if any, these mycoplasmas play in some disease processes. There is still much to be learned about this delicate host-parasite relationship and the additional factors important in tipping the balance toward disease manifestation. Given the number of variables that are unknown, the practitioner must make decisions based on present knowledge. The decisions regarding treatment and management of disease are influenced by herd history, clinical findings, laboratory results and economic impact and often must be tailored for the individual situation.

#### Biologic Properties and Taxonomic Considerations

Members of the class Mollicutes, often referred to as the mycoplasmas, are distinct from other bacteria of their small genomic size and the fact that they lack a cell wall or even the genetic coding to form one.<sup>8</sup> Because of the absence of a cell wall, mycoplasmas exhibit a high degree of plasticity and pleomorphism. The cell diameter ranges from 0.3 to 0.8  $\mu$ . Their plasticity allows them to pass easily through filters with pore sizes of 0.45  $\mu$  or less. The cell make-up of mycoplasmas is

fairly simple, essentially ribosomes and DNA bound by a cytoplasmic membrane. The trilaminar cytoplasmic membrane is composed of phospholipids, sterols and proteins. The sterols play a role in maintaining membrane fluidity. Some species such as *Mycoplasma mycoides* subsp. *mycoides* and *M. dispar* also possess a polysaccharide capsule. *Ureaplasma* spp. (T-mycoplasmas) are unique in their requirement of urea for growth.

Of the five recognized genera in the Mollicutes, the significant bovine pathogens belong to the genera *Mycoplasma* and *Ureaplasma*. The most commonly encountered bovine pathogens are listed in Table 1. Species designation is ultimately dependent on determination of serologic relatedness to the type species. Most of the bovine mycoplasmas exhibit a fairly high degree of host specificity, although this is not absolute.

Table 1. Bovine mycoplasma species and associated clinical manifestations.

Mycoplasma Species	Clinical Manifestations
<i>Mycoplasma alkalescens</i>	arthritis, mastitis
<i>Mycoplasma arginini</i>	mastitis
<i>Mycoplasma bovis</i>	abortions, abscesses, arthritis, mastitis, pneumonia
<i>Mycoplasma bovigenitalium</i>	endometritis, mastitis, vulvitis, seminal vesiculitis
<i>Mycoplasma bovirhinis</i>	pneumonia?
<i>Mycoplasma bovoculi</i>	conjunctivitis
<i>Mycoplasma californicum</i>	mastitis
<i>Mycoplasma canadense</i>	abortions, mastitis
<i>Mycoplasma dispar</i>	bronchiolitis, alveolitis
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i>	contagious bovine pleuropneumonia
<i>Ureaplasma diversum</i>	abortions, pneumonia, conjunctivitis, vulvitis, seminal vesiculitis

## Host-Organism Interaction

The bovine mycoplasmas require close interaction with their host because of their fastidious nature and requirement for a source of lipids and nucleic acid precursors for growth. Both the pathogenic and commensal mycoplasmas have the ability to adhere to the surface of hosts' mucous membranes.

Various factors can transform this interaction from a harmonious commensal relationship to one of serious disease. The site affected is an important factor in determining how this host-parasite relationship will manifest. Concurrent infections with other pathogens may predispose animals to developing mycoplasma infections. Conversely, mycoplasma infections may allow opportunistic agents to establish and further contribute to the disease process. The importance of various factors in pathogenesis of mycoplasma infections is still largely unknown.

The host's immune response to mycoplasmas is an important component in the disease process. The ability of mycoplasmas to incorporate host antigens within the cytoplasmic membrane (capping) is thought to play a role in helping mycoplasmas evade the host immune system, while incorporation of mycoplasma antigens onto the surface of host cells may cause an adverse immunologic response that is directed against the host. Specific components of mycoplasma cells have been shown to affect the host's immune system. Capsular polysaccharide of *M. dispar* prevents activation of bovine alveolar macrophages and suppresses production of cytokines such as tumor necrosis factor and interleukin-1.<sup>1</sup> Other studies have shown that *M. bovis*, which does not possess a capsule, also has a nonspecific suppressing effect on lymphocyte proliferation as measured by the lymphocyte blastogenesis tests.<sup>7</sup> This general suppression of the immune response could play a role in persistence of infections and could be beneficial to other pathogens in mixed infections.

