Feedlot Session

Moderators: Bill DuBois, Tom Portillo

Mycoplasma bovis – Unique Features, Pathogenesis and Lesions Update

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Abstract

In Canadian and probably most US feedlots, *Mycoplasma bovis* pneumonia with or without concurrent arthritis is found in a high percentage of antibiotic non-responsive, chronically sick cattle. Extensive multifocal yellow areas of caseation-like necrosis with sequestra formation are characteristic lesions. Subacute to chronic fibrinous or fibrosing pleuritis may accompany the lung lesions. Arthritis with similar necrotizing lesions of adjacent soft tissues, including tendon sheaths, are present in some cases. Characteristic gross lesions, unique histologic lesions and confirmation by IHC demonstration of *M. bovis* in these lesions are the best diagnostic tools in our experience. This chronic pneumonia and polyarthritis syndrome (CPPS) is a very costly syndrome in the feedlot industry, and management changes will probably be needed to control the problem. Vaccines are unlikely to be the solution to this problem. Prevention of transient BVDV infections is a scheme that may be beneficial, but more experimental work is required to confirm this.

Introduction

Mycoplasma bovis pneumonia and arthritis is being increasingly diagnosed as an infectious disease complex of feedlot cattle throughout the world.[6] Initially isolated from North American cattle in 1962, *M bovis* is now recognized to be responsible for a significant proportion of subacute to chronic, non-antibiotic-responsive feedlot cattle pneumonia and/or arthritis cases. Some parts of North America are also experiencing serious financial losses due to *M. bovis* mastitis in dairy cattle herds.[5] Other miscellaneous *M. bovis* infectious include otitis media, decubitus ulcers/abscesses, laryngeal necrosis, conjunctivitis, meningitis, endometritis, salpingitis, oophoritis, seminal vesiculitis and orchitis.[9] In Canada CPPS is now the accepted terminology for *M. bovis* infections of feedlot cattle.[4] The lung lesions are quite different than so-called "cuffing pneumonia" as seen in veal calf enzootic pneumonia for which *M. bovis* is considered to be at least partially responsible.

Classification, Virulence Factors and Special Features of *M. bovis*

M. bovis belongs to the family Mycoplasmataceae and is the second most pathogenic member of the *Mycoplasma* genus. Contagious bovine pleuropneumonia (CBPP) is caused by *M. mycoides* subspecies *mycoides* (small colony type), which is the most pathogenic of the bovine mycoplasmas, and was exterminated from North American cattle in 1892. It is still endemic in parts of Africa and Asia in cattle and domestic water buffalo. However, the subacute to gross lesions of CPPS pneumonia as seen in feedlot cattle are remarkably similar to the subacute and chronic lesions of CBPP.

Many features of *M. bovis* make it well armed to cause extensive lesions, as either a primary or secondary pathogen and especially under feedlot management conditions. Like other mycoplasma species, it has no cell wall, thus beta-lactam antibiotics (penicillins, cephalosporins, carbopenems and monobactams) are of no value for therapy. Tilmicosin, tylosin, tetracyclines, lincomycin, spectinomycin and florfenicol have the most potential for therapy, but extensive field response studies and recent *in vitro* sensitivity tests are showing poor to no response with these antibiotics. Lack of a cell wall also means that Gram stains are of no value to identify the organism on direct smears or formalin fixed and paraffin embedded sections, and special culture media and conditions are required to culture the organism. Diagnostic laboratories therefore do not routinely moni-

tor for *M. bovis* in field specimens. In our hands, histopathology along with immunohistochemistry (IHC) on fixed tissues works extremely well, and IHC in particular is the basis for my abundant experience with the lesions of this organism in the lung, joint and other soft tissues that extends now over 20 years. The ability to visualize the organisms relative to the distinct and unique lesions of this organism is the huge advantage of IHC. Sensitivity and specificity of the IHC technique is essentially 100% for *M. bovis*. The IHC technique has other advantages, including being able to do retrospective studies on stored paraffin sections, convenience for the veterinarian (only fixed tissue needs to be submitted) and the stained sections are permanently stored along with the H and E stained slides. Culturing the organism and PCR techniques on lung and other tissues are less reliable because *M. bovis* is a commensal of the bovine upper and lower respiratory tract, especially in feedlot cattle. Research work and identification of this organism in dairy herds with mastitis problems, however, make culture and PCR techniques necessary and very valuable.

