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Mannheimia haemolytica and bovine respiratory disease

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Abstract

Mannheimia haemolytica is the principal bacterium isolated from respiratory disease in feedlot cattle and is a significant component of enzootic pneumonia in all neonatal calves. A commensal of the nasopharynx, *M. haemolytica* is an opportunist, gaining access to the lungs when host defenses are compromised by stress or infection with respiratory viruses or mycoplasma. Although several serotypes act as commensals, A1 and A6 are the most frequent isolates from pneumonic lungs. Potential virulence factors include adhesin, capsular polysaccharide, fimbriae, iron-regulated outer membrane proteins, leukotoxin (Lkt), lipopolysaccharide (LPS), lipoproteins, neuraminidase, sialoglycoprotease and transferrin-binding proteins. Of these, Lkt is pivotal in induction of pneumonia. Lkt-mediated infiltration and destruction of neutrophils and other leukocytes impairs bacterial clearance and contributes to development of fibrinous pneumonia. LPS may act synergistically with Lkt, enhancing its effects and contributing endotoxic activity. Antibiotics are employed extensively in the feedlot industry, both prophylactically and therapeutically, but their efficacy varies because of inconsistencies in diagnosis and treatment regimes and development of antibiotic resistance. Vaccines have been used for many decades, even though traditional bacterins failed to demonstrate protection and their use often enhanced disease in vaccinated animals. Modern vaccines use culture supernatants containing Lkt and other soluble antigens, or bacterial extracts, alone or combined with bacterins. These vaccines have 50–70% efficacy in prevention of *M. haemolytica* pneumonia. Effective control of *M. haemolytica* pneumonia is likely to require a combination of more definitive diagnosis, efficacious vaccines, therapeutic intervention and improved management practices.

Keywords: bovine pnemonia, pneumonic pasteurellosis, *Mannheimia haemolytica*, leukotoxin, respiratory vaccines

Introduction

Mannheimia haemolytica is a bovine pathogen of considerable economic importance to the global cattle industry and in particular to the North American feedlot industry. Although the organism naturally exists as a commensal of the upper respiratory tract and nasopharynx of healthy ruminants (Frank, 1989; Carter *et al*., 1995), it is also associated with the diseased state, pneumonic pasteurellosis, and is considered the major bacterial agent of bovine respiratory disease complex. The organism may cause disease in young calves as a component of enzootic pneumonia of beef and dairy calves (Kiorpes *et al*., 1988; Van Donkersgoed *et al*., 1993a; Ames, 1997); however, its greatest impact occurs in recently weaned beef calves shortly after entry to feedlots (Jubb and Kennedy, 1970; Mosier *et al*., 1989; Wilson, 1989). Economic losses to the North American feedlot industry due to respiratory disease have been estimated to be as high as 1 billion dollars annually (Whiteley *et al*., 1992). Despite improved management practices and extensive use of vaccination programs, bovine respiratory disease continues to be a major cause of losses in feedlot cattle.

Clinically, cattle suffering from *M. haemolytica* respiratory infections may have fever, nasal discharge, cough

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and respiratory distress along with inappetance and weight loss (Friend *et al.*, 1977). The major cause of death is acute fibrinous pleuropneumonia. Necropsy findings include obstruction of bronchioles with fibrinous exudate, accumulation of neutrophils, macrophages and fibrin in the alveoli, and thrombosis and distention of lymphatic vessels.

The organism

M. haemolytica is a Gram-negative, non-motile, non-spore-forming, fermentative, oxidase-positive, facultative anaerobic coccobacillus (Quinn, 1994; Hirsh and Zee, 1999). The organism is a member of the family *Pasteurellaceae*, genus *Mannheimia*. First named *Bacterium bipolare multocidum* by Theodore Kitt (Kitt, 1885), it was renamed *Pasteurella haemolytica* in 1932, to reflect its weakly hemolytic phenotype on sheep's blood agar plates (Newsome and Cross, 1932) and was historically classified into 16 serotypes, based on an indirect hemagglutination test using extractable capsular surface antigens (Biberstein, 1978). The genus was further divided into distinct biotypes (A and T) based on the ability to ferment arabinose or trehalose, respectively (Smith, 1961; Lo and Shewen, 1991). Twelve A serotypes and four T serotypes were identified. Later, Younan and Fodor (1995) characterized a new serotype of *M. haemolytica*, A17, isolated from sheep in Syria. Through DNA–DNA hybridization studies and 16S RNA sequencing, all but one of the A biotypes were assigned the species designation *M. haemolytica* (Angen *et al.*, 1999a). *P. haemolytica* T biotypes were renamed *Pasteurella trehalosi* (Bingham *et al.*, 1990; Sneath and Stevens, 1990), then *Bibersteinia trehalosi* (Blackall *et al.*, 2007). The remaining A11 serotype was renamed *Mannheimia glucosida* (Angen *et al.*, 1999b).