Toxins, some of which are membrane associated, have also been described for some mycoplasmas. These are complex polysaccharides and have a role in activating complement and in increasing vascular permeability. In addition, other cell products such as hemolysins, proteases and nucleases, may also have toxic effects on host cells, particularly ciliated cells, and may contribute to the tissue damage observed in some mycoplasma infections.

## Clinical Manifestations

### Mastitis

Mycoplasma mastitis is the most economically significant manifestation of bovine mycoplasma infections in the United States. The route of infection is thought to be via the teat canal, although a systemic phase is

also recognized. Mycoplasma species that cause mastitis are frequently isolated from the respiratory tract of cows from many dairies without a history of mycoplasma mastitis, suggesting that other factors are needed to precipitate a mycoplasma mastitis outbreak. Spread among cows during outbreaks is greatly influenced by management practices. *Mycoplasma bovis* is the most common cause of mycoplasma mastitis followed by *M. californicum*. Other species have also been reported to be responsible for mastitis outbreaks. Mycoplasma mastitis usually presents as severe mastitis, although subclinical infections are recognized. The affected gland is swollen and firm. Sometimes all four quarters are involved and the entire udder is swollen. Systemic signs are not encountered commonly, although arthritis is occasionally a sequelae. The appearance of the milk can vary. Frequently, the milk is viscid or composed or floccular deposits in a clear or yellow-tinged supernatant. Initially, the cellular response is granulocyte with a lymphocytic infiltrate predominating as the infection becomes chronic. Progressive fibroplasia of the gland often ensues. Upon resolution of active infection, the affected gland rarely returns to the previous level of production and organisms may be shed intermittently for long periods.

### Arthritis

*Mycoplasma bovis* is the most common cause of mycoplasma arthritis in calves. Infection occurs as a result of bacteremia from the lungs with localization to the synovial membrane. Mastitic milk may be another source. One limb is usually affected; the stifle, hock and elbow are the most commonly affected joints. A fibrinous, cream-colored exudate is found in the joint cavity. Histologically there is a mononuclear cell infiltrate with focal ulceration of the synovial membrane. Extension of the lesion to subchondral bone can cause an osteomyelitis.

### Respiratory Disease

*Mycoplasma mycoides* subspecies *mycoides* (small-colony type) is the most notorious of the mycoplasmas responsible for respiratory-tract infections in cattle. It is the cause of contagious bovine pleuropneumonia (CBPP). Fortunately, CBPP is not presently recognized to be in the United States. It is endemic in Africa and Asia. CBPP causes a fibrinonecrotic bronchopneumonia and serofibrinous pleuritis. Necrotic areas of lung, resulting from vascular thromboses, become sequestered in fibrous capsules. Both chronic and acute manifestations of the disease exist. The disease is spread predominantly from animal to animal by respiratory aerosols.

Other mycoplasmas, including *M. bovis*, *M. dispar* and *Ureaplasma* spp., are frequently isolated from cases of enzootic pneumonia in calves. *Mycoplasma bovis* is

the most pathogenic of these mycoplasmas. Often more than one species of mycoplasma is present. The predominant pathology associated with mycoplasma pneumonia is a bronchitis, bronchiolitis and bronchopneumonia. In more advanced cases, foci of coagulation necrosis occur. Chronic, low-grade inflammatory forms also occur. By itself, mycoplasma pneumonia is rarely fatal. In most cases other bacterial pathogens such as *Pasteurella haemolytica* and *P. multocida* are also found and *Mycoplasma* is considered to be but one of a number of pathogens involved in this respiratory disease complex.