Toxins produced by *M. bovis* have not been demonstrated, but many other virulence factors and features allow this organism to produce extensive lung and other soft tissue lesions. *M. bovis* induces IL-1, IL-6 and TNF from macrophages and cytotoxic T lymphocytes, resulting in an endotoxin-like effect. In addition, the organism survives severe inflammatory reactions by reducing the respiratory burst of neutrophils. It also destroys T lymphocytes by apoptosis and thus results in immunosuppression via its effects on both humoral and cellular immune responses. *M. bovis* also has numerous extensively studied surface lipoproteins (Vsp) that can rapidly be activated or deactivated, resulting in rapid antigenic variation and thus allowing it to evade the immune system and contribute to chronic persistent infections. These lipoproteins may also contribute to different tissue tropisms with different "strains" of the organism.[3] The Vsp is a reason why vaccines may not be successful against this organism. By mechanisms not yet clearly understood, *M. bovis* can readily enter the bloodstream from within the lower respiratory tract and this is undoubtedly responsible for the common coexisting joint, tenosynovitis and sometimes other soft tissue lesions seen. The normal mucociliary system of the lung, trachea and larynx probably needs to be damaged for this invasion to occur and this may explain why other concurrent infections (eg. BVDV) and stressors such as crowding, transport and adverse weather conditions are considered important predisposing factors for *M. bovis* infections. Peroxide and superoxide oxygen products produced by *M. bovis* are also thought to play a role in tissue destruction in the lung and other soft tissues. The organism's ability to survive in cool, moist environments, be a commensal in nonclinical carrier cattle, and rapid spread by aerosol transmission are also important in feedlot situations.

Case Definition of CPPS

CPPS cases are calves with persistent low grade fever, poor to no response to antibiotic therapy, frequent lack of weight gain or progressive weight loss, and usually are transferred to a convalescent or chronic pen. Severe lameness is usually present in those with progressive weight loss and eventually they may remain recumbent. Distinct respiratory signs are often not obvious unless 75% or more of total lung parenchyma is obliterated, at which time severe weakness due to hypoxemia and inability to feed properly become clinically apparent. Days on feed from initial treatment for bovine respiratory disease (BRD) until death or euthanasia is often 30 to 120 days or longer.[7]

Gross Post Mortem, Histopathologic and Immunohistochemical Lesions of CPPS

The lung lesions of *M. bovis* (CPPS) vary considerably from case to case depending on chronicity, cause of death (natural or euthanasia), other concurrent infections and therapy. Unique to *M. bovis* pneumonia is that death often does not occur until 75% or more of both lungs have been destroyed. With such extensive lesions the animal either dies after severe respiratory distress or dies suddenly with cor pulmonale. Both lungs are usually involved but it is common to have more extensive lesions of the right lung than the left. Concurrent acute fibrinous to very chronic fibrosing pleuritis is also not uncommon, and those cases with extensive pleuritis usually clinically progress to death more rapidly. The necrotizing lesions characteristic of *M. bovis* consistently involve the cranial and middle lung lobes, but in more severe cases involvement of the caudal lobes is also common. The necrotic areas of lung vary tremendously in size, but in less severe or early cases are small 2-4 mm circular foci of yellow to yellow-white material that commonly project above the pleural surface as palpable or visible nodular lesions as well as being scattered throughout multiple lobules on the cut surfaces. These yellow necrotic areas tend to be dry or caseous-like, but in some larger lesions purulent material may be visible in the more peripheral zones of these necrotic lesions. Tuberculosis always has to be considered as a differential diagnosis and the lesions indeed look remarkably similar. It is also common to see elongated or linear areas of similar yellow necrotic lesions, and these represent interlobular lymphatics stuffed with necrotic material and laden with masses of *M. bovis* organisms by immunohistochemistry. Characteristically

these necrotic lung lesions have minimal odor unless coexisting *Arcanobacterium pyogenes* or *Fusobacterium necrophorum* infections are also present. As the disease progresses, very large irregular ghost-like necrotic areas, involving often multiple lobules of lung parenchyma, occur and the outer zones may show white areas of fibrosis and sometimes with intervening purulent material. Large masses of necrotic lung, separated from the adjacent lung by purulent material, often dislodge easily or can be plucked out with the knife tip, and these plugs of dry, firm necrotic lung are called sequestra (pl.) or a sequestrum.[1,2] These necrotic masses of lung parenchyma may actually involve entire lung lobes and in fact often the entire right lung is necrotic.