M. haemolytica comprises 12 capsular serotypes based on those originally assigned to *P. haemolytica* (A1, A2, A5, A6, A7, A8, A9, A12, A13, A14, A16 and A17) (Angen *et al.*, 1999b). Both serotypes A1 and A2 colonize the upper respiratory tract of cattle and sheep. Pneumonia in cattle is mainly associated with isolation of serotype A1 from lungs at necropsy (Frank and Smith, 1983; Allan *et al.*, 1985), even though healthy cattle frequently carry both serotypes A1 and A2 in the nasopharynx. Davies *et al.* (2001) state that 'serotype A1 and A6 strains account for almost all cases of bovine pneumonic pasteurellosis', and recent surveys in Germany (Ewers *et al.*, 2004) and the USA (Purdy *et al.*, 1997; Al-Ghamdi *et al.*, 2000) document that serotype A6 constitutes 30% of the total number of serotyped isolates. Interestingly, apart from the capsule structure, serotype A1 and A6 are extremely similar structurally (Davies and Donachie, 1996; Morton *et al.*, 1996). Serotype A2 is a common cause of pneumonic pasteurellosis in sheep (Shewen and Conlon, 1993) but there is also an increased prevalence of serotypes A5, A6 and A7 (Ewers *et al.*, 2004).

M. haemolytica resides in the nasopharynx (Babiuk and Acres, 1984) and tonsils (Frank and Briggs, 1992; Frank *et al.*, 1995) of healthy calves. As a commensal organism, *M. haemolytica* inhabits the nasopharynx and maintains a symbiotic relationship with its host; however, key inciting events including stress of weaning, adverse weather conditions, changes in feed, transportation over long distances, mixing of cattle and infection with other microorganisms (including viruses and mycoplasma sp.) cause this benign coexistence to become a fulminating disease state (Farley, 1932; Blood *et al.*, 1983).

Tonsillar tissue has been identified as a reservoir for *M. haemolytica* (Frank and Briggs, 1992; Frank *et al.*, 1995). Calves may be negative for *M. haemolytica* on culture of nasal swabs, but positive on culture of the tonsils (Frank *et al.*, 1994). Not only does the frequency of isolation of *M. haemolytica* A1 increase as calves move to the feedlot, but the number of bacteria also increases rapidly (Frank, 1984). When high numbers of *M. haemolytica* are present on the nasal mucosa of calves, the bacteria are inhaled into the lungs (Grey and Thomson, 1971). In healthy calves, lung clearance of inhaled *M. haemolytica* is highly efficient, with elimination of 90% of an administered dose within 4 h (Lillie and Thomson, 1972) (Fig. 1A).

Several studies have attempted to understand the mechanisms associated with the switch from commensal to pathogen. Cold stress (chilling of calves with cold water) and transportation were studied for their effect on immunosuppression. Cold stress was demonstrated to transiently increase plasma cortisol levels, but had no effect on histamine or bradykinin levels (Slocombe *et al.*, 1984). Transportation was found to cause a transient elevation of plasma cortisol levels, suppressing *in vitro* lymphocyte blastogenesis (Filion *et al.*, 1984). Serum complement activity has also been noted to be decreased in calves that are purchased at auction and moved into the feedlot (Purdy *et al.*, 1991). These alterations in immune function could impact the ability of the calf to maintain normal homeostasis with the commensal organism.