Otitis media due to mycoplasma, usually *M. bovis*, can be a sequelae to chronic respiratory-tract infections in calves. Calves often present with head tilts and the ears may droop. Infection ascends the eustachian tube. Frequently other respiratory pathogens are also found, although mycoplasma may be the only agent present. Chronic lesions tend to be caseous in nature.

### *Urogenital Tract Infections*

Interpreting of culture results for mycoplasmas or ureaplasmas from the urogenital tract is difficult because of the frequent isolation in the absence of clinical signs. *Mycoplasma bovis genitalium* has been associated with granular vulvitis.<sup>4</sup> Such lesions have been reproduced experimentally with *M. bovis genitalium*. *Mycoplasma bovis genitalium* has also been isolated from bulls with seminal vesiculitis and has been used to reproduce experimentally this condition.

The potential for fertility disorders associated with mycoplasmas in semen has been a subject of investigation. Because mycoplasma can adhere to sperm, some investigators speculate that presence of *M. bovis genitalium* in semen may adversely affect semen quality. A variety of antibiotics appear to control or kill mycoplasma in semen. The potential for contaminated sperm to directly affect the cows' fertility is also of concern. Both *M. bovis* and *M. bovis genitalium* have been shown to adhere to intact zona pellucida.<sup>5</sup> Neither standard washing procedures nor treatment with antibiotics or trypsin was effective in eliminating adherent mycoplasmas. Experimentally, adverse effects of mycoplasmas on explants of ciliated epithelial cell have been demonstrated raising concern about *in vivo* effects on ciliated cells, such as those in the oviduct.

Although *M. bovis* infects the reproductive tract less often, under experimental conditions the disease consequences are more severe than are *M. bovis genitalium* infections. *Mycoplasma bovis*, as well as other mycoplasma species, has been isolated from aborted bovine fetuses. Mycoplasma abortions in cattle, however, are not considered (or at least not recognized) as common.

*Ureaplasma diversum* is a common inhabitant of the vagina of the cow and prepuce and urethra of the

bull. Ureaplasmas have been associated with granular vulvitis, salpingitis, endometritis and abortions. The vulvitis associated with ureaplasmas presents with copious mucopurulent discharge, which helps differentiate it from infectious pustular vulvovaginitis. The cow does not appear systemically ill. The relationship of granular vulvitis and infertility is unclear, although some studies suggest an adverse affect on fertility. Lesions of granular vulvitis consist of small, elevated papules primarily located on the vulva. Microscopically, the lesions consist of lymphoid aggregations below the squamous epithelium. Ureaplasmas have also been isolated from cows with salpingitis and endometritis, both of which have been reproduced experimentally. Effects of *U. diversum* on the cilia of the oviduct are thought to adversely affect fertility.

Ureaplasmas are generally considered to be a rare cause of abortion in cattle, although some geographic locations report a fairly high percentage of the abortion due to ureaplasma. Ureaplasma abortions occur in the last trimester and can cause premature delivery. The pathology associated with these abortions is a multifocal-to-confluent necrosis on the inner surface of the amnion with vasculitis, fibrosis and occasionally mineralization. An interstitial pneumonia with monocytic airway cuffing frequently occurs.

### *Conjunctivitis*

Both *Mycoplasma bovoculi* and *Ureaplasma* spp. have been associated with conjunctivitis in cattle. Experimentally, both organisms can cause conjunctivitis. The infection usually is self-limiting. Mycoplasma conjunctivitis should be differentiated from other ocular infections such as those caused by IBR virus and *Moraxella bovis* (infectious bovine keratoconjunctivitis). Some investigators suggest that conjunctival infection with *M. bovoculi* is a predisposing factor to developing infectious bovine keratoconjunctivitis.<sup>6</sup>

### *Abscesses*

Decubital abscesses caused by *M. bovis* are a recently recognized manifestation of mycoplasma infections in dairy calves.<sup>3</sup> Abscesses are associated with confinement in hutches and develop in pressure areas such as the brisket region, dorsal aspect of the carpus and lateral aspect of the hock. The lesions contain a thick, yellow coagulated material and are encapsulate in thick, fibrous tissue. Although concurrent mycoplasma pneumonia has been found in some cases, no involvement of the joints adjacent to affected areas has been detected. The initial source of *M. bovis* is unknown, although in some cases a practice of feeding mastitic milk was employed.

