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Update on bacterial pathogenesis in BRD

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Abstract

Mannheimia haemolytica, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis* and *Arcanobacterium pyogenes* are all frequently implicated in bovine respiratory disease (BRD). *M. haemolytica* is considered the most important of the group. These bacteria are commensals in the nasopharynx and establish infection in the lungs of cattle that are subjected to a variety of stresses. Factors that permit adherence to and proliferation in the lungs and factors that cause tissue destruction and inflammation have been identified as having major roles in pathogenesis. These virulence factors include protein adhesins, capsular polysaccharide, outer membrane proteins, iron-binding proteins, lipopolysacharide or lipooligosaccharide, enzymes and toxins. These bacterial products function to evade the immune system, damage the immune system and induce a severe inflammatory response.

Keywords: *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Arcanobacterium pyogenes*, *Bibersteinia trehalosi*, *Mycoplasma bovis*

Introduction

In his overview of pathogenesis of pneumonia in feedlot cattle in the 1983 symposium on bovine respiratory disease (BRD), Thomson (1984) described only *Mannheimia haemolytica* and *Pasteurella multocida* as bacterial pathogens in the BRD complex. Today, we recognize that *M. haemolytica*, *P. multocida*, *Histophilus somni*, *Arcanobacterium pyogenes*, *Mycoplasma bovis* and most recently *Bibersteinia trehalosi* are associated with severe bacterial pneumonia frequently seen in dairy calves (enzootic pneumonia) and in feedlot cattle (shipping fever). All of these bacteria are ubiquitous in the cattle population as commensals in the nasopharynx and, following stress or viral infection, can proliferate and be inhaled into the lungs. Each has its own cadre of known virulence factors such as adhesins, toxins and enzymes that enhance its ability to colonize, cause tissue destruction and incite an intense inflammatory response. This article reviews the virulence factors, pathogenesis and lesions associated with these bacteria. The reader is referred to several excellent reviews on these bacteria (Czuprynski *et al.*, 2004; Jost and Billington, 2005; Caswell and Archambault, 2007; Corbeil, 2007; Dabo *et al.*, 2007; Rice *et al.*, 2007).

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M. haemolytica (formerly *Pasteurella haemolytica* Biotype A)

Of the pathogenic bacteria discussed herein, *M. haemolytica* is arguably the most important of the group and has traditionally been associated with the acute fulminating pleuropneumonia seen within the first weeks of cattle feeding. This Gram-negative bacterium has 12 representative capsular serotypes, but serotype 1 (S1) and S6 are the most prevalent in bovine pneumonia. In early life, calves acquire *M. haemolytica* through contact with their dams and other cattle. The bacterium resides in the nasopharynx and tonsils with the latter considered the major reservoir (Rice *et al.*, 2007).

Virulence factors are many and include protein adhesins, capsular polysaccharide, lipopolysaccharide (LPS), iron-binding proteins, secreted enzymes and a ruminant-specific RTX toxin – leukotoxin (LKT). Specific adhesins include a glycoprotein, N-acetyl-D-glucosamine, that mediates adherence to tracheal epithelial cells and activates the oxidative burst of bovine neutrophils. Heat-modifiable outer membrane protein A (OmpA) and the

surface lipoprotein 1 (Lpp1) mediate *M. haemolytica* binding to bronchial epithelial cells. In addition, the *M. haemolytica* capsule may function as an adhesin in addition to its antiphagocytic properties. Neuraminidase and sialoglycoprotease modify cell surfaces and may enhance bacterial adhesion. *M. haemolytica* LPS has typical endotoxic and proinflammatory properties, causes vasculitis, and complexes with LKT enhancing LKT-receptor production and augmenting LKT activity. LKT induces dose-related changes in bovine leukocytes. At high LKT concentrations, cells undergo rapid osmotic swelling, membrane pore formation and necrosis. At reduced doses, LKT can induce leukocyte apoptosis or release of proinflammatory cytokines, oxygen free radicals and cellular proteases. The sum result of these virulence factors is alveolar and vascular damage with pulmonary inflammation (Confer *et al.*, 1995; Czuprynski and Welch, 1995; Czuprynski *et al.*, 2004; Rice *et al.*, 2007).

M. haemolytica-induced pneumonia is characterized by acute cranioventral fibrinous to fibrinopurulent pleuropneumonia. On the cut surface, there is characteristic 'marbling' of the lung with lobules varying from normal, gray, or red. Whole lobules undergo hemorrhage or coagulation necrosis. Interlobular septa are usually distended with fibrin-rich edema fluid, and interlobular lymphatics may contain fibrin thrombi. Histologically, alveoli are flooded with fibrin-rich exudate, a variable neutrophil and macrophage infiltrate, and the traditional 'oat cells' that are flattened and streaming necrotic macrophages within the affected alveoli. Lobules undergoing coagulation necrosis appear as infarcts with thick neutrophilic infiltrates around the periphery of the necrotic lobule. Vasculitis with fibrin thrombi is frequently seen. Bronchioles often are spared or contain fibrin-rich exudate that may extend from alveoli (Caswell and Williams, 2007).