The effects of mixing of calves (during transportation, in auction barns or after sorting at the feedlot) on the prevalence of respiratory disease may result from the stresses of interacting with strange calves or may reflect increased opportunities to contact infectious agents (Jericho, 1979). Viral and bacterial agents break down the antimicrobial barrier of β-defensins, anionic peptides and serous and mucous secretions of the respiratory tract, allowing *M. haemolytica* to be released from its commensal status (Brogden *et al.*, 1998). Infection with bovine herpes virus 1 (BHV-1) parainfluenza virus 3 (PI-3) and bovine viral diarrhea virus (BVDV) leads to proliferation of the bacteria in the nasopharynx, interferes with normal clearance from the lungs (Lopez *et al.*, 1976) and impairs the ciliary activity of epithelial cells in the trachea (Rossi and Kiesel, 1977). BVDV infection also

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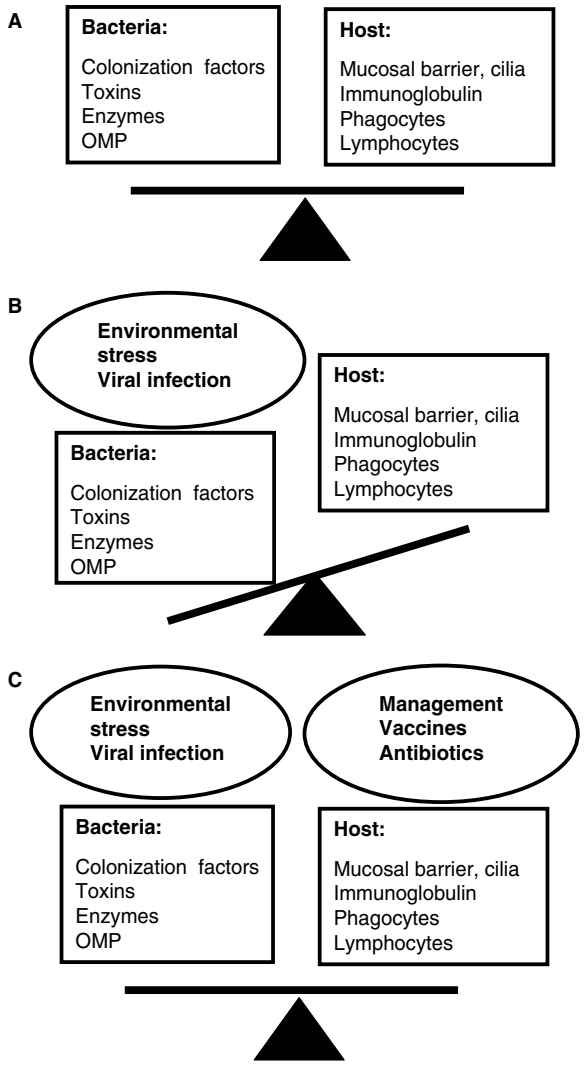

Fig. 1. Host–bacterium–environment interactions in *Mannheimia haemolytica* pneumonia of cattle. (**A**) Homeostasis in the commensal state; (**B**) environmental factors tip the balance to favor lung colonization and disease; (**C**) intervention strategies restore homeostasis.

leads to impaired neutrophil and lymphocyte functions (Brown *et al*., 1991) predisposing to bacterial pneumonia. Immunosuppression in the case of bovine respiratory syncytial (BRS) virus (Woldehiwet and Sharma, 1992) and BHV-1 diminishes the activity of T lymphocytes, B lymphocytes, monocytes and macrophages (Brown and Ananaba, 1988). All of these factors tend to impair innate immune defenses and can provide an opportunity for the organism to gain access to deeper structures of the respiratory tract (Ribble *et al*., 1995a) (Fig. 1B).