### *Spongiform encephalopathies???*

Although most investigators subscribe to the prion



hypothesis for the spongiform encephalopathies (including mad cow disease), some researchers have speculated on the possible role of bacteria, specifically spiroplasmas, as the cause.<sup>2</sup> Spiroplasmas are spiral mycoplasmas isolated most commonly from plants. Evidence cited for the role of spiroplasmas includes (1) presence of spiral inclusions in brain tissue of spongiform encephalopathy cases that are morphologically similar to spiroplasmas, (2) the presence of detergent-extractable internal fibrils in spiroplasmas that appear identical to scrapie-associated fibrils, and (3) internal fibrils from spiroplasmas antigenically crossreact with scrapie-associated fibrils in immuno-blot studies. While these findings are intriguing, the actual role of spiroplasmas in spongiform encephalopathies is far from conclusive and will require much additional study.

### Diagnosis of Mycoplasma Infections

Direct gram stain of clinical material generally does not facilitate diagnosis of mycoplasma infections because the organisms stain poorly, if at all. Isolation remains the most common means used for diagnosing bovine mycoplasma infections. Most pathogenic bovine mycoplasmas are isolated readily on suitably enriched, artificial media. Serum, usually of horse origin, is a requirement for a source for cholesterol incorporation into the cell membrane. Other components (including yeast extract as a source of nucleotides) are also fundamental to mycoplasma medias. Some species, such as *M. dispar*, require special media formulation. Because the mycoplasmas do not possess a cell wall, penicillin or other antibiotics that affect peptidoglycan synthesis are often incorporated in media to reduce bacterial contamination. Thallium acetate is often included to suppress fungi and some gram-negative bacteria. Colonies are usually present in 3-4 days, however, cultures should be held for a week or longer. Pre-enrichment in a mycoplasma broth for 48 hours may improve the recovery rate, especially when only small numbers of organisms are present. Colonies are small and best visualized with the aid of a stereomicroscope. The typical, fried-egg colony is suggestive of a mycoplasma, however, not all mycoplasma species produce this morphology. A dienes stain helps to differentiate members of the Mycoplasmatales from other bacteria. Sensitivity to digitonin can be used to differentiate non-pathogenic *Acholeplasma* spp. (a common environmental mycoplasma) from potentially pathogenic *Mycoplasma* spp. Certain biochemical tests (glucose fermentation and arginine hydrolysis) can be used in identifying individual species; however, final identification of a specific mycoplasma species requires immunologic methods such as indirect fluorescent antibody tests, immunoperoxidase tests and growth inhibition tests.

Serology is not routinely used as a diagnostic tool for bovine mycoplasma infections, except for testing for CBPP. Alternative methods such as PCR and cellular fatty acid analysis are currently being investigated as ways of differentiating bovine mycoplasma species. Direct fluorescent antibody tests and immunoperoxidase staining on tissue sections have been used for in situ identification of some species such as *M. dispar* and *M. bovis*.

Isolation of *Ureaplasma* sp. is somewhat more difficult and requires a special media containing urea. Because of this species sensitivity to pH changes, sample dilutions or frequent subculturing of ureaplasma broths is required for successful isolation. Ureaplasma colonies on agar media appear brown or black from deposition of divalent cations.

Culture attempts for mycoplasmas and ureaplasmas are not practiced routinely in abortion workups. Since the placenta is frequently not available for evaluation, pathology in the fetal lung consisting of a mononuclear infiltrate in the alveolar duct may be the first suggestion of a *Ureaplasma*-related abortion. A lymphocytic infiltrate in the lamina propria of fetal conjunctiva with goblet cell metaplasia may also suggest a *Ureaplasma* abortion. Recovery of *Ureaplasma* from the fetus alone, without accompanying pathology, is not sufficient for diagnosis.