Cases with concurrent *M. bovis* arthritis that are euthanatized for humane reasons often have much less extensive lung lesions, and in our experience not more than 10-15% of cattle with *M. bovis* pneumonia have arthritis as well. However, I have yet to diagnose *M. bovis* arthritis in which I could not find at least focal *M. bovis* lung lesions. The right middle and accessory lung lobes often have at least a few lung lesions in these mild cases with concurrent severe arthritis and tenosynovitis. One, two or more joints may be involved, but one or both stifle joints are involved in a high percentage of cases. Clinically the joints may not be severely enlarged and it is common for only one stifle to be involved. When there is stifle involvement, within the abdomen the corresponding internal iliac lymph nodes lateral to the abdominal pelvic inlet are usually very enlarged and should remind you to open one or both stifle joints. The joint exudates are abundant, typically yellow and primarily fibrinous, but some cloudy to slimy thick exudate is often present as well. The foul odor and creamy green to gray purulent exudates characteristic for *A. pyogenes* and other pyogenic bacteria are not present. Periarticular soft tissue involvement including tenosynovitis is common, and very consistent is extension of necrosis around the peroneus tertius and long digital extensor tendon that originate on the extensor fossa of the femur. This tendon passes through the joint space and into the extensor muscles below the stifle and just lateral to the tibial crest. Necrosis following this tendon and muscle as far as the hock is quite a consistent feature of *M. bovis* stifle infection. Other surrounding tissues, including skeletal muscles and ligaments, are often similarly involved. Involvement of coxofemoral, tarsus, carpus, elbow, scapulohumeral and even occipitoatlantal joints are sometimes seen.[8] The areas of necrosis in all soft tissues look the same as in the lung, being somewhat dry and yellow. Local necrotic lesions of the laryngeal vocal folds and heart wall or pericardial sac involvement are also not rare.

Histologically the lesions in all tissues are best described as brightly eosinophilic areas of coagulation necrosis. The necrotic areas are a very amorphous, hypereosinophilic mass of cells which are often necrotic neutrophils, judging by having examined hundreds of lesions in varying stages of development. In the larger lung lesions, central ghost-like outlines of necrotic alveolar parenchyma are distinctly visible. The outer edges of necrotic zones usually show macrophages, low numbers of lymphocytes and plasma cells, but lymphoid hyperplasia and intact neutrophils are not common. Sequestra lesions always have a peripheral thick outer zone of basophilic degenerate neutrophils and cell debris (the purulent zones seen grossly) separating the central necrotic areas from the adjacent lung tissue. Earlier, more acute or mild *M. bovis* lesions will show large numbers of intact to degenerate neutrophils in bronchiolar and alveolar lumens and contain *M. bovis* organisms on IHC, but these lesions are not seen grossly until they are at least 1mm in diameter. Interlobular lymphatics, when involved, are distended with similar necrotic neutrophils, some visible fibrin and often lightly basophilic masses of degenerate mycoplasma organisms. Inter-lesional fibrosis and/or collapsed lung parenchyma are common changes as well. Lungs with pleuritis show acute fibrinous exudation, but in chronic cases this fibrin gradually becomes organized and replaced by fibrosis. Immunohistochemistry using either polyclonal or *M. bovis* specific monoclonal antibodies shows strong positive staining confined to the eosinophilic coagulation necrosis areas, but the densest population of organisms is in the outer edges of the necrotic zones. Other bacteria that may be present in the same lesions and that the *M. bovis* reagents do not cross react with include *M. haemolytica*, *Pasteurella multocida*, *A. pyogenes*, *F. necrophorum* and *H. somni*.

The earliest histologic lesions of *M. bovis* in the lung begin like any other bronchopneumonia, with organisms mixed with neutrophils in bronchiolar lumens and variable numbers of surrounding alveoli, but even at this earliest stage necrotic eosinophilic masses of neutrophils with no bacteria visible on H and E sections are a hallmark feature of *M. bovis*. Concurrent lung lesions of other bacteria are not uncommon as well, but antibiotic therapy up to the time of death or euthanasia has often eliminated these other organisms. It is my impression that many if not all of the milder lung *M. bovis* lesions begin with growth of the organism within the lumens of airways damaged by other infections and often, viral agents.

In my experience with CPPS cases, a careful histologic evaluation of multiple sections of heart, lung, ileum and sometimes kidneys will often reveal typical lymphocytic and/or necrotizing vasculitis or chronic proliferative arterial lesions of a transient BVDV infection, and IHC will show positive IHC staining for BVDV in these vessels. Positive BVDV staining most often occurs

if vascular tunica media necrosis is still actively occurring. The Peyer's patches follicles are always severely atrophic and depleted, but how much is due to immunosuppression by *M. bovis* itself or to BVDV infection is impossible to establish in natural cases.

