**Pasteurella multocida** and bovine respiratory disease

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**Abstract**

*Pasteurella multocida* is a pathogenic Gram-negative bacterium that has been classified into three subspecies, five capsular serogroups and 16 serotypes. *P. multocida* serogroup A isolates are bovine nasopharyngeal commensals, bovine pathogens and common isolates from bovine respiratory disease (BRD), both enzootic calf pneumonia of young dairy calves and shipping fever of weaned, stressed beef cattle. *P. multocida* A:3 is the most common serotype isolated from BRD, and these isolates have limited heterogeneity based on outer membrane protein (OMP) profiles and ribotyping. Development of *P. multocida*-induced pneumonia is associated with environmental and stress factors such as shipping, co-mingling, and overcrowding as well as concurrent or predisposing viral or bacterial infections. Lung lesions consist of an acute to subacute bronchopneumonia that may or may not have an associated pleuritis. Numerous virulence or potential virulence factors have been described for bovine respiratory isolates including adherence and colonization factors, iron-regulated and acquisition proteins, extracellular enzymes such as neuraminidase, lipopolysaccharide, polysaccharide capsule and a variety of OMPs. Immunity of cattle against respiratory pasteurellosis is poorly understood; however, high serum antibodies to OMPs appear to be important for enhancing resistance to the bacterium. Currently available *P. multocida* vaccines for use in cattle are predominately traditional bacterins and a live streptomycin-dependent mutant. The field efficacy of these vaccines is not well documented in the literature.

**Keywords:** *Pasteurella multocida*, cattle, shipping fever, enzootic pneumonia, immunity, pathogenesis

**Basic taxonomy of Pasteurella multocida**

The taxonomy and nomenclature of *Pasteurella* have undergone many changes since first isolated by Pasteur as the causative agent of fowl cholera in 1880 (Fajfar-Whetstone et al., 1995). Nearly a half century later (in 1929), bacteria with common biochemical and morphological features were grouped together as *Pasteurella septica* and thereafter (in 1939) as *Pasteurella multocida* (Weber et al., 1984; Fajfar-Whetstone et al., 1995). Later, Mutters et al. (1985) reclassified the members of the genus *Pasteurella* into 11 species including *P. multocida* within which three subspecies (subsp.) *multocida*, *septica* and *gallicida* were defined based on sugar (D-sorbitol and dulcitol) fermentation. Molecular techniques based on M13 core fingerprinting and α-glucosidase activity were also developed to better differentiate between *P. multocida* subsp. *multocida* and subsp. *septica* (Hunt Gerardo et al., 2001). *P. multocida* subsp. *multocida*, the most important pathogenic member of the genus *Pasteurella sensu stricto*, has a broad host spectrum that includes many wild and domestic animal species. *P. multocida* subsp. *gallicida* and *septica* are specifically isolated from fowl and domestic pets (cats and dogs), respectively (Mutters et al., 1985). To define isolates associated with tiger bites, a fourth subspecies (tigris) was recently proposed by Capitini et al. (2002) on the basis of phenotype and 16S rRNA gene sequence comparison.

The capsule structure and lipopolysaccharide (LPS) composition of *P. multocida* are the basis for the classification of the bacterium into serogroups and
P. multocida association with BRD

It is clear that P. multocida is one of the primary bacterial pathogens associated with the clinical syndromes defining BRD complex including neonatal calf (enzootic) pneumonia and beef cattle pneumonia (shipping fever) (Lillie, 1974; Watts et al., 1994; Fulton et al., 2000; Welsh et al., 2004). Both syndromes are considered multifactorial; that is, multiple infectious and non-infectious factors are involved in development of disease (Ames, 1997; Mosier, 1997). Unfortunately, many epidemiological studies of these conditions failed to differentiate among the variety of pathogens involved.

Strong evidence exists to link P. multocida to respiratory disease in dairy calves. This includes culture from lung tissue (Van Donkersgoed et al., 1993; Sivula et al., 1996a; Singer et al., 1998; Hirose et al., 2003), from nasopharyngeal swabs (NPS) (Catry et al., 2006) and from transtracheal washes (TTW) (Singer et al., 1998; Virtala et al., 2000). The significance of P. multocida in calf pneumonia is more apparent than with shipping fever. Multiple studies involving dairy calves have identified P. multocida in much higher prevalence than Mannheimia haemolytica (Bryson et al., 1978; Virtala et al., 2000; Nikunen et al., 2007). Studies conducted by Nikunen et al. (2007) on 84 calves with BRD in Finland found a significant relationship between the presence of P. multocida in calves with clinical signs of respiratory disease and the elevated concentrations of serum acute phase proteins (i.e. fibrinogen, haptoglobin, serum amyloid-A, LPS-binding protein and α1-acid glycoprotein), suggesting a strong pathogenic role for P. multocida in respiratory disease of calves. No significant relationship was observed for other pathogens including Mycoplasma sp., which were isolated in over 90% of calves as compared to 14% for P. multocida. Recent examination of 396 calves from 280 different dairy farms for occurrence of bacterial, mycoplasmal and viral pathogens found that P. multocida was the most commonly isolated bacterial pathogen and was detected in 26.4, 32.6 and 42.3% of tracheobronchial lavage samples from healthy, suspected or diseased calves, respectively (Autio et al., 2007). Seventy-five percent of the P. multocida isolates were indole-negative phenotypes, underlining the need to investigate the role of indole-negative P. multocida isolates in calf respiratory disease. Nonetheless, the presence of the bacterium does not equal disease, as seroconversion to P. multocida occurred in control calves, confirming the ubiquitous nature of the organism and the multifactorial nature of the disease (Virtala et al., 1996).

Evidence for involvement of P. multocida in shipping fever has been obtained by several methods. The bacterium was isolated from lungs of fatal cases in feedlot cattle by numerous investigators (Watts et al., 1994; Fulton et al., 2000; Storz et al., 2000a; Welsh et al., 2004; Gagea et al., 2006). Indeed, M. haemolytica is the most common isolated bacterium from shipping fever, but the proportion of fatal cases of respiratory disease in feedlot cattle attributable to P. multocida appears to be increasing (Welsh et al., 2004). Potential causes for this shift include changes in virulence among the pathogens, efficacy of antimicrobial agents, and changes in identification and/or management of sick cattle (Welsh et al., 2004). P. multocida was cultured from nasal or NPS, TTW and/or bronchoalveolar lavage (BAL) of sick animals (Allen et al., 1991; DeRosa et al., 2000; Fulton et al., 2002). A positive correlation was found between the species of bacteria isolated from multiple sites (e.g. NPS and TTW) (DeRosa et al., 2000). The presence of P. multocida in BAL fluid accounted for ~40% of respiratory disease (Allen et al., 1992a). However, the presence or absence of P. multocida in the nasal pharynx did predict disease (Allen et al., 1991; Fulton et al., 2002). Similarly, seroconversion occurred in a high percentage of calves and thus was not predictive of disease (Virtala et al., 2000). Nonetheless, low antibody concentration to P. multocida at feedlot entry correlated with decreased value to the owner (carcass value – total feedlot expenses) (Fulton et al., 2002). Therefore, these various findings suggest that P. multocida is ubiquitous and can be found in both healthy and sick cattle; and not all calves harboring or exposed to the bacterium will develop pneumonia. However, its presence (particularly in large numbers) in the nares, trachea or bronchioles of a sick calf strongly suggests its involvement in the disease process.

Although knowledge of P. multocida serogroup and serotype specificity from BRD cases is limited, the
reported data clearly singled out *P. multocida* serogroup A strains as the most frequently isolated from BRD cases (Blanco-Viera et al., 1995; Ewers et al., 2006; Autio et al., 2007; Nikunen et al., 2007). Studies by Blanco-Viera et al. (1995) showed that 61 and 25% of *P. multocida* isolated from pneumonic lung lesions belonged to capsular groups A and D, respectively, with serotype 3 (77%) being the most frequent. Kumar et al. (2004) investigated the prevalence of *P. multocida* serotypes in samples from different animal species in India. Cattle samples found to be positive for *P. multocida* were predominantly serotype A:3, in agreement with reports by Frank (1989). Studies in our laboratories found that 80% of *P. multocida* isolates from cattle with pneumonia were serotype A:3 (Dabo et al., 1997), and recently, more than 92% of *P. multocida* isolated from cattle were found to be serogroup A (Ewers et al., 2006).