Bacterial virulence factors

Multiple products and components of *M. haemolytica* A1 have been proposed as virulence factors, including an adhesin (Jaramillo *et al*., 2000), capsular polysaccharide (Confer *et al*., 1989; Conlon and Shewen, 1993; Brogden *et al*., 1995), fimbriae (Morck *et al*., 1987, 1989), iron-regulated outer membrane proteins (OMPs) (Squire *et al*., 1984; Morck *et al*., 1991; Gatewood *et al*., 1994), leukotoxin (Lkt) (Gentry *et al*., 1985; Shewen and Wilkie, 1985, 1988), lipopolysaccharide (LPS) (Confer and Simons, 1986), lipoproteins (Cooney and Lo, 1993; Nardini *et al*., 1998), neuraminidase (Frank and Tabatabai, 1981; Straus and Purdy, 1994; Straus *et al*., 1998), a serotype-specific antigen (Gonzalez-Rayos *et al*., 1986; Lo *et al*, 1991), sialoglycoprotease (Abdullah *et al*., 1992; Lee *et al*., 1994) and transferrin-binding proteins (Ogunnariwo and Schryvers, 1990; Potter *et al*., 1999). The adhesin protein, capsular polysaccharide, fimbriae, sialoglycoprotease and neuraminidase may have roles in the attachment of *M. haemolytica* and its colonization of cells of the respiratory tract of calves (Babiuk and Acres, 1984; Morck *et al*., 1988, 1989; Whiteley *et al*., 1992). The capsular polysaccharide also has antiphagocytic properties (Chae *et al*., 1990) and increases directed migration of neutrophils (Czuprynski *et al*., 1989). The sialoglycoprotease, by cleaving bovine IgG1, may reduce the effectiveness of opsonizing antibodies (Lee and Shewen, 1996). Transferrin-binding proteins and other iron-regulated proteins enable *M. haemolytica* to proliferate *in vivo* in spite of the low iron environment normally maintained by the host. LPS as an inducer of inflammation has a central role in the development of vascular lesions in lung tissue (Whiteley *et al*., 1992).

The virulence factor believed to be pivotal in the pathogenesis of *M. haemolytica* is the ruminant-specific Lkt. The effects of Lkt range from impairment of function to lysis of ruminant leukocytes (Baluyut *et al*., 1981; Berggren *et al*., 1981; Shewen and Wilkie, 1982; Gentry *et al*., 1985; Clinkenbeard *et al*., 1989; DeBey *et al*., 1996). Lkt is a heat labile protein, actively secreted by all serotypes of the bacterium during the logarithmic phase of *in vitro* growth (Shewen and Wilkie, 1985). It is a member of the RTX (repeats in toxin) family of multidomain exotoxins (Lo, 1990) and contains six highly conserved regions, glycine-rich nonapeptide repeats, near the C-terminal end of its structure (Lo, 1990; Coote, 1992; Jeyaseelan *et al*., 2002). Strathdee and Lo (1987) found Lkt to be very similar genetically to *Escherichia coli* α-hemolysin, and Lkt has been shown to lyse sheep erythrocytes (Murphy *et al*., 1995). Like the α-hemolysin, the Lkt is encoded by four genes in the RTX toxin operon, designated C, A, B and D (Lo *et al*., 1985, 1987; Strathdee and Lo, 1987). The A gene (*lktA*) codes for the structural toxin. The product of the C gene is involved in toxin activation in conjunction with an acyl carrier protein. The product of the *lktA* gene is biologically inactive until modified post-translationally by fatty acid acylation (Issartel *et al*., 1991). The products of B and D genes are involved in toxin secretion (Chang *et al*., 1989; Highlander *et al*., 1989; Strathdee and Lo, 1989).

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Through sequence analysis of the *lkt* genes and studies of polymorphism, it has been shown that different serotypes produce different Lkt types and that LktA may vary between bovine and ovine isolates of the same serotype (Davies *et al.*, 2002). Serotypes A1, A5, A6, A8, A9 and A12 have very similar Lkts (LktA1.1, LktA1.2 and LktA1.3), whereas serotype A2 isolates may express any of four Lkt types (LktA2, LktA3, LktA8 and LktA10) (Davies *et al.*, 2001; Davis and Baillie, 2003). Even though these differences exist, polyclonal antibodies raised to one Lkt cross-neutralize Lkt produced by other serotypes, although antisera neutralize homologous Lkt more efficiently (Shewen and Wilkie, 1983; Lainson *et al.*, 1996). The epitope associated with neutralizing activity has been identified for *M. haemolytica* serotype 1 and is located at the C-terminal end of Lkt A (Lainson *et al.*, 1996).

