Pasteurella haemolytica complicated respiratory infections in sheep and goats

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Review article

Pasteurella haemolytica complicated respiratory infections in sheep and goats

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Abstract – Respiratory infections which commonly occur in sheep and goats often result from adverse physical and physiological stress combined with viral and bacterial infections. Inevitably, *Pasteurella haemolytica* pneumonia occurs as a result of these interactions. In this review, we present recent advances in research on the complex etiology of pneumonia involving *P. haemolytica*. Initially stress, induced by factors such as heat, overcrowding, exposure to inclement weather, poor ventilation, handling and transport is a major predisposing factor. Respiratory viruses including parainfluenza 3 (PI-3) virus, adenovirus type 6 and respiratory syncytial virus (RSV), and to a lesser extent bovine adenovirus type 2, ovine adenovirus types 1 and 5, and reovirus type 1 cause respiratory infections and pneumonia. More importantly these viruses also dramatically increase the susceptibility of sheep and goats to secondary *P. haemolytica* infection. Primary infection of the lower respiratory tract, with *Mycoplasma ovipneumoniae* and *Bordetella parapertussis* can increase the susceptibility of sheep and goats to secondary *P. haemolytica* infection. It is possible that initial infections with viral or primary bacterial agents break down the antimicrobial barrier consisting of β defensins and anionic peptides found in epithelial cells, resident and inflammatory cells, and serous and mucous secretions of the respiratory tract. Loss of barrier integrity may release *P. haemolytica* from its usual commensal status. Once in the lung, *P. haemolytica* becomes opportunistic. To grow and colonize, *P. haemolytica* uses extracellular products like O-sialoglycoprotein endopeptidase, neuraminidase and RTX leukotoxin, as well as cell-associated products such as capsular polysaccharide, lipopolysaccharide, outer membrane proteins, proteins involved in iron acquisition and a periplasmic superoxide dismutase. In lambs and kids, pneumonic pasteurellosis can be acute, characterized by fever, listlessness, poor appetite and sudden death. Sheep and goats that survive the acute stage may recover or become chronically affected showing reduced lung capacity and weight gain efficiency and sporadic deaths may occur. This infection is detrimental to sheep and goats throughout the world and flocks and herds of small ranches, dairy operations, or large feedlots are all affected.

sheep / pneumonia / virus / *Pasteurella haemolytica* / respiratory infection

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Résumé – Infections respiratoires aggravées par *Pasteurella haemolytica* chez le mouton et la chèvre. Les infections respiratoires, qui se produisent couramment chez le mouton et la chèvre, résultent souvent de stress physique et physiologique associés à des infections virales et bactériennes. Inévitablement, l'interaction de ces facteurs conduit à la pneumonie à *Pasteurella haemolytica.* Dans cette revue de la littérature, nous présentons les avancées récentes sur l'étiologie complexe de la pneumonie à *Pasteurella haemolytica.* À l'origine, le stress, induit par des facteurs tels que la chaleur, la surpopulation, l'exposition à un climat rude, une mauvaise ventilation, la manipulation et le transport, est un facteur majeur de prédisposition. Les virus respiratoires, comprenant le parainfluenza 3 (PI-3), l'adénovirus de type 6, et le virus syncytial respiratoire, et, dans une moindre mesure, l'adénovirus bovin de type 2, les adénovirus ovins de types 1 et 5, et le réovirus de type 1, causent des infections respiratoires et des pneumonies. Ces virus augmentent également de manière très importante la sensibilité des moutons et des chèvres aux infections secondaires à *Pasteurella haemolytica.* Les infections primaires du tractus respiratoire inférieur à *Mycoplasma ovipneumoniae* and *Bordetella parapertussis* peuvent augmenter la sensibilité des moutons et des chèvres aux infections secondaires à *P. haemolytica.* Il est possible que les infections initiales à virus ou à bactéries primaires détruisent la barrière composée de défensines β et de peptides anioniques trouvés dans les cellules épithéliales, les cellules résidentes et inflammatoires, et les sécrétions séreuses et mucosales du tractus respiratoire. La perte de l'intégrité de cette barrière peut faire perdre à *P. haemolytica* son statut commensal habituel. Une fois dans la cavité pulmonaire, *P. haemolytica* devient opportuniste. Afin de grossir et de coloniser, *P. haemolyoca* utilise des produits extracellulaires tels que l'O-sialoglycoprotéine endopeptidase, la neuraminidase, ou la leucotoxine RTX, ainsi que des produits associés à la cellule tels que le polysaccharide capsulaire, le lipopolysaccharide, des protéines de la membrane externe, des protéines impliquées dans l'acquisition de fer, et une superoxyde dismutase périplasmique. Chez l'agneau et le chevreau les pasteurelloses pneumoniques peuvent être aiguës, caractérisées par de la fièvre, une apathie, un faible appétit et une mort soudaine. Les moutons et les chèvres qui survivent à cette phase aiguë peuvent guérir ou devenir chroniquement malades, ce qui se traduit par une réduction de la capacité pulmonaire et de l'efficacité du gain pondéral. Des morts sporadiques peuvent également se produire. Cette infection est préjudiciable pour les moutons et les chèvres dans le monde entier, et les troupeaux et cheptels de toutes tailles, aussi bien laitiers que pour la production de viande, en sont tous affectés.