**Clinical disease: shipping fever**

The term ‘undifferentiated bovine respiratory disease’ is often considered synonymous with ‘shipping fever’, although BRD may also be considered to include other disease entities, including enzootic calf pneumonia. For this discussion, respiratory disease in beef cattle will be referred to as shipping fever, which affects post-weaning beef calves, most commonly after transport to a stocker or feedlot operation. Diagnostic criteria are often vague, but typically include depression, inappetence, cough, nasal discharge and fever (or some combination of these). Most epidemiologic investigations relied upon producer treatment as a proxy for diagnosis. This frequently is no more exclusive than ‘animals that are pulled for treatment and found to have no signs referable to a system other than the respiratory tract’ (Bagley et al., 2003; Cusack, 2004). Studies have shown limited correlation between treatment and more objective measures such as mortality, BAL cytology and lung lesions at slaughter (Allen et al., 1992a, b; Griffin et al., 1994; Ribble et al., 1995a; Wittum et al., 1996; Bryant et al., 1999). Clearly the limitations of field-based diagnosis contribute significantly to the difficulty in studying shipping fever (Kelly and Janzen, 1986).

Morbidity and mortality due to shipping fever average approximately 14 and 1%, respectively (USDA National Animal Health Monitoring System (NAHMS)). However, both vary widely, with reported ranges for morbidity from 4.6% (Snowder et al., 2006) to 89% (Storz et al., 2000b) and for mortality from 1% (Snowder et al., 2006) to 13% (Storz et al., 2000b). Outbreaks are common, with initial cases appearing 7–10 days after arrival and typically peaking prior to 20 days (Jensen et al., 1976; Alexander et al., 1989). Mortality appears shortly after initiation of the outbreak, peaking at 16 days (Ribble et al., 1995b). However, this timeframe can be lengthened by continued introduction of new animals into the herd or pens (Alexander et al., 1989). Although disease occurs throughout the year, shipping fever has seasonal variation, peaking in fall to winter (Jensen et al., 1976; Ribble et al., 1995a; Loneragan et al., 2001).

Numerous predisposing factors are frequently cited for shipping fever. The most well documented of these are viral infections, which will be discussed in regard to their specific association with *P. multocida*. Other proposed factors are generally termed ‘stressors’ and include transportation, co-mingling with other cattle, dust, cold, sudden and extreme changes of temperature and humidity, dehydration, hypoxia, endotoxin, cold coupled with wetness, and acute metabolic disturbances (Lillie, 1974; Irwin et al., 1979). These are thought to alter the respiratory mucosa or hinder cattle’s immune system, either directly or through the effects of endogenous agents such as cortisol, making the animals more susceptible to opportunistic infections. The intuitive nature of these suggestions is appealing, and they have become widely accepted and repeated throughout the literature. However, limited epidemiologic investigations have been done to confirm or refute the significance of these factors, and studies done thus far have been inconclusive. The most consistent finding in epidemiologic studies of shipping fever is the immense variability in occurrence, from operation to operation and year to year. This makes interpretation of interventions difficult, particularly in multi-year studies.

Several predisposing factors have been conclusively demonstrated. Newly received cattle are at greater risk for disease (Jensen et al., 1976; Kelly and Janzen, 1986; Martin and Meek, 1986; MacVean et al., 1986; Alexander et al., 1989; Ribble et al., 1995b). Calves purchased through a salebarn are more at risk than those arriving directly from farm sources (Wilson et al., 1985; Gumnow and Mapham, 2000). However, it is possible that other factors commonly associated with salebarn calves are responsible for the increased risk, rather than the salebarn process itself. These may include co-mingling of cattle from multiple sources, as well as the stress of repeated unloading, loading, handling, etc. Marketing through a salebarn may also reflect different management practices (e.g. better managers market direct, while poorer managers rely upon the salebarn). When examining what strategies can prevent disease, metaphylaxis (administration of parenteral antimicrobials to calves that are at high risk for respiratory disease) has consistently reduced morbidity (Van Donkersgoed, 1992; Frank et al., 2000, 2002; Macartney et al., 2003; Cusack, 2004), while results of vaccination have been equivocal at best (Mosier et al., 1989; Perino and Hunsaker, 1997).

**Clinical disease: enzootic calf pneumonia (ECP)**

Similar to shipping fever, the term ‘enzootic calf pneumonia’ (ECP) is more a description than a diagnosis. In some ways, the distinction between ECP and shipping
fever is arbitrary, with ECP occurring prior to shipping fever and after weaning. However, pneumonia is infrequent in pre-weaned beef calves on pasture. Thus, ECP usually refers to respiratory disease in dairy and veal calves raised in confinement. Diagnostic criteria for ECP are similar to those for shipping fever, both in content and vague nature. Studies have shown relatively poor correlation between producer-diagnosed ECP and that detected by a veterinarian (Van Donkersgoed et al., 1993; Virtala et al., 1999; Svensson et al., 2006). Nevertheless, most field studies relied upon treatment by the producer as a proxy for disease, greatly complicating the conclusions that can be reached from such research.

ECP has traditionally been defined as developing between 2 and 6 months of age (Ames, 1997). Studies looking at younger calves found peak occurrence from nearly 4 weeks to 6 weeks of age (Van Donkersgoed et al., 1986; Curtis et al., 1988a; Van Donkersgoed et al., 1993; Virtala et al., 1996). Reported morbidity varies significantly. Two studies found 6–8% of calves affected (Curtis et al., 1988a; Sivula et al., 1996b), whereas two other studies found morbidity rates of 15% (Waltner-Toews et al., 1986) to 29% (Van Donkersgoed et al., 1993). Mortality ranged from approximately 5% (Waltner-Toews et al., 1986) to nearly 10% (Sivula et al., 1996b).

Fewer studies have been done on the epidemiology of ECP compared to shipping fever. Waltner-Toews et al. (1986) and Van Donkersgoed et al. (1993) found larger herds had higher incidence of ECP than smaller herds. This relationship, however, was not identified by Curtis et al. (1988b). None of these studies included farms that would be considered large by today’s standards (Waltner-Toews et al., 1986). Waltner-Toews et al. (1986) and others (Bryson et al., 1978; Svensson et al., 2006) found a higher incidence in fall and winter but Curtis et al. (1988b) did not. Calves that had diarrheal disease may be at an increased risk for ECP (Curtis et al., 1988b; Van Donkersgoed et al., 1993; Svensson et al., 2006). However, Van Donkersgoed et al. (1993) found that relationship was no longer significant after adjusting for clustering, and other studies did not identify a relationship between scour and respiratory disease (Virtala et al., 1999). Failure of passive transfer (FPT) is an intuitive potential predisposing factor, but investigations of a possible relationship between FPT and ECP yielded conflicting results (Van Donkersgoed et al., 1993; Virtala et al., 1996, 1999). The diagnostic criteria for FPT may account for part of the discrepancies. Virtala et al. (1999) determined that immunoglobulin of less than 1200 mg dl$^{-1}$ provides the best prediction of disease and thus the best break point for FPT. In contrast, Van Donkersgoed et al. (1993), Sivula et al. (1996a) and Virtala et al., (1996) used values less than 800 mg dl$^{-1}$ for diagnosing FPT. Another potential confounder is the diagnosis of respiratory disease. Van Donkersgoed et al. (1993) found no correlation between FPT and owner-diagnosed respiratory disease but found a positive association with that diagnosed by a veterinarian. Prado et al. (2006) found collostral antibodies to P. multocida to be short-lived, largely being undetectable by 2 months of age. Thus, their impact on ECP would likely be minimal.

Housing of calves was a significant influence on ECP in several studies. Several studies (Martin and Meek, 1986; Waltner-Toews et al., 1986; Virtala et al., 1999) found hutch to be better than inside pens, whereas Svensson et al. (2006) found housing calves individually better than group housing. Sivula et al. (1996a) were not able to demonstrate a reduction in ECP attributable to housing in hutch. Using the same data set, the authors applied objective criteria to define ‘adequate ventilation of calf housing’ and assessed its relationship with ECP (Sivula et al., 1996b). There was a positive correlation between adequate ventilation of housing and average daily gain, but no relationship between either of these and ECP. These findings again could reflect the inadequacy of producer-diagnosed respiratory disease. Such an assertion is bolstered by the finding that producer-diagnosed death due to pneumonia only had a 58.3% sensitivity (Sivula et al., 1996b). Numerous other factors have been potentially implicated in ECP. These include sire, vaccination of dam prior to calving, calves born in loose housing, time of day when born, how and when colostrum is provided, navel treatment, weaning based on size rather than age, use of non-medicated milk replacer, and provision of water and mineral.