Once the bacterium gains entry into deeper respiratory structures, Lkt plays a major role in lung injury and also in allowing bacteria to survive by evading phagocytic cell destruction. Tatum *et al.* (1998) generated *M. haemolytica* deficient in Lkt through gene knock-out. Although the mutant retained the ability to colonize the upper respiratory tract, it could not induce lung lesions. Fedorova and Highlander (1997) created a mutant strain that secreted an antigenic proLkt that was not leukotoxic or hemolytic, confirming that the *lktC* gene was required for activation of proLkt to mature Lkt. Later, Highlander *et al.* (2000) demonstrated that a strain that secretes inactive Lkt had attenuation of virulence in a calf model.

RTX toxins attach to cells through passive adsorption, which does not always lead to cell lysis, and through specific cell surface receptors. The receptor for Lkt has been identified as the transmembrane receptor CD18, the constant β-subunit of the β_2-integrin family. CD18 complexed with CD11a forms the lymphocyte-function-associated antigen 1 (LFA-1), which is responsible for the high affinity adhesion of Lkt to ruminant leukocytes and platelets (Bailly *et al.*, 1995; Gahmberg, 1997; Lally *et al.*, 1997; Wang (JF), 1998a; Ambagala *et al.*, 1999; Li *et al.*, 1999; Deshpande *et al.*, 2002; Berman *et al.*, 2003; Dassanayake *et al.*, 2007). The repeating nanopeptide of the LktA molecule is the ligand for LFA-1 on host cells (Lally *et al.*, 1999).

The effect of Lkt on bovine cells is dose-dependent. At very low concentrations, the toxin activates target cells triggering respiratory burst and degranulation. As the concentration of Lkt is increased, target cells are stimulated to undergo apoptosis (Lally *et al.*, 1999). At high Lkt concentrations, necrosis of target cells occurs as a consequence of the formation of pore-like structures in the plasma membrane (Clarke *et al.*, 1998), which leads to K^+ efflux and Ca^{2+} influx, colloidal osmotic swelling and eventual cell lysis (Orrenius *et al.*, 2003). The size of the pore varies among RTX toxins; in the case of *M. haemolytica* Lkt, the transmembrane pore is 0.6–1.0 nm in diameter (Clinkenbeard *et al.*, 1989). Extensive *in vitro* studies have been successful in reproducing neutrophil necrosis utilizing purified Lkt (Wang (Z), 1998b; Ambagala *et al.*, 1999; Sun *et al.*, 1999, 2000; Jeyaseelan *et al.*, 2000, 2001; Cudd *et al.*, 2001; Davies and Baillie, 2003). At subcytolytic concentrations, Lkt enhances the inflammatory response by activating cells to produce mediators and release reactive oxygen metabolites and proteases. The lesions seen in infected lungs, including fibrinous exudate and thrombosis of lymphatic vessels, result, in part, from effects of the toxin on neutrophils (Slocombe *et al.*, 1985; Breider *et al.*, 1988) and from lysis of platelets (Clinkenbeard and Upton, 1991).

Another critical virulence factor is the lipid A component of the LPS of the cell wall of the organism. The lipid A fraction is responsible for endotoxic effects, such as pyrexia, macrophage activation, release of tumor necrosis factor and induction of hypotensive shock (Keiss *et al.*, 1964), and plays a role in the vascular lesions seen in diseased lung tissue (Whiteley *et al.*, 1992). In addition, LPS forms complexes with Lkt, which may enhance cytotoxicity (Li and Clinkenbeard, 1999). Although LPS has been reported to be a major antigenic determinant (Confer *et al.*, 1986), antibody titers to LPS do not correlate with resistance to experimental pneumonia (Confer *et al.*, 1989; Mosier *et al.*, 1995).

Lipoproteins have also been identified (Cooney and Lo, 1993; Nardini *et al.*, 1998) and are present in most serotypes. A surface-exposed 45-kDa OMP, designated PlpE, was sequenced and cloned by Pandher *et al.* (1999). Mosier *et al.* (1989) reported this protein to be immunogenic in cattle and Pandher *et al.* (1998) found that antibodies to PlpE were associated with complement-mediated killing of *M. haemolytica*. A recombinant PlpE was highly immunogenic when injected subcutaneously in vaccination studies (Confer *et al.*, 2003). The same group demonstrated that the surface-exposed immunodominant epitope between amino acids 26 and 76 conferred protection from challenge (Ayalew *et al.*, 2004). A recent report from these researchers suggests that addition of recombinant PlpE to existing commercial vaccines enhances protection against experimental challenge (Confer *et al.*, 2006).