Conclusions

With extensive post-mortem experience on large numbers of cases, the feedlot veterinarian can soon become comfortable with a gross diagnosis of CPPS, but in at least initial stages of this experience, confirmation by a diagnostic laboratory is needed. Immunohistochemistry is the most efficient way for the diagnostic pathologist to become comfortable making this diagnosis, but both gross and histopathologic lesions of *M. bovis* in at least subacute to chronic cases of CPPS are remarkably distinctive and allow a diagnosis of *M. bovis* pneumonia to be reliably made.

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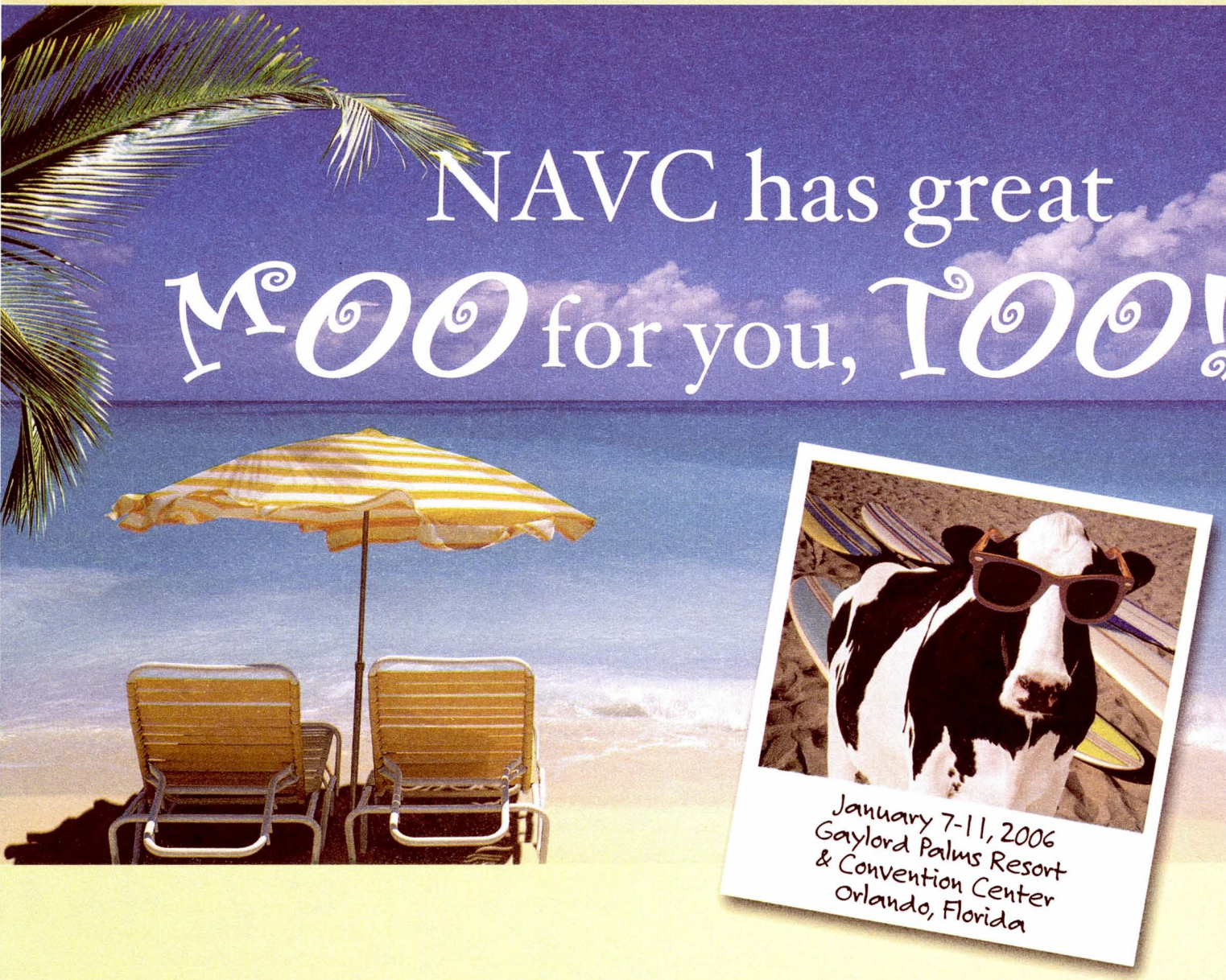

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