### Pathology of *P. multocida* pneumonia in cattle

Bovine 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 such as *M. haemolytica* and Histophilus somni. Dungworth (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 of these pathogens from pneumatic lungs in conjunction with respiratory viruses and/or *Mycoplasma* spp. occurs frequently (Gagea et al., 2006). This has led authors to question if *P. multocida* is a primary pathogen in bovine pneumonia (Lopez, 2007). Experimental reproduction of bovine pneumonia with *P. multocida* alone and its isolation from cases of naturally acquired bovine pneumonia without evidence of other infectious agents indicate that it can likely occur as a primary pathogen in young or stressed cattle.

The gross pulmonary pathological changes have been described differently by various authors, and those designations probably reflect the age of the lesion, whether or not other infectious agents were involved but not identified, and descriptive preferences of the various authors. The lesion is a typical cranioventral
bronchopneumonia and has been characterized simply as bronchopneumonia by some authors (Haritani et al., 1989; Mathy et al., 2002; Ewers et al., 2006; Lopez, 2007) or as a bronchopneumonia with various descriptive modifiers denoting lesion age and type of exudate. These include acute fibrinosuppurative (Gagea et al., 2006), subacute to chronic fibrinopurulent (Mosier, 1997), fibrinous to fibrinopurulent (Dungworth, 1985), suppurrative (Tegtmeier et al., 1999) and fibrino-necrotizing (Tegtmeier et al., 1999). The presence of fibrinous to fibrinopurulent pleuritis, distended interlobular septa with edema or fibrin and/or abscesses is variable with \textit{P. multocida} infection. Lopez (2007) designated the \textit{P. multocida}-associated lesion as bronchointerstitial to bronchopneumonia depending on whether or not there was concurrent viral infection.

Histologic changes typically consist of bronchi and bronchioles filled with neutrophils, macrophages and necrotic epithelium interspersed with a small amount of fibrin (Dungworth, 1985; Jericho and Carter, 1985; Haritani et al., 1989; Dowling et al., 2004; Lopez, 2007). Neutrophil morphology ranges from intact to necrotic. Similar exudate is present in alveoli with peribronchial alveoli mostly severely affected particularly in the early phases of infection. The character of the exudate varies with time with acute lesions having a higher percentage of neutrophils and lower percentage of macrophages compared to chronic lesions. There is usually a variable amount of intra-alveolar hemorrhage, and \textit{P. multocida} cells and antigen can be detected within alveolar lumens and within neutrophils and macrophages. Both intact and degenerating bacteria are seen within phagosomes of phagocytes (Haritani et al., 1989), and intracellular survival of \textit{P. multocida} within phagocytes has been demonstrated (Dowling et al., 2004). Interlobular lymphatics can be dilated with fibrinous to fibrinopurulent exudate. Large foci of necrosis and abscesses have been described occasionally after experimental challenge (Dowling et al., 2002; Mathy et al., 2002; Ishiguro et al., 2004). Immunohistochemical studies of natural cases of bovine pneumonia demonstrated, however, that abscesses were more often associated with \textit{M. haemolytica}, \textit{Arcanobacterium pyogenes} or \textit{Mycoplasma bovis} infections and not with \textit{P. multocida} (Haritani et al., 1990; Adegboye et al., 1995; Gagea et al., 2006).

\textbf{Epidemiology of \textit{P. multocida} pneumonia}

True epidemiologic investigation of \textit{P. multocida}-associated respiratory disease is scant. One reason for this is the ubiquitous nature of the organism. Its mere presence in the upper respiratory tract is not diagnostic of disease; whereas confirmation of its involvement in pneumonia requires post-mortem culture or invasive measures (TTW or BAL), which are not practical for large numbers of cattle. It appears that \textit{P. multocida} has a synergistic relationship with \textit{Mycoplasma} species. This was particularly clear for dairy calf pneumonia, as isolation of both from clinical cases is more common than isolating either alone (Virtala et al., 2000; Hirose et al., 2003). Co-infection with other pathogens has also been noted, including \textit{H. somni} (Gagea et al., 2006), \textit{M. haemolytica} (Van Donkersgoed et al., 1993; Fulton et al., 2000), coronavirus (Storz et al., 2000a; Autio et al., 2007), adenovirus (Autio et al., 2007), parainfluenza-3 virus and bovine respiratory syncytial virus (Van Donkersgoed et al., 1993; Autio et al., 2007). The ability of \textit{P. multocida} to cause disease in the absence of other pathogens has been debated (Bryson et al., 1978). In a Finnish study, \textit{P. multocida} was one of only three pathogens to be isolated more commonly from BAL of diseased than from healthy and suspect cattle (Autio et al., 2007). However, no association was found between the isolation of \textit{P. multocida} and clinical disease when no other pathogen was present (Autio et al., 2007). This led them to accept the conclusion of Maheswaran et al. (2002) that \textit{P. multocida} is an opportunistic pathogen and not a primary causative agent. In contrast, another Finnish study found \textit{P. multocida} to be the only agent positively associated with clinical signs and elevated serum acute phase proteins (Nikunen et al., 2007). Those authors concluded that there is ‘a strong pathogenic role for \textit{P. multocida} in respiratory disease in calves in Finland, at least in situations where other known pathogens are absent’.

Finland is free of bovine viral diarrhea virus and \textit{M. bovis}; therefore, findings there may not reflect disease where interaction between \textit{P. multocida} and those agents may occur.

A major epidemiologic question is whether there is an inherent difference in pathogenic versus commensal populations of \textit{P. multocida} or whether \textit{P. multocida}-associated disease is strictly a change in host–bacteria relationship. Bacteriological characterizations have not identified an inherent difference between pathogen and commensal isolates, but the ability to detect such differences may be the limiting factor. Davies et al. (2003a) found that different subpopulations are responsible for progressive atrophic rhinitis and pneumonia in swine. Similarly, \textit{P. multocida} isolates from ovine respiratory and reproductive tracts were often distinguishable (Davies et al., 2003b). Therefore, differences (perhaps quite subtle) may exist between commensals and pathogens.

The entire \textit{P. multocida} genome has now been sequenced, albeit from a single avian isolate, Pm70 (May et al., 2001). This should enable relatively rapid characterization of genetic differences among various isolates and has in fact already yielded valuable information. Several virulence factors have been identified and are discussed elsewhere in this article. This section will focus on the efforts to use bacterial products or genes encoding these products as markers for epidemiologic investigation. Hunt et al. (2001) identified several
genes whose expression is up-regulated in vivo, including membrane proteins and metabolic and biosynthetic pathways. However, those were derived from bacteria inoculated into the bloodstream of mice and may not reflect events occurring in the upper or lower respiratory tract of healthy or sick cattle. Additionally, only one isolate of *P. multocida* was used; thus no comparative analysis was possible to determine what genes may promote virulence. Boyce and Adler (2006) concluded that ‘true virulence genes might be constitutively expressed, upregulated only during initial stages of infection’. Thus, assaying for expression of virulence factors may be confounded by the progression of disease and interaction of host and pathogen. A more relevant comparative study examined the genotypes of a wide range of isolates from multiple host species (cattle, sheep and goats, bison, swine, rabbits, poultry, cats, dogs and humans), with some of the isolates being from diseased animals and others from clinically normal ones (Ewers et al., 2006). They identified several genes that were more common in bovine isolates than from isolates obtained from other species. They also had modest success in identifying genes more commonly associated with isolates from diseased animals.

Another question relates to transmission, i.e. is *P. multocida* pneumonia contagious? The ubiquitous presence and commensal nature of *P. multocida* means that finding the organism in multiple animals does not demonstrate transmission. Instead, to assess transmission requires a way of discriminating between bacterial isolates. Classic means of distinction, including serotyping and serogrouping, are inadequate (Wilson et al., 1992). Purdy et al. (1997) employed an extensive assessment of enzymatic activity to compare 61 *P. multocida* isolates collected from multiple locations over the course of 8 years. The profiles that resulted showed no evidence of clustering, suggesting that this practice offered little discriminatory power (Purdy et al., 1997).