Acute pulmonary infection in feedlot cattle is characterized by a fibrinosuppurative and necrotizing inflammatory response. Parenchymal necrosis is most likely caused by Lkt and LPS, as well as inflammatory factors released by neutrophils and other cells of the acute inflammatory process. Neutrophil infiltration during *M. haemolytica* pneumonia is associated with alveolar epithelial cell damage and necrosis. Slocombe *et al.* (1985) demonstrated the contribution of neutrophils to parenchymal damage of the lung in pasteurellosis. Depletion of neutrophils prior to inoculation with the bacteria protects calves from the gross fibrinopurulent pneumonic and pleuritic lesions (Weiss *et al.*, 1991; Ulevitch and Tobias, 1995) but less severe changes still

occur (Breider *et al*., 1988). Pathognomonic for bovine pneumonic pasteurellosis is necrosis of the alveolar epithelium due to the strong influx of neutrophils and accumulation of fibrin in the lungs. Depending on the size and distribution of the fibronecrotizing lesions, this pneumonia may result in death.

Feedlot management practices and therapeutic intervention

It has been questioned whether pneumonic pasteurellosis in feedlot calves should be considered a highly contagious disease (Thomson, 1984). Although morbidity rates as high as 69% have been reported in the first weeks after feedlot arrival (Kelly and Janzen, 1986), it has been observed that 'fibrinous pneumonia does not sweep through the feedlot like an epizootic but rather it centres on certain pens' (Thomson, 1984). This would suggest that characteristics of the calves in the various pens rather than mere exposure to *M. haemolytica* are critical in determining disease outcomes. In an experimental study, 50% of non-challenged control calves (naive to *M. haemolytica*) in contact with animals challenged with *M. haemolytica* developed clinical signs of respiratory disease and responded serologically (Gibbs *et al*., 1984), but it is unclear how well this reflects transmission under field conditions.

In reality, pneumonic pasteurellosis is a management disease resulting from an incompatibility between the biology of calves (and their pathogens) and the managing and/or marketing systems devised by humans. Calves that move directly from a ranch into feedlots without moving through saleyards, and without mixing with calves from other sources, have an expected morbidity of less than 5% (Radostits *et al*., 1994). While vaccines and antibiotics can be useful in controlling pneumonia, basic changes in when and how calves are weaned, sold and transported to feedlots would have a profound impact on the prevalence of disease.