mouton / pneumonie / virus / *Pasteurella haemolytica* / infection respiratoire

Plan

1. INTRODUCTION

Small ruminants are susceptible to complicated respiratory infections brought on by physical and physiological stresses combined with a variety of infectious agents of both exogenous and endogenous origin. Depending upon the agent and circumstances, acute viral pneumonia, mild proliferative pneumonia, acute bacterial pneumonia, or chronic proliferative pneumonia may result (Martin, 1996) often involving *Pasteurella haemolytica*. In lambs and kids, pneumonic pasteurellosis can be acute with fever, listlessness, dyspnea, poor appetite and sudden death. Those animals which survive the acute stage may recover or become chronically affected with reduced lung capacity and weight gain efficiency, and sporadic deaths may occur. Pneumonic pasteurellosis is important to sheep and goats throughout the world. Flocks and herds of small ranches, dairy operations, or large feedlots are all affected.

In the past few years, research on respiratory tract disease in small ruminants has progressed at a remarkable rate. The etiology of complex respiratory infections is being identified and the role of each individual agent, and its determinants for pathogenicity are being analysed. Subsequently, data are being assembled to explain the complex mechanisms which result in pulmonary damage. In this review, we will present recent advances in research on the complex etiology of pneumonic pasteurellosis in sheep caused by *P. haemolytica*. However, in some areas of research, progress has lagged and information is lacking. In those instances, we have used research findings from similar work in cattle and other animals to substantiate the possible situation in sheep.

2. INNATE PULMONARY IMMUNITY

Pasteurella haemolytica has sometimes been thought to be an opportunistic pathogen incapable of inducing disease. Challenge models with the organism alone have generally been inconsistent (Gilmour et al., 1980). The reasons for this are not known. It is possible that stress or initial infections with viral or primary bacterial agents break down innate pulmonary immune barriers. A breakdown of barrier integrity at any level may release *P. haemolytica* from its usual commensal status in the nasopharynx and allow it to colonize and proliferate throughout the upper respiratory tract and induce tissue damage in the lung.

2.1. Innate immune mechanisms

Inhaled air and aspirated aerosols of upper respiratory secretions contain large quantities of microorganisms often approaching 10^8–10^9 bacteria per milliliter

(Bartlett, 1981). Despite the continuous, constant exposure to both environmental and commensal organisms, the respiratory tract remains remarkably free from infections. This is generally thought to be a result of overlapping mechanical, chemical and cell-mediated pulmonary innate clearance mechanisms (Coonrod, 1986; McNabb and Tomasi, 1981; Sherman, 1992) shown in *table I*. Innate defenses in the mucus, airway surface fluid, and epithelial cells of the trachea and changes or destruction that favor infection and colonization by *P. haemolytica* are shown in *figure 1*.

2.1.1. Mucociliary clearance

Mucociliary clearance is important in removing organisms that reach the lower respiratory tract and conditions that generally affect ciliated epithelial cells and tracheal mucous velocity may result in increased respiratory infections. For example, calves exposed to cold temperatures and then exposed intranasally with *P. haemolytica* were found to have a higher pulmonary concentration of *P. haemolytica* than controls (Diesel et al., 1991). Also, nasal mucus velocity, measured in four nonanesthetized calves at ambient temperature of 2–4 °C, was 24 % lower. It was thought that cold exposure increases pulmonary deposition of pathogens, while simultaneously decreasing mucociliary clearance of the upper airways, thus predisposing cold-exposed calves to respiratory tract infections. Similarly, animals infected with respiratory viruses, which destroy and denude tracheal epithelium, are very susceptible to secondary bacterial infection (Jakab, 1982). Reduced clear-

Table I. Innate mechanisms of pulmonary defense.