Another phenotypic approach to the question of transmission is assessment of antimicrobial resistance. Catry et al. found tetracycline-resistant *P. multocida* in the nasopharynx of 5 calves on 1 farm, while no resistant *P. multocida* were found in the other 56 calves from 15 farms (Catry et al., 2006). No tetracycline had been used on any farms. The authors suggested that the five isolates were clonal, indicating horizontal transmission. A large retrospective study found evidence of regional differences in antimicrobial resistance among *P. multocida* isolates, with clustering in specific regions (Singer et al., 1998). No further examination was done to assess whether isolates were clonal or even if the mechanism of antimicrobial resistance was consistent. A smaller study examined the genotypic basis for tetracycline resistance (Kehrenberg et al., 2005). Six *Pasteurella* and *Mannheimia* isolates were obtained from a single farm where oxytetracycline had been given prophylactically. The same gene encoding resistance was found in multiple bacterial species, with little or no mutation. The authors concluded that *Pasteurella* and *Mannheimia* likely had been transmitted between calves. However, they acknowledged that horizontal transmission of resistance genes could also account for the findings. Because the same genetic material was found in multiple species of bacteria, it is possible that only the genes were passed from animal to animal (within another microbial species) and were subsequently taken up by the resident bacteria.

Genotypic analysis should be more insightful than the phenotypic methods described above for understanding transmissibility of *P. multocida*. Several methods have been used to examine isolates from several host species reviewed in Hunt et al. (2000). Molecular techniques that have been successfully used include restriction endonuclease analysis (REA), ribotyping, sequencing of rRNA, random amplification of polymorphic DNA (PCR fingerprinting), pulse field gel electrophoresis (PFGE) and multilocus sequence analysis as well as using PCR for capsular typing and analysis for the presence of the gene coding for dermonecrotokin. Each of these techniques has both positive attributes and limitations: PFGE is considered the ‘gold standard’ of molecular epidemiology (Olive and Bean, 1999); however, few researchers have employed it in examining *P. multocida*, perhaps due to its time-consuming nature and relatively high equipment costs. Ribotyping and rRNA sequencing are less sensitive than other approaches in identifying differences among *P. multocida* isolates (Davies, 2004; Dziva et al., 2004), whereas numerous researchers have employed PCR fingerprinting for epidemiologic investigation of *P. multocida* in several animal species (Chaslus-Dancla et al., 1996; Zucker et al., 1996; Dabo et al., 1999a, b, 2000; Dziva et al., 2001, 2004). Due to the relative low cost and ease of conducting PCR fingerprinting, this is arguably the technique of choice.

Most studies have used a combination of the above approaches and/or cellular characterization methods (whole cell protein profiles, outer membrane protein (OMP) profile, etc.). Davies et al. (2003a) used OMP profiles, capsular typing and the presence of dermonecrotokin to compare *P. multocida* isolates from porcine pneumonia and found limited diversity among isolates. They offered two potential explanations: either there is little diversity among the commensal population of *P. multocida* with little potential for diversity in disease state or there are a few virulent clones that account for disease under most circumstances. Similar techniques (capsular typing and OMP profiles) using avian isolates, however, demonstrated wide diversity of strains, suggesting that avian disease more likely represents opportunistic infection (Davies et al., 2003b).

Davies et al. (2003a) also examined bovine isolates, using a combination of multilocus sequence analysis, OMP profiling and rRNA sequencing. They found only a limited number of OMP types predominated among bovine isolates, even from isolates obtained from diverse
geographical regions. They, therefore, speculated that few clones have a marked capacity to cause disease (similar to the proposed situation in swine). Using ribotyping, we (Dabo et al., 1999a) found little heterogeneity among isolates from diverse geographical areas, but the significance of this is uncertain considering the limitations of ribotyping. All of Davies isolates were from diseased animals, whereas we used isolates from healthy and diseased cattle. While we (Dabo et al., 1999a) did not identify which isolates were obtained from healthy versus sick animals, some variation was found in ribotypes of isolates obtained from lung which were from diseased animals compared to nasal passage, which were potentially from healthy or diseased animals. This could be consistent with differences existing between commensal and pathogenic isolates. DeRosa et al. (2000) found only one ribotype among Pasteurella multocida isolates obtained from both TTW and NPS of sick calves. They concluded that this method is not sensitive enough to discern relatedness. However, given the findings of others (Dabo et al., 1999a; Davies et al., 2003a), it is possible that all isolates in DeRosa’s population were clonal in origin. DeRosa also performed antibiograms to compare isolates obtained from the two sources (TTW or NPS). The authors state that ‘although a few paired isolates exhibited identical ribotypes, their antibiotic susceptibility profiles were different’. However, the study included both Pasteurella multocida and M. haemolytica, and there is no clarification of which species demonstrated the divergent ribotyping and antibiogram results. When taken together, these findings indicate that an inherent difference exists between commensals and pathogens. It is clear, though, that additional work is required to confirm this, preferably using a consistent methodology with a more sensitive molecular approach. This final point is reinforced by several studies that found different grouping patterns among the same isolates when using multiple molecular or cellular techniques (Dabo et al., 1999a; Blackall et al., 2000; Davies, 2004; Davies et al., 2004).

The primary involvement of Pasteurella multocida in BRD is well established. Nonetheless, the understanding of the disease entities and the bacterial mechanisms in causing the diseases is still rudimentary. Improved methods of diagnosis of shipping fever and ECP will likely be needed before progress can be made in clarifying the host and environmental factors that contribute to the diseases. In contrast, it is possible that the tools needed for understanding the pathogen’s role already exist. Hopefully there will soon be an answer as to what (if anything) distinguishes the commensal Pasteurella multocida from that which causes disease.

**P. multocida virulence factors**

The potential Pasteurella multocida virulence factors and features of the bacterium important in BRD infection are limited. The review below will focus on the virulence factors associated with Pasteurella multocida serotype A isolates.

**Adherence and colonization factors**

Attachment is a primary prerequisite for bacterial infections of a host, and ligands that are involved in such adherence are potential virulence factors. Several studies examined the adherence of different Pasteurella multocida isolates to different cell types, tissues or organs and reflected serogroup-specific host preference and pathogenicity (Glorioso et al., 1982; Jacques et al., 1988; Ackermann et al., 1991; Letellier et al., 1991; Dugal et al., 1992; Esslinger et al., 1994; Isaascon and Trigo, 1995; Al-Haddawi et al., 2000; Borrathybay et al., 2003; Ali et al., 2004a). Fimbriae, as adherence mediators in Pasteurella multocida serotype A, have been reported (Glorioso et al., 1982; Rebers et al., 1988; Ruffolo et al., 1997). Pasteurella multocida serotype A specifically bound HeLa and rabbit pharyngeal cells in vivo and in vitro with fimbriae as putative adhesins (Glorioso et al., 1982). Pasteurella multocida serogroup A, B, D and B:2 strains are known to possess type IV fimbriae (designated PfA) (Ruffolo et al., 1997; Doughty et al., 2000; Siju et al., 2007), and sequence comparison revealed variation within the gene among isolates (Doughty et al., 2000). However, the role of type IV fimbriae in Pasteurella multocida serotype A adherence to host cells has not been reported. A 39 kDa OMP, lipoprotein B (lpB) from Pasteurella multocida serotype A isolates was shown to be an adherence factor and stimulated cross-protective immunity in mice and poultry (Borrathybay et al., 2003; Ali et al., 2004a, b; Tabatabai and Zehr, 2004; Harper et al., 2006).

The Pasteurella multocida genome contains several adhesinandrelated genes including homologs of Bordetella pertussis filamentous hemagglutinin (pfaB1 and pfaB2) and Haemophilus influenzae surface fibril (Hsf1 and Hsf2) proteins (May et al., 2001). Application of SPAAN (Sachdeva et al., 2005) to Pasteurella multocida whole genome revealed several uncharacterized novel adhesions. *B. pertussis* FHA family of proteins are known or suspected to play a crucial role in bacterial adherence to and colonization of host cells (Barenkamp and St Geme, 1994; Barenkamp, 1996; Barenkamp and St Geme, 1996a, b; Ward et al., 1998). We previously cloned and sequenced in bovine Pasteurella multocida strains a gene encoding a protein related to *B. pertussis* FHA proteins (Dabo and Confer, 2001; PmFHA, GenBank accession no. AY035342). *H. influenzae* type b Hsf is a major vitronectin-binding protein that contributes to serum resistance of that bacterium (Hallstrom et al., 2006). Both Pasteurella multocida FHA and Hsf proteins were found to be transcriptionally activated under nutrient-deficient conditions (Paustian et al., 2002). Additionally, signature-tagged mutagenesis (SMT) identified four adhesin-related genes in bovine Pasteurella multocida strains that attenuate...
Table 1. Potential sites for Pasteurella multocida adherence in various diseases and lesions