Antibiotics

Injectable antibiotics are employed extensively in the feedlot industry in North America in attempts to prevent and treat bovine respiratory disease. Antibiotics are rarely selected on the basis of *in vitro* sensitivity of isolates from nasal or pharyngeal swabs. Isolates from these sources do not accurately reflect organisms present in the lower respiratory tract of the same animal (Allen *et al*., 1991). Necropsy specimens from antibiotic-treated calves also do not provide reliable information as to the antibiotic sensitivities of the organisms initiating the pneumonia. Most published studies examining the efficacy of antibiotics against disease in feedlots make no attempt to distinguish pneumonic pasteurellosis from respiratory conditions caused by other bacteria or by viruses (Mechor *et al*., 1988; Libersa *et al*., 1995; Vogel *et al*., 1998). The term 'undifferentiated fever' has been considered synonymous with 'bovine respiratory disease complex' by some workers (Jim *et al*., 1999; Booker *et al*., 2007; Schunicht *et al*., 2007). Thus a succession of antimicrobial drugs have been examined for efficacy against undifferentiated respiratory disease under field conditions, including penicillin (Mechor *et al*., 1988; Bateman *et al*., 1990), oxytetracycline (Mechor *et al*., 1988; Bateman *et al*., 1990; Harland *et al*., 1991), trimethoprim/sulfadoxine (Mechor *et al*., 1988; Bateman *et al*., 1990; Harland *et al*., 1991), ampicillin (Bentley and Cummins, 1987; Libersa *et al*., 1995), tilmicosin (Gorham *et al*., 1990; Schumann *et al*., 1990; Hoar *et al*., 1998), florfenicol (Libersa *et al*., 1995; Hoar *et al*., 1998; Jim *et al*., 1999) and tulathromycin (Booker *et al*., 2007; Schunicht *et al*., 2007; Wellman and O'Connor, 2007). Although all of these antimicrobials have been efficacious for treatment of bovine respiratory disease, many isolates of *M. haemolytica* are now resistant to penicillin, ampicillin, tetracycline, sulfonamides and tilmicosin (Bateman, 1993; Watts *et al*., 1994; Apley, 1997; Welsh *et al*., 2004). Penicillin and oxytetracycline have been used at higher dosage rates than recommended by the manufacturers in attempts to improve efficacy without resorting to the use of more expensive antibiotics (Bateman, 1993), prompting concerns about antibiotic residues in meat (Mechor *et al*., 1988). The use of antibiotics at dosage rates, or by routes, or in species not approved by governmental agencies, has led to legislation in the USA to curtail 'extra-label' use of antibiotics in food-producing animals (Apley, 1997). The availability of ceftiofur (1988), tilmicosin (1992), florfenicol (1996) and most recently tulathromycin (2005) for use in cattle has provided several drugs efficacious at label dosage rates against bovine respiratory pathogens (Bateman, 1993; Apley, 1997; Hoar *et al*., 1998; Jim *et al*., 1999; Schunicht *et al*., 2007), at least for the time being. Current use of antibiotics in treatment of bovine respiratory disease has been reviewed (Apley, 1999). Product formulations providing sustained blood levels of antibiotics for 48–72 h or more [oxytetracyclines (Bateman, 1993), experimental sustained release ceftiofur (Kesler and Bechtol, 1999) and high-dose florfenicol (Varma *et al*., 1998)] are especially valued for use in feedlots as a means of reducing handling and treatment stresses of sick animals and of minimizing labor costs.

In a meta-analysis of 107 field trials of prophylactic mass medication of feedlot cattle, it was concluded that the parenteral administration of tilmicosin or long-acting oxytetracycline preparations on arrival was associated with significant reductions in morbidity (Van Donkersgoed, 1992). Available data were considered inadequate to judge the efficacy of mass medication administered in water or feed. Another meta-analysis of 14 field trials found that treatment of bovine respiratory disease with tulathromycin was associated with approximately 50%

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reduction in the risk of re-treatment compared to treatment with tilomicosin (Wellman and O'Connor, 2007). Several investigators have promoted the concept of 'metaphylactic' (Young, 1995; Jim *et al.*, 1999) use of parenteral antibiotics, meaning the use of mass medication at therapeutic doses before overt signs of disease are evident (Vogel *et al.*, 1998, Booker *et al.*, 2007). A single dose (most commonly oxytetracycline, tilmicosin or tulathromycin) is administered to all calves on arrival, or at some later time point chosen on the basis of observed signs of disease in a minority of animals or on the basis of historical patterns of disease. This practice is considered standard procedure in some parts of North America (Jim *et al.*, 1999). With current concerns about the development of antibiotic-resistant bacteria through excessive agricultural use of antibiotics, antibiotic residues in food products, and animal welfare, it is imperative that efficacious means that are not antibiotic-dependent be developed to prevent pneumonic pasteurellosis.

Vaccines

Vaccines intended for prevention of respiratory disease in feedlot cattle have been manufactured for over a century (Mosier *et al.*, 1989). Commercial vaccines available in Canada (Bowland and Shewen, 2000) and the USA (Hjerpe, 1990) for prevention of bovine respiratory disease have been reviewed. Evaluation of vaccine efficacy in beef calves involves considerable difficulties (Martin, 1989; Ribble, 1989), in part because of difficulties in defining objective outcome measures (Ribble, 1989), in part because of difficulties in achieving adequate group sizes to obtain acceptable statistical power (Wilson, 1989), and in part because of the unpredictability of morbidity and mortality rates from year to year (Van Donkersgoed *et al.*, 1994; Ribble *et al.*, 1995b). It is considered that a single field trial is inadequate to assess the economic impact of a particular vaccine on respiratory disease in feedlots (Wilson, 1989).