P. multocida

P. multocida has been isolated from up to 40% of the cases of enzootic and shipping fever pneumonia (Welsh *et al.*, 2004). This Gram-negative bacterium is acquired at an early age from the dam and is a common nasopharyngeal commensal. Serotype A:3 is the most common *P. multocida* isolate from bovine pneumonia, and pneumonia associated with *P. multocida* infections (whether in young dairy calves, in weaned and shipped beef cattle, or in calves experimentally challenged) is often difficult to discern from pneumonia associated with other bovine bacterial pathogens, i.e. *M. haemolytica* and *H. somni*. Dungworth (1985) indicated that 'less fulminating . . .bronchopneumonias tend to be more often caused by *P. multocida* than by *P.* [*Mannheimia*] *haemolytica*'. In fact, isolation of more than one bacterial pathogen from pneumonic lungs in conjunction with respiratory viruses and/or *Mycoplasma* spp. occurs frequently (Gagea *et al.*, 2006b).

P. multocida A:3 virulence factors are less numerous than those identified in *M. haemolytica*. Several adhesins, a thick polysaccharide capsule and LPS are the major factors. Adhesins responsible for adherence of the bacterium to cell surfaces include type IV fimbriae, OmpA, neuraminidase and filamentous hemagglutinin (FHA); the latter is similar to FHA found in *Bordetella* spp. In addition, OmpA, various iron-binding proteins such as hemoglobin-binding protein A and transferrin-binding protein A bind fibronectin and other extracellular matrix proteins suggesting that they aid in invasion. *P. multocida* LPS induces classic signs of endotoxic shock and has biological and chemical properties and R-type LPS structure (with no polymeric O-antigen) similar to those found in many Gram-negative bacteria (Harper *et al.*, 2006). *P. multocida* LPS is a potent stimulator of inflammatory cytokines and a predominant inciter of pulmonary inflammation. The importance of capsule as a virulence determinant in the pathogenesis of *P. multocida* infection is due to its antiphagocytic properties (Dabo *et al.*, 2007).

Various authors have described the *P. multocida*-associated gross pulmonary pathological changes differently, and those designations probably reflect (1) the age of the lesion, (2) whether or not other infectious agents were involved but not identified, and (3) descriptive preferences of the various authors. The lesion is a typical cranioventral bronchopneumonia and has been characterized simply as bronchopneumonia or as a bronchopneumonia with various descriptive modifiers including acute fibrinosuppurative, subacute to chronic fibrinopurulent, fibrinous to fibrinopurulent, suppurative and fibrino-necrotizing (Dabo *et al.*, 2007). The presence of fibrinous to fibrinopurulent pleuritis, distended interlobular septa with edema or fibrin, and/or abscesses is variable with *P. multocida* infection (Caswell and Williams, 2007).

H. somni (formerly *Haemophilus somnus*)

H. somni, a Gram-negative bacterium, is associated with numerous pathological processes including pneumonia, septicemia, myocarditis, abortion, thrombotic meningoencephalomyelitis and synovitis. The bacterium is associated with enzootic or shipping fever pneumonia with epidemiological studies demonstrating marked variation in its prevalence compared to *M. haemolytica* or *P. multocida* in shipping fever pneumonia (Corbeil, 2007).

H. somni are non-encapsulated, and the virulence factors include lipooligosaccharide (LOS) and various Omps – especially transferrin-binding proteins and immunoglobulin-binding proteins (IgBPs). Studies of adhesion proteins are generally lacking. Of particular

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interest with respect to virulence are LOS and IgBPs. LOS can mediate endothelial cell apoptosis, and through antigenic phase variation can assist the bacterium to escape the host immune response. IgBPs are surface-exposed fibrillar protein networks that bind the Fc domain of bovine IgG2 and are responsible for resistance to complement-mediated serum killing, a characteristic of pathogenic strains of *H. somni* (Corbeil, 2007). Recently, studies demonstrated *H. somni* wild-type strain was cytotoxic for macrophages and inhibited phagocytosis of microspheres through disruption of the actin-filament structure. Conversely, cytotoxicity and phagocytosis inhibition were absent in an IgBPA – deficient mutant (Hoshinoo *et al.*, 2009). *H. somni* produces histamine, which in conjunction with anti-major Omp IgE, may account for early respiratory lesions (Corbeil, 2007). Recently, biofilm production by *H. somni* within the host has been documented, and FHA proteins may be involved in that process (Sandal *et al.*, 2009).