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Animal species (Disease)</th>
<th>Adherence sites</th>
</tr>
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<tbody>
<tr>
<td>Upper and lower respiratory disease</td>
<td>• Cattle (Shipping fever or neonatal dairy calf)</td>
<td>• Upper respiratory mucosa</td>
</tr>
<tr>
<td></td>
<td>• Swine bronchopneumonia</td>
<td>• Lower respiratory mucosa</td>
</tr>
<tr>
<td></td>
<td>• Swine atrophic rhinitis</td>
<td>• Pneumocytes</td>
</tr>
<tr>
<td></td>
<td>• Rabbits (Snuffles)</td>
<td>• Alveolar interstitial ECM</td>
</tr>
<tr>
<td></td>
<td>• Birds (Fowl cholera)</td>
<td>• Upper respiratory ECM</td>
</tr>
<tr>
<td>Septicemia</td>
<td>• Cattle (Hemorrhagic septicemia)</td>
<td>• ECM</td>
</tr>
<tr>
<td></td>
<td>• Rabbits (Septicemic snuffles)</td>
<td>• Endothelium</td>
</tr>
<tr>
<td></td>
<td>• Birds (Septicemic fowl cholera)</td>
<td>• ECM</td>
</tr>
</tbody>
</table>

ECM, extracellular matrix.

virulence in a murine septicemia model (Fuller et al., 2000); these include the two pfbaB1 and pfbaB2 genes, one gene (pfbaC) that is similar to hemolysin accessory gene of B. pertussis FbaC, and one gene (tadD), a homolog of Actinobacillus actinomycetemcomitans tadD protein (Fuller et al., 2000). TadD is part of flp (fimbria-like protein) operon, a widespread locus in bacteria known to be essential for virulence in A. actinomycetemcomitans and Haemophilus ducreyi (Kachlany et al., 2000, 2001). The expression of P. multocida tad genes was also up-regulated under starvation conditions (Paus- tian et al., 2001). Studies by Tatum et al. (2005) showed that P. multocida pfbaB2 mutant was highly attenuated in turkeys following intranasal challenge, suggesting a role in initial colonization by P. multocida; however, pfbaB2 mutant and parent strain were both resistant to complement killing. Recently, Ewers et al. (2006) showed that only 46% of the cattle isolates investigated harbored the fba gene, and a significant association was reported between disease status and bovine P. multocida strains harboring fba gene. Hypothetically, P. multocida FHA may also function as a serum resistance factor based on sequence homology to H. somni immunoglobulin-binding p76 protein (Cole et al., 1993). A related immunoglobulin-binding protein (designated Pm76) was previously identified and sequenced in bovine P. multocida strains (Dabo and Confer, 2001–PmFHAA, GenBank accession no. AAK61596).

The mechanisms of P. multocida adherence (not colonization) to bovine cells or the bacterial factors involved in such adherence in BRD infections have been poorly addressed. Many pathogens including members of the family Pasteurellaceae exploit host extracellular matrix (ECM) molecules for virulence through adherence and colonization (Duensing and van Putten, 1997, 1998; Molinari et al., 1997; Duensing et al., 1999; Bresser et al., 2000). The broad host range characteristic of P. multocida suggests recognition of host cell surface components (ECM molecules) common to multiple animal species and tissue types (Table 1). Recent studies in our laboratories investigated the binding of P. multocida to ECM molecules and demonstrated that bovine P. multocida isolates bind to several ECM molecules including fibronectin (Fn) (Dabo et al., 2005). Bacteria bound both soluble and immobilized Fn and preferentially bound the N-terminal heparin-binding fragment (Hep-1) of Fn, similar to that described for other bacterial pathogens (Westerlund and Korhonen, 1993; Patti et al., 1994; Joh et al., 1999; Dabo et al., 2005). The P. multocida proteins identified as putative Fn-binding proteins include P. multocida OmpA, the TonB-dependent receptor HgbA (hemoglobin-binding protein A), the transferrin-binding protein A (TbpA) and Pm10769 (Dabo et al., 2003, 2005). P. multocida OmpA bound Madin–Darby bovine kidney (MDBK) via heparin and/or Fn bridging (Dabo et al., 2003), and monoclonal antibodies against OmpA significantly reduced or abolished bacterial binding to and invasion of MDBK cells (S. M. Dabo and A. W. Confer, unpublished data). The OmpA gene from bovine P. multocida strains exists as an eight-stranded transmembrane anti-parallel β-barrel that displays four surface-exposed loops with putative roles in adherence and serum resistance (Dabo et al., 2005). In Escherichia coli, OmpA has been associated with virulence, functions as a multifunctional protein displaying phage receptor and porin activities (Datta et al., 1977; Schweizer and Henning, 1997), is involved in the bacterial invasion of host cells (Prasadarao, 2002; Prasadara et al., 1996) and contributes to serum resistance (Weiser and Gotschlich, 1991). Two P. multocida regulatory genes have also been reported as potential virulence factors: DNA adenine methylase (Dam) and the Rci recombinase (rci) gene (Fuller et al., 2000; Chen et al., 2003). In Salmonella Typhi, Rci protein inverted the DNA in the C-terminal region of the type IV pili, resulting in the inhibition of the minor pilus protein expression and the promotion of bacterial self-association (Morris et al., 2003; Tam et al., 2004). Whether Rci of P. multocida has a similar action on the bacterium type IV pili is unknown. The dam gene regulated the expression of virulence genes in several Gram-negative bacteria including P. multocida (Blyn et al., 1990; Chen et al., 2003; Heusipp et al., 2007). Overexpression or deletion of dam gene in several organisms resulted in attenuation for mice (Heithoff et al., 1999; Chen et al., 2003; Badie et al., 2007). P. multocida serotype A harboring a plasmid-mediated alteration of dam expression was highly attenuated in mice (Chen et al., 2003). However, a Klebsiella pneumoniae dam mutant showed only partial attenuation in mice (Mehling et al., 2007).
Neuraminidase (sialidase) is prevalent among *P. multocida* strains and is expressed *in vivo* by *P. multocida* A:3 strains associated with bovine pneumonia (White et al., 1995; Straus et al., 1996; Ewers et al., 2006); both cell-bound and extracellular sialidases were reported (White et al., 1995). Its role in BRD, however, remains largely unknown. Evidence of neuraminidase function in *P. multocida* disease suggested that the protein is protective in mice (Ifeanyi and Bailie, 1992), is associated with virulence ( Muller and Krassmann, 1974) and may contribute to bacterial adherence, colonization and persistence (Mizan et al., 2000). However, the relationship between the ability of *P. multocida* to produce the enzyme and to cause pneumonia in cattle has not been established. Two sialidase genes (*nanB and nanH*) were reported in 100 and 88.5% of *P. multocida* bovine strains investigated, respectively (Mizan et al., 2000). Mutation in nanH resulted in a bacterium that was not deficient in sialidase production but had reduced enzyme activity (Mizan et al., 2000). As reported by Steenbergen et al. (2005) and reviewed by Harper et al. (2006), Pm70 genome database contains several putative sialometabolic gene products, and the uptake, not catabolism, of sialic acid appeared essential for virulence in mice by protecting bacteria from innate host defense mechanisms that may promote persistence (Steenbergen et al., 2005; Harper et al., 2006).

In summary, determining the *P. multocida* genes involved in bacterial adherence, primarily during the early stage of infection, can assist in our understanding of the molecular pathogenesis of *P. multocida*. Changes in host–pathogen interaction resulting from changes in environmental signals are most likely the cause of differences between commensal and pathogenic *P. multocida*. The molecular differences between commensal and pathogenic *P. multocida* are not at present understood, but the mechanisms clearly involve the ability of the latter to induce inflammatory responses most likely through toll-like receptor (TLR) signaling. TLR signaling is crucial to innate host defense, which provides the first line of defense against infection through bacterial identification and killing and activation of adaptive immunity. Because a growing number of bacterial adhesins are reported to depend on TLR for induction of proinflammatory cytokines (Abramson et al., 2001; Hajishengallis et al., 2002; Ogawa et al., 2002; Lorenz et al., 2004; Inatsuka et al., 2005; Fischer et al., 2006), future studies should focus on the identification of proinflammatory *P. multocida* adhesins as potential vaccine candidates.