Early bacterins were prepared from cultures of *Bacillus boviseptica* (which has since been divided into the species *M. haemolytica* and *Pasteurella multocida*). Following field trials, their efficacy was questioned (Miller *et al.*, 1927) or they were found to have detrimental effects (Farley, 1932). More recently, bacterins prepared from *M. haemolytica* have been reported to have no clear beneficial effect (Hamdy *et al.*, 1965; Martin, 1983) or to have detrimental effects (Schipper and Kelling, 1971; Friend *et al.*, 1977; Wilkie *et al.*, 1980). Live *M. haemolytica* vaccines have been investigated (Mosier *et al.*, 1989; Babiuk and Campos, 1993) and marketed (Hjerpe, 1990), but protection studies have yielded variable results (Smith *et al.*, 1985; Purdy *et al.*, 1986; Mosier *et al.*, 1998). Use of antibiotics in cattle vaccinated with live *M. haemolytica* (within 7 days after or 3 days before vaccination) is not recommended, due to inhibitory effects on replication of vaccine organisms (Hjerpe, 1990). The widespread use of antibiotics in calves at feedlot arrival makes the use of live bacterial vaccines impractical.

Identification of the importance of the Lkt, produced during logarithmic phase growth, in the pathogenesis of pneumonic pasteurellosis (Shewen and Wilkie, 1982, 1985; Gentry *et al.*, 1985) led to the development of a commercial, cell-free, *M. haemolytica* A1 culture supernatant vaccine (Shewen and Wilkie, 1988; Shewen *et al.*, 1988). In experimental trials using two doses of vaccine at an interval of 21 days, efficacy against moderate to severe pneumonia ranged from 60 to 70% (Shewen *et al.*, 1988). Results from field trials were variable (Bateman, 1988; Jim *et al.*, 1988; Thorlakson *et al.*, 1990). Numerous other antigens of *M. haemolytica* have been documented in logarithmic phase culture supernatant, including surface antigens involved in agglutination reactions (Shewen and Wilkie, 1988), capsular polysaccharide (Conlon and Shewen, 1993), lipoproteins (Cooney and Lo, 1993) and sialoglycoprotease (Abdullah *et al.*, 1992), and these antigens may contribute to vaccine efficacy.

Since this initial *M. haemolytica* subunit vaccine, various companies have introduced other subunit or subunit-enriched vaccines consisting of outer membrane extracts (Srinand *et al.*, 1996), or extracted antigens enriched with recombinant Lkt (Babiuk and Campos, 1993; Van Donkersgoed *et al.*, 1993b, 1994, 1995), or bacterins with added culture supernatant antigens (Babiuk and Campos, 1993; Srinand *et al.*, 1996). Most recently (1999), a vaccine based on antigens expressed under iron-limiting conditions has been licensed (Gilmour *et al.*, 1991; Donachie, 1999). Serological responses to selected commercial *M. haemolytica* vaccines have been compared (Srinand *et al.*, 1996; Confer *et al.*, 1998).

Recent studies have focused on the induction of immunity to *M. haemolytica* by mucosal delivery of antigen. Induction of local immunity in the nasopharynx could reduce colonization by *Pasteurella* spp. Viral vectors (replicating in the upper respiratory tract) expressing antigens of *M. haemolytica* (Babiuk and Campos, 1993), oral administration of *M. haemolytica* antigens encapsulated in alginate microspheres (Bowersock *et al.*, 1999) and oral administration of modified live *M. haemolytica* are under consideration (Briggs and Tatum, 1999). Researchers have also engineered plants for expression of *M. haemolytica* antigens, with the ultimate aim of producing a transgenic edible vaccine (Lee *et al.*, 2001)

Conclusion

Given the complex etiology of respiratory disease attributed to *M. haemolytica*, it is unlikely that any single strategy will be completely effective in preventing

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disease. A combination of more definitive diagnostic methods, more efficacious vaccines, improved therapeutic agents and more rational management practices will be needed (Fig. 1C) and the search for solutions is likely to involve veterinary researchers for some time to come.

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