Mycoplasmas are isolated readily from acute cases of arthritis. Isolation is more difficult from chronic lesions. Culturing of the synovial membrane rather than joint fluid may yield better results.

### Control and Treatment

Most mycoplasmas are sensitive *in vitro* to tetracyclines, macrolides (tylosin, tilmicosin), and quinolones. Despite this *in vitro* susceptibility, treatment failures are common. Superficial, localized infections may respond adequately to topical antibiotic application.

Cows with mycoplasma mastitis generally respond poorly, at best, to antibiotic therapy. Control efforts should be directed towards improved management to minimize spread among cows, as with other contagious mastitis pathogens. Some individuals recommend whole-herd cultures, with removal of all culture-positive cows. This may not be economically feasible; furthermore, removal of clinically affected animals and improved management may be an effective control. Bulk tanks should be monitored routinely for the presence of mycoplasmas.

Many cases of mycoplasma arthritis eventually resolve. Early intervention with antibiotics may be helpful. Once articular damage has occurred, chronic unresponsive arthritis may ensue.

Granular vulvitis may not require any specific treatment, although a myriad of treatments have been tried. Infusion of tetracycline solution into the lower

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genital tract is a common treatment. Infusion of caustics have also been reported to be beneficial. Use of artificial insemination (AI) may decrease spread among cows. Care should be taken with AI to prevent the transfer of organisms from the lower genital tract into the uterus.

Since mycoplasma-associated pneumonias usually involve other pathogens, treatments must be directed at the spectrum of potential etiologic agents. Management changes to minimize stress factors associated with pneumonia are also important. Antibiotics that act on cell walls, commonly used for treatment of respiratory diseases, will not affect mycoplasmas. Early recognition and treatment of respiratory-related problems minimizes the development of otitis media.

Decubital abscesses do not respond to antibiotic treatment; they persist despite repeated lancing of the abscesses.

## Bovine Salmonellosis

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The role of *Salmonella* in feedlot and stocker calf disease complex is probably more significant than is routinely recognized. Some of the recognition problem is that the clinical presentation in older calves is not the same as those observed in younger nursing calves which usually have an acutely fatal severe hemorrhagic diarrhea with septicemia. Additionally, some salmonella infections/isolates may in fact be secondary to a primary disease process but still play a significant role in the outcome of the primary disease. Some salmonella serotypes are of minimal pathogenicity and may have little or no clinical significance even though they are readily isolated.

Unfortunately, *Salmonella* serotyping frequently requires several weeks. Because of the many opportunities for confusion, the significance placed on a salmonella isolate can be inaccurate.

Signs of salmonella in older calves are more subtle and they often appear as poor doers or chronics. Chronic salmonella infection of the large intestine may or may not produce significant changes in the character of the stool. However, a persistent infection causing mucosal damage, with the concurrent loss of protein and the continual absorption of low levels of salmonella endotoxin, results in poor doers and chronics. Calves in this compromised state are more susceptible to many of the common viruses and bacteria present in multiple origin

cattle. *Salmonella* infected cattle appear to be unable to recover from these infections in a normal manner. Subsequently, the typical "sweat out" never seems to end and cattle continue to be pulled and repulled from salmonella infected pens.

In older calves, a concurrent BVD infection may be associated with salmonella infection making determination of the primary infection difficult.

At necropsy, the more prominent lesions are often related to other concurrent infections with pneumonia being the more prominent and readily observable necropsy lesion. If the large intestine is examined in chronically infected calves, close examination will reveal changes in the mucosa of the cecum and large intestine, ranging from slight thickening and hyperemia to extensive thickening, with focal erosions and ulcerations. A diagnosis requires culture and collection and fixation of at least three sections of the large intestine for histopathological evaluation. Culture is often negative because many of these calves are clinically ill a few days prior to death and have been treated with multiple antibiotics. In the absence of a positive salmonella culture, histological lesions of mucosal ulceration and necrosis with vascular thrombosis of the large intestine are consistent with a tentative diagnosis of salmonellosis in stocker and feeder calves.