**LPS**

*P. multocida* LPSs have similar properties (biological and chemical) and structure to R-type LPS (with no polymeric O-antigen) found in many Gram-negative bacteria (Lugtenberg et al., 1984; Rimler, 1990; Confer, 1993; Harper et al., 2006). These molecules induce classic signs of endotoxic shock (Rimler and Rhoades, 1989). The LPS structure has been determined for three *P. multocida* serotype A strains (VP161, X73 and Pm70) (St Michael et al., 2005a, b, c) and revealed a highly conserved inner but variable outer core (Harper et al., 2006). The investigation of the *P. multocida* Pm70 genome also identified gene clusters that may be responsible for LPS biosynthesis (St Michael et al., 2005c). Pathogenic bacteria expressing similar LPS include *H. influenzae*, *Campylobacter jejuni*, and *Neisseria* spp., and changes in the outer core oligosaccharides of these bacteria are often the result of phase-variation strategies in key LPS genes (Raetz and Whitfield, 2002). No such evidence was found in LPS genes in the *P. multocida* genome (Harper et al., 2007). Clusters of several putative glycosyltransferase genes, not always found in this genus, were identified in *P. multocida* Pm70 genome (May et al., 2001; St Michael et al., 2005c).

A novel finding of *P. multocida* LPS is the identification of two predicted heptosyl-1-transferases (designated *HptA* and *HptB*) expressing a single or two Kdo residue(s), respectively (St Michael et al., 2005a). *hptA* is annotated as *opsX* in *P. multocida* Pm70 genome for its homology to the same gene in *H. influenzae*, and *hptB* is a homolog to *waac* of *E. coli* and *K. pneumoniae* (St Michael et al., 2005a; Harper et al., 2007). Virulence studies show that the *hptB* mutant was fully virulent (Harper et al., 2007), whereas the *hptA* mutant was fully attenuated (Harper et al., 2004, 2007). Therefore, *hpta* may be critical for the survival of the organism in the host.

Purified *P. multocida* LPS is highly antigenic and capable of producing widespread vascular alterations and death, but it is poorly immunogenic (Heddleston and Rebers, 1975; Tsuji and Matsumoto, 1988; Ryu and Kim, 2000). Generally, *P. multocida* LPS is immunogenic or protective only when complexed with proteins (Ganfield et al., 1976; Tsuji and Matsumoto, 1988; Ryu and Kim, 2000) or ribosomes (Phillips and Rimler, 1984). Ryu and Kim (2000) demonstrated complete protection in mice vaccinated with *P. multocida* type A:3 LPS–protein complex; however, neither the LPS nor protein alone nor the LPS and protein as a mixture provided protection. The authors also showed that the LPS–protein complex induced both humoral and cell-mediated immunity (Ryu and Kim, 2000). Previously, a bactericidal monoclonal antibody against LPS from a *P. multocida* serotype A isolate completely protected mice against homologous challenge (Wijewardana et al., 1990; Confer, 1993). Sutherland et al. (1993) also found that polyclonal anti-idiotypic antibodies that mimic a linear *P. multocida* LPS molecule protected mice against homologous challenge (Sutherland et al., 1993). However, studies by Lu et al. (1991) failed to show protection in mice against *P. multocida* infection following passive immunization with affinity-purified rabbit anti-LPS serum. An opsonic
but not bactericidal anti-LPS monoclonal antibody only partially protected against *P. multocida* infection in mice (Ramdani and Adler, 1991; Confer, 1993).

LPS is also known as a stimulator of host defense against *P. multocida* infection (Iovane et al., 1998; Galdiero et al., 2000). Purified *P. multocida* LPS stimulated the expression and release of several cytokines in mice including the inflammatory (IL-1β, IL-6 and TNF-α) and immunoregulatory cytokines (IFN-γ and IL-12), which are known to play an important role in host defense (Iovane et al., 1998). Galdiero et al. (2000) also demonstrated a role for *P. multocida* LPS in neutrophil adhesion and migration through bovine endothelial cell monolayers. In another study, LPS from a *P. multocida* serotype A strain enhanced humoral and cell-mediated immune responses in chickens (Maslog et al., 1999).

**Capsule and the capsule biosynthetic genes**

The genetic organization of the capsule biosynthetic loci from the five *P. multocida* serogroups was determined and reviewed (Chung et al., 1998; Boyce et al., 2000a; Townsend et al., 2001; Harper et al., 2006). Comparative analysis of the five capsular biosynthetic regions identified serogroup-specific regions confirming a genetic basis for the serological differences between the observed strains (Carter, 1952; Rimler and Rhoades, 1989; Townsend et al., 2001).

The importance of capsule as virulence determinants in the pathogenesis of *P. multocida* has been clearly established; however, the focus to date has been primarily on *P. multocida* capsular types A and B from fowl cholera and hemorrhagic septicaemia infections, respectively (reviewed in Harper et al., 2006) (Boyce and Adler, 2000; Chung et al., 2001; Harper et al., 2006). Studies on the specific role of bovine *P. multocida* capsule in BRD infections are non-existent. Virulence studies in mice indicated that isogenic serotype A mutants were attenuated as compared to their encapsulated wild types (Jacques et al., 1993; Boyce and Adler, 2000; Chung et al., 2001). Transposon insertion into a hyaluronan synthase gene of bovine *P. multocida* strain attenuated virulence in a septicemic mouse model (Fuller et al., 2000). Watt et al. (2003) reported the loss of virulence of unencapsulated variants of a *P. multocida* serotype A strain and the restoration of virulence with the capsulated phenotype in mice. The capsular dissociation was not due to alteration in capsule gene nor was it regulated by the two-component (histidine kinase/response) regulator of capsule synthesis which was absent in *P. multocida* isolates under investigation as well as the Pm70 genome database.

Although *P. multocida* capsules are implicated in the virulence of *P. multocida*, vaccination of mice with capsule did not stimulate protection (Esslinger et al., 1993, 1994; Gutierrez-Pabello et al., 1995; Pruimboom et al., 1996). In general, many virulent strains of *P. multocida* express capsule consisting of hyaluronic acid (HA) (types A and B), chondroitin (type F) or heparin (type D) (Carter and Chengappa, 1980; Muniandy and Mukkur, 1993; Boyce et al., 2000b; DeAngelis and Padgett-McCue, 2000; DeAngelis and White, 2002; DeAngelis et al., 1998, 2002) that are similar to molecules found naturally in the various hosts (Snipes et al., 1987; Rimler and Rhoades, 1989; Tsuji and Matsumoto, 1989; DeAngelis, 1996). This ‘molecular mimicry’ prevents the mounting of a strong antibody response to capsular materials, impairs phagocytosis, reduces the action of complement killing and increases adherence to and survival in the host (Harmon et al., 1991). Paradoxically, only *P. multocida* capsular B strains, and more specifically B:2 strains, produced hyaluronidase, an enzyme that specifically cleaves HA between its repeating disaccharides (N-acetylglucosamine and D-glucuronic acid) (Carter and Chengappa, 1980; Rimler and Rhoades, 1994; Harper et al., 2006).

Antiphagocytic activity of *P. multocida* was reported and associated with the presence and thickness of capsule (Truscott and Hirsh, 1988; Harmon et al., 1991; Pruimboom et al., 1996). Decapsulation of *P. multocida* serotype A:3 with hyaluronidase increased phagocytosis by macrophages, whereas an unencapsulated variant of the bacteria was not internalized (Pruimboom et al., 1996). Moreover, isogenic non-encapsulated *P. multocida* serotype A strains had significantly increased sensitivity to phagocytosis than the encapsulated wild-type strain (Boyce and Adler, 2000). Serum resistance correlated with encapsulation in *P. multocida* serotype A, because spontaneous, isogenic acapsular mutants or decapsulated strains were generally sensitive to complement-mediated killing compared to wild-type strains (Snipes and Hirsh, 1986; Hansen and Hirsh, 1989; Chung et al., 2001). In contrast, acapsular *P. multocida* B2 mutants were resistant to complement-mediated killing (Boyce and Adler, 2000).

Conflicting reports exist with regard to the role of *P. multocida* capsule in adherence, which may be dependent both on the bacterial strain and on the type of host cells used in the studies. Pruimboom found that *P. multocida* adhesion to macrophages was mediated by capsular HA (Pruimboom et al., 1996). Enzymatic depolymerization of the capsule decreased *P. multocida* serotype A adherence to HeLa cells and macrophages (Esslinger et al., 1994). Reduced adherence to macrophages was reported for a spontaneous *P. multocida* serotype A mutant (Pruimboom et al., 1996). *P. multocida* serotype A strains also had reduced adherence to peripheral blood monocytes as compared to air sac macrophages, and the exposure of monocytes to anti-HA-binding cell surface proteoglycan (CD44) monoclonal antibody decreased bacterial binding (Pruimboom et al., 1999). In contrast, Glorioso et al. (1982) showed increased adherence of *P. multocida* serogroup A to HeLa cells and rabbit pharyngeal cells following enzymatic
depolymerization of the capsule. Others demonstrated that the absence of capsule increases *P. multocida* adherence to and colonization of lung and tracheal by cells (Jacques et al., 1994).