Innate defense	Reference
Mechanical	
mucociliary clearance	(Diesel et al., 1991)
Humoral	
immunoglobulins	(Lamm et al., 1995)
pulmonary surfactant	(Brogden, 1992)
SP-A	(Downing et al., 1995)
	(Manz-Keinke et al., 1992)
	(Turner, 1996)
	(van Iwaarden et al., 1994)
SP-D	(Schelenz et al., 1995)
	(Kishore et al., 1996)
	(Brown-Augsburger et al., 1996)
	(Kuan et al., 1992)
SAAP	(Brogden et al., 1996)
Cellular	
alveolar macrophages	(Crystal, 1991)
α defensins	(Lehrer et al., 1983)
epithelium	
epithelial cell desquamation	
β defensins	(Diamond et al., 1991)
	(Diamond et al., 1992)
	(Schonwetter et al., 1995)

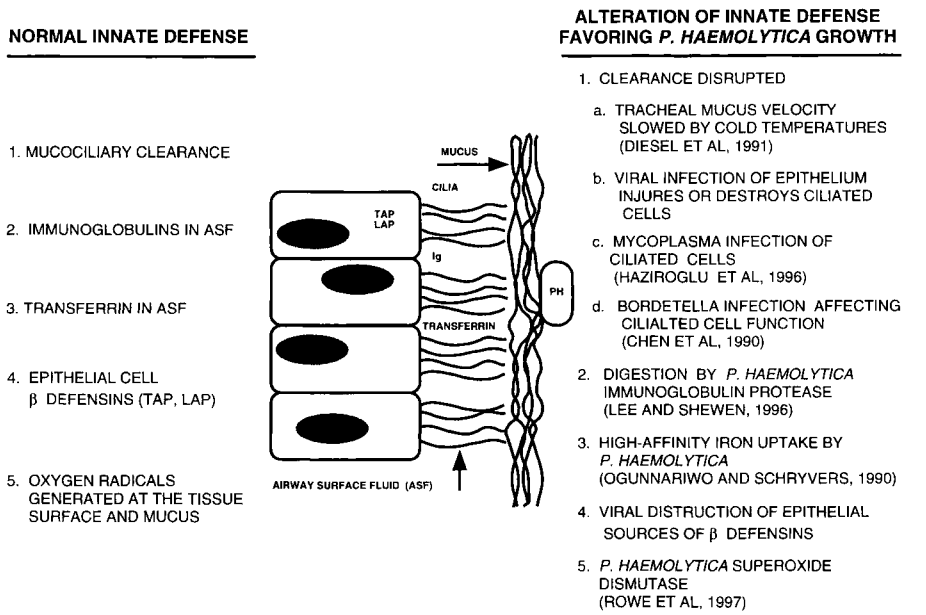


Figure 1. Innate defenses in the mucous, airway surface fluid and epithelial cells of the trachea and changes or destruction that favor infection and colonization by *Pasteurella haemolytica.*

ance of aspirated upper respiratory secretions containing microorganisms and cellular debris results in conditions favoring bacterial growth.

2.1.2. Immunoglobulins

Immunoglobulins in mucosal secretions generally serve as an immune barrier to exclude foreign matter, including microorganisms, from mucosal surfaces (McNabb and Tomasi, 1981). IgA (Mazanec et al., 1993) and IgG can both be found in pulmonary secretions and in some instances, resistance to pneumonic pasteurellosis can be correlated with specific titers of both IgA and IgG antibodies (Donachie et al., 1986; McBride et al., 1996; McVey et al., 1990; Nelson and Frank, 1989). Therefore, the ability of *P. haemolytica* to cleave immunoglobulins would allow it to colonize and proliferate in the ovine respiratory tract. Recently, Lee and Shewen (1996), detected IgG1 protease activity in partially purified *P. haemolytica* culture supernatant. Bovine IgG1 was hydrolysed sequentially into three distinct fragments of approximately 39, 12 and 7 kDa. This preparation also partially hydrolysed IgG2 but not IgA nor IgM.

2.1.3. Pulmonary surfactant

Pulmonary surfactant is antimicrobial for a number of bacterial species (Coonrod, 1986; LaForce and Boose