Gross lesions of acute fulminating pneumonia due to *H. somni* are similar to those seen with *M. haemolytica* infection, i.e. a cranioventral fibrinous pleuropneumonia with hemorrhage and coagulation necrosis that can involve entire lobules. Pulmonary vasculitis is often seen, and those vessels may contain fibrin thrombi. In several studies, *H. somni* was isolated later in the feeding period than was *M. haemolytica*, and in *H. somni* cases, the pneumonic lesions included fibrosis, fibrous pleural adhesions and bronchiolitis obliterans (Caswell and Williams, 2007).

M. bovis

This bacterium has been the subject of considerable investigation; however, its primary role in bovine bacterial pneumonia is controversial. Much of the controversy is because many healthy feedlot cattle shed *M. bovis* from the nasal passages with approximately 50% of cattle positive upon feedlot entry and nearly 100% positive by day 12 (Allen *et al.*, 1992). In addition, *M. bovis* has been isolated from up to 45% of grossly and histologically normal bovine lungs (Gagea *et al.*, 2006b). The bacterium is predominately an extracellular pathogen that is present on respiratory epithelial surfaces. *M. bovis* antigen is present within macrophages of infected lung, and that antigen is most likely phagocytized debris (Caswell and Archambault, 2007).

Virulence factors include variable surface proteins (VSPs) that function as adhesins, are responsible for phenotypic variation among *M. bovis* strains, and allow for evasion of host immune responses. Several other surface proteins have been demonstrated that assist in colonization. A polysaccharide toxin has been described; however, the role or even existence of that toxin remains controversial. *M. bovis* strains may produce hydrogen peroxide, which forms oxygen free radicals and causes host lipid peroxidation. Heat shock proteins are produced, but their role in virulence is not known. The formation of biofilm has been associated with numerous *M. bovis* strains, and that trait enhances immune and antimicrobial resistance and colonization (Caswell and Archambault, 2007).

Transmission of *M. bovis* is through nasal shedding and fomites; however, direct contact with others is the most common route, because survival outside the host is limited. Lesions attributable to *M. bovis* are a cranioventral caseonecrotic bronchopneumonia that may have abscesses, bronchiectasis and sequestration (Gagea *et al.*, 2006a). Arthritis may accompany respiratory disease. The chronic nature of the pneumonia is apparent at the gross and microscopic levels. In addition, the highest percentages of *M. bovis* pneumonia are in cattle previously treated for BRD. Death often occurs after 4–6 weeks in the feedlot (Booker *et al.*, 2008). Histologically, *M. bovis* antigen is present within and surrounding the caseous foci (Khodakaram-Tafti and López, 2004).

A. pyogenes (formerly *Actinomyces pyogenes*)

A. pyogenes is a Gram-positive, rod-shaped bacterium, which is a common pathogen in bovine abscesses. In the lungs, *A. pyogenes* is associated with chronic abscessing pneumonia. *A. pyogenes* is an inhabitant of many mucosal surfaces in cattle including the nasopharynx and appears to be a secondary invader into a lung already pneumonic due to other infectious agents. Factors precipitating its proliferation and inhalation are not known; however, Catry *et al.* (2008) recently demonstrated that fluoroquinolone administration to healthy cattle diminished upper respiratory *P. multocida*, whereas *A. pyogenes* became the predominant nasopharyngeal isolate. Therefore, it may survive and proliferate in cattle treated for BRD.

A. pyogenes has several important virulence factors. These include a collagen-binding protein (CbpA) that allows it to bind collagen and promote adhesion to host cells (Pietrocola *et al.*, 2007). A cholesterol-dependent cytolysin (pyolysin) that is a pore-forming cytolysin for immune cells and is also a hemolysin has been characterized (Rudnick *et al.*, 2008). Several adhesins have been identified including two neuraminidases, which cleave sialic acids and expose cell receptors, and type 2 fimbrae. Several extracellular matrix-binding proteins that bind to collagen or fibronectin and exoenzymes (DNAse and proteases) assist in invasion of tissue and degradation of proteins and nucleic acids. In addition, *A. pyogenes* can evade host defenses by invasion of epithelium, intracellular survival in macrophages and formation of biofilm (Jost and Billington, 2005).

Transmission of *A. pyogenes* occurs at a young age with calves acquiring the bacterium from their dams. Pulmonary lesions ascribed to *A. pyogenes* are primarily severe abscesses within areas of chronic bronchopneumonia or

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chronic pleuropneumonia (Lopez, 2007). These abscesses are typically characterized by liquefactive necrosis surrounded by a thick fibrous connective tissue band, whereas they are often larger and less caseous than *M. bovis*-induced lesions.

B. trehalosi (formerly *Pasteurella trehalosi*)

B. trehalosi was originally classified as *P. haemolytica* Biotype T and is primarily a sheep pathogen, especially associated with septicemia (Blackall *et al.*, 2007). *B. trehalosi* has been associated with severe pneumonia in Bighorn sheep and domestic sheep (Goodwin-Ray *et al.*, 2008). Most recently, *B. trehalosi*-associated pneumonia in young dairy calves has been described, though not documented in the refereed literature.

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