**OMPs**

Several *P. multocida* OMPs possess virulence attributes including surface exposure, *in vivo* expression, immunogenic and bactERICidal properties (Confer, 1993; Confer et al., 1996; Marandi and Mittal, 1996; Dabo et al., 1997; Vasfi Marandi and Mittal, 1997; Gatto et al., 2002; Borrathbay et al., 2003; Davies et al., 2004). Our studies of bovine *P. multocida* serogroup A isolates identified several surface-exposed and *in vivo*-expressed OMPs, including an *E. coli* OmpA and a *H. influenzae* type b (Hib) P1 homolog proteins (Dabo et al., 1997). *P. multocida* OmpA is a major immunogenic and antigenic OMP that is conserved, surface-exposed and expressed *in vivo* (Dabo et al., 1997, 2003). High antibody responses to this and several other OMPs consistently correlated with resistance of cattle to challenge with virulent *P. multocida* (Dabo et al., 1997, 2003).

OmpH is another major antigenic, surface-exposed and conserved OMP porin that is detected in 100% of bovine isolates investigated (Lugetenberg et al., 1984, 1986; Chevalier et al., 1993; Ewers et al., 2006) and has potential as a vaccine candidate (Luo et al., 1997, 1999; Vasfi Marandi and Mittal, 1997). The protein provided protection against *P. multocida* challenge in mice (Vasfi Marandi and Mittal, 1997) and chickens (Luo et al., 1997, 1999), and its expression was shown to be negatively regulated by iron and glucose. Electrophoretic analysis showed that OmpH levels increase in *P. multocida* fur mutant compared to wild-type strain (Bosch et al., 2001). The expression of OmpH–lacZ fusion in the wild-type strain increases in the presence of chelating agent 2,2′-dipyridyl (DPD), and there was a 5-fold decline in the expression of the gene in the wild type as compared to the fur mutant when grown in the presence of glucose. Given the importance of OmpH as a potential immunogen and the observed inhibitory effect on OmpH expression, those authors recommended growing bacteria in the absence of glucose for bacterin production (Bosch et al., 2001).

Several *in vivo* studies identified potential *P. multocida* virulence genes and OMP-associated cross-protection factors (Rimler, 1994; Wang and Glisson, 1994a, b; Hunt et al., 2001). When using antisera produced against live *P. multocida* isolates, Wang and Glisson (1994a, b) found four high-molecular-weight proteins (ranging from 204 to 153 kDa) that stimulate cross-protection. Hunt et al. (2001) used *in vivo* expression technology (IVET) to identify two lipoproteins (PM0554 and PM14440) with homology to conserved and widely distributed PCP (peptidoglycan-associated lipoprotein (Pal) cross-reaction protein), and protein D of non-typeable *H. influenzae*, respectively. The *H. influenzae* protein D mediates the binding to IgD, a form of immune evasion, and stimulates cross-protection against heterologous challenge in rats (Akkoyunlu et al., 1996). Anti-PCP serum was bactericidal against *H. influenzae* clinical isolates (Deich et al., 1990). *P. multocida* PCP was found to be surface-exposed, while protein D (designated GlpQ) was not (Lo et al., 2004); however, neither protein stimulated protection against challenge with live *P. multocida* in mice and chickens. Boyle et al. (2006) found significant up-regulation of Pm0803 and the ef-Tu (elongation factor-Tu) during growth *in vivo* and significant down-regulation of OmpW. ef-Tu (tufA and tufB) is cytoplasmic in location but may be targeted to the outer membrane since its homologs bind host fibronectin in *Mycoplansma pneumoniae* and host mucin in *Lactobacillus johnsonii* (Boyce et al., 2006). While the major *P. multocida* surface proteins have been studied, little is known about the less abundant proteins and their roles in the pathogenesis of *P. multocida* infections in general.

### Iron-regulated and acquisition proteins – IROMPs

Iron acquisition and uptake are essential for bacterial survival and pathogenic bacteria have developed different strategies for that uptake. *P. multocida* produces both iron-chelating siderophores and outer membrane receptors for the iron-binding host molecules (transferrin, hemoglobin and hemin) (Choi-Kim et al., 1991; Ogunnariwo et al., 1991). Early studies showed that some *P. multocida* isolates produce siderophores (Choi-Kim et al., 1991) or contained only one specific Tbp (TbpA) (Ogunnariwo and Schryvers, 2001; Ogunnariwo et al., 1991). *P. multocida* grown under iron-depleted media or *in vivo* expressed three iron-regulated OMPs (IROMPs) with molecular masses of 76, 84 and 94 kDa, with all three having affinity for siderophore binding (Choi-Kim et al., 1991). The *P. multocida* *tbpA* gene was absent in avian isolates and detected only in ruminants (cattle, 70%; sheep, 80%; and buffalo, 57.1%) (Ewers et al., 2006). TbpA from *P. multocida* serotype B:2 strain was predicted to be a gated pore with several membrane-spanning regions (Shivachandra et al., 2005). The specific role of TbpA in *P. multocida* virulence is still unknown.

Analysis of attenuated mutants by Fuller et al. (2000) identified two loci containing potential virulence genes with homology to iron-acquisition-related genes *exbB* and *hgbA* of *H. influenzae*. *exbB*, along with *exbD*, are part of the *tonB* operon in *P. multocida* and are independently transcribed (Bosch et al., 2002a). In a related study, Bosch et al. (2002b) demonstrated that three ORFs (PM0298, PM0299 and PM300) of *P. multocida* constitute a single transcriptional operon. Mutants defective in PM0298 or PM0299 demonstrated that these genes were essential for the viability of the pathogen (Bosch et al., 2002b).
et al., 2002b). The product of PM300 (designated HgbA) bound hemoglobin (Bosch et al., 2002b) and hemin (Bosch et al., 2004), but binding and virulence of the bghA-mutant strain were not affected (Bosch et al., 2002b). Similarly, inserional inactivation of hgbB did not affect the ability of P. multocida to bind hemoglobin (Cox et al., 2003), suggesting the presence of additional hemoglobin receptor proteins in the P. multocida genome. Consistent with that finding, comparative analysis of the P. multocida Pm70 genome (May et al., 2001) identified nine iron-acquisition-related genes (including hgbA) encoding immunogenic proteins (Bosch et al., 2004). Six proteins bound both hemin and hemoglobin and the other two bound either hemin or hemoglobin. These proteins did not stimulate protective immunity in mice against P. multocida infection when inoculated alone (Bosch et al., 2004). P. multocida HbpA (hemin-binding protein A), another iron acquisition gene, was shown to be immunogenic and regulated through a Fur-independent mechanism; HbpA-specific antibodies were not protective in mice (Garrido et al., 2003). The prevalence of tonB, hbpA, hgbA and hgbB genes in P. multocida bovine strains is reported to be 100, 70.2, 95.2 and 57.7%, respectively (Ewers et al., 2006), and there was significant association between disease status and the presence of hgbB and hbpA as single virulence-associated genes in P. multocida bovine isolates. Our characterization of a heme absorption system receptor (HasR) in a P. multocida bovine isolate demonstrated that the protein, which is 98% identical to its homolog in the P. multocida Pm70 genome sequence, is surface-exposed, is conserved among most P. multocida isolates and is an immunodominant IROMP (Prado et al., 2005). Finally, a recent comparative transcriptional response to iron limitation between M. haemolytica and P. multocida found few homologous genes induced in both organisms and include the hemoglobin receptor bmbR2, the hbpAB, yfeABCD ABC-type systems, tonB and exbD (Roehrig et al., 2007). The hemin and hemoglobin transporter genes were among the genes uniquely found up-regulated in P. multocida, an indication of its capacity to utilize iron from blood sources (Roehrig et al., 2007).

**Extracellular enzymes**

Several extracellular enzymes such as proteases and lipases have also been reported as possible virulence factors in P. multocida (Negrete-Abascal et al., 1999; Pratt et al., 2000). The role of lipases in pathogenic bacteria is probably nutritional, whereas proteases may assist pathogens against host defense degrading host IgG and reducing opsonization. Negrete-Abascal (1999) reported the secretion of proteases into the culture supernatant of P. multocida from different species including bovine. Those proteases were capable of degrading host IgG in a wide range of pH (Negrete-Abascal et al., 1999). Pratt et al. (2000) demonstrated the production of lipases by clinical isolates of P. multocida from different species. The role, if any, these enzymes play in the pathogenesis of P. multocida infections is unknown.

**Immunity against P. multocida serogroup A in cattle**

Immunity of cattle to P. multocida has been investigated most extensively with respect to hemorrhagic septicemia of cattle and buffalo involving serotypes B:2 and E:2 (Confer, 1995). Studies on immunity in cattle pneumonia caused by P. multocida serotype A isolates are limited. This section of the review will focus on current knowledge of bovine immunity against P. multocida serotype A isolates.

Important antigens responsible for immunity against P. multocida serotype A isolates in cattle are not completely understood. Adler et al. (1999) reviewed candidate vaccine antigens and genes in P. multocida related to various diseases of production animals and indicated that LPS can provide some protection against homologous serotypes. They further reviewed several putative immunologically important antigens by focusing on studies that focused on antigens expressed under in vivo conditions. These include OMP Oma87, which is a homologue of the D15 protective antigen of H. influenzae; type 4 fimbria and its subunit protein PtfA; capsule; and IROMPs, such as the Tbp TbpA. None of these putative vaccine candidates were directly investigated with respect to stimulating immunity in cattle against P. multocida serotype A. The gene for Oma87 has been cloned and expressed; however, vaccination against that protein failed to protect chickens against an experimental challenge (Mitchison et al., 2000; Chaudhuri and Goswami, 2001). The ptfA gene was demonstrated in most bovine P. multocida isolates and has been cloned (Adler et al., 1999; Ewers et al., 2006); however, immunogenicity was not reported. The potential for TbpA to be a good vaccine candidate is questionable, because approximately 30% of bovine P. multocida strains lack the tbpA gene (Ewers et al., 2006; Roehrig et al., 2007). We suggested that OMPs from bovine P. multocida serotype A strains may be good vaccine candidates based on correlations between high antibodies and resistance against experimental bovine P. multocida-induced pneumonia (Confer et al., 1996). However, Abdullahi et al. (1990), using bovine P. multocida serotype A isolates, questioned the potential efficacy of an OMP vaccine based on data obtained from vaccination of mice with whole bacterial cell vaccines followed by intraperitoneal challenge.

Immunity in bovine respiratory pasteurellosis is presumed to be antibody-mediated. Anti-P. multocida antibodies were demonstrated in dairy cow colostrum by quantitative indirect immunofluorescence and
agglutination assays (Gresham et al., 1984). Passive antibodies to *Pasteurella multocida* serotype A were demonstrated in sera from newborn dairy and beef calves, and those passively acquired antibodies were short-lived in sera compared to values of 4–6 months for antiviral antibodies demonstrated in one of these studies (Virtala et al., 1996; Fulton et al., 2004; Step et al., 2005; Prado et al., 2006). Virtala et al. (1996) found no correlation between postcolostral antibody titers in dairy calves with and without naturally acquired respiratory disease, usually associated with *P. multocida* and/or *Mycoplasma dispar* infection. Passively acquired anti-*P. multocida* OMP antibodies were demonstrated in dairy calf sera within 24 h of birth (Fulton et al., 2004; Step et al., 2005). Step et al. (2005) demonstrated that while dairy calves were isolated in hutches, *P. multocida* antibody concentrations declined to a nadir between 47 and 73 days of age. Those calves were subsequently removed from hutches, grouped together and spontaneously seroconverted to *P. multocida*, most likely from exposure to the bacterium from other calves and from fomites or from stress-related factors stimulating commensal *P. multocida* proliferation. In beef calves, similar passive/acquired anti-*P. multocida* antibody responses were found in calves from two different herds (Prado et al., 2006). Serum concentrations of anti-*P. multocida* antibodies in neonatal calves varied greatly between the two herds. In both herds, however, passively acquired IgG1 antibodies declined rapidly and reached their nadir between 60 and 90 days of age. This was followed by a rapid rise in anti-*P. multocida* IgM and IgG2 antibodies that reached peak concentrations between 150 and 180 days of age. Although, at this time, protection against respiratory disease has not been associated with passively acquired anti-*P. multocida* antibodies, their short-lived nature negates any potentially protective role after approximately 3 months of age.

As indicated above, active antibody responses to *P. multocida* develop in calves at a young age at least as measured by ELISA (Virtala et al., 1996; Step et al., 2005; Prado et al., 2006). Whether antibodies acquired naturally or from vaccination truly protect cattle against *P. multocida*-associated pneumonia is not well documented. Serum anti-*P. multocida* antibody concentrations were associated with several cattle production parameters in a study of cattle shipped from 24 different ranches to a feedlot without going through the order buyer/sale barn marketing system (Fulton et al., 2002). In that study, high antibodies to *P. multocida* OMPs at the time of feedlot entry were associated with increased average daily gain during feeding, whereas low *P. multocida* antibodies were associated with a decreased net value to owner, decreased gross margin and increased total costs of finishing. There was, however, no association between health or treatment costs and *P. multocida* antibody concentrations. Few of the represented herds had been vaccinated with *P. multocida* vaccines. Therefore, it is assumed that the antibodies measured were from prior natural exposure.

Enhanced resistance to respiratory *P. multocida* challenge has been demonstrated in several studies using live vaccines. These include wild-type bacteria, streptomycin-dependent mutant and chemically modified *P. multocida*. (Kucera et al., 1981; Panciera et al., 1984; Chengappa et al., 1989; Confer et al., 1996; Mathy et al., 2002). In addition, Kadel et al. (1985) demonstrated greater weight gains, less severe clinical signs of pneumonia and lower death rates in calves vaccinated with both live streptomycin-dependent *P. multocida* and live streptomycin-dependent *M. haemolytica* when compared to non-vaccinated calves. In several studies, a correlation was demonstrated between high serum *P. multocida* antibodies and resistance against experimental challenge in live *P. multocida*-vaccinated cattle. Compared to controls, cattle vaccinated with live *P. multocida* via aerosol or subcutaneous routes had significant reduction in lesion scores (Panciera et al., 1984) or reduced histologic lesions, reduced neutrophil influx, reduced IL8 and IL1ß, and increased IL2 responses (Mathy et al., 2002). Live *P. multocida*-vaccinated calves also developed serum antibodies against the whole bacteria (Panciera et al., 1984) or to outer membrane preparations (Confer et al., 1996) using quantitative indirect immunofluorescence assay or ELISA, respectively. In both studies, there was a significant correlation (P<0.05) between high antibodies against *P. multocida* and low lesion scores following transthoracic challenge. In addition, using Western blotting and densitometric analyses, high serum antibodies against six specific OMPs (approximately 100, 90, 85, 74, 53, 35 and 16 kDa) significantly correlated with less severe lesions following *P. multocida* challenge (Confer et al., 1996). Amino-terminus sequencing demonstrated that the 35 kDa protein is a heat modifiable (28–35 kDa) OMP from the OmpA family of proteins (Dabo et al., 1996). Another *P. multocida* OMP shown to be of importance in stimulating resistance to infection in animals is OmpH, an approximately 33.6–38.5 kDa protein (Vasfi Marandi and Mittal, 1997; Davies and Lee, 2004). That protein, however, has not been studied immunologically in cattle.

Studies of vaccination of cattle with non-living *P. multocida* vaccines have also been limited. Early studies suggested some protection of cattle against shipping fever using *Pasteurella* spp. bacterins (reviewed in Collins, 1977). Vaccination of calves with a vaccine containing modified-live-Parainfluenza 3 virus, killed *M. haemolytica* and killed *P. multocida* vaccine enhanced resistance against experimental challenge involving stress and aerosol exposure to all three infectious agents (Matsuoka et al., 1966). In more recent years, however, neither aerosol nor intratracheal vaccination of calves...
with formalin-killed *P. multocida* resulted in protection against experimental pulmonary challenge (Confer et al., 1996; Dowling et al., 2004; Prado et al., 2005). In our laboratory, we demonstrated that calves vaccinated with Freund's incomplete adjuvant–outer membrane preparations from *P. multocida* cultured in the presence or absence of an iron chelator (iROMPs or OMPs, respectively) developed antibodies to OMPs and iROMPs, as detected by ELISA (Prado et al., 2005). Vaccination with OMPs or iROMPs resulted in significant decrease in lesion scores following *P. multocida* challenge compared to vaccination with adjuvant alone. Of specific interest was that vaccinated calves developed intense antibody responses to a 96 kDa protein band, and correlation between high anti-96 kDa antibodies and low lesion scores approached significance (*P* < 0.06). The 96 kDa OMP was a homologue to the iron-regulated protein HaSR, a heme acquisition receptor protein. The hasR gene, however, was not demonstrated in all *P. multocida* isolates examined. Therefore, its potential immunological significance is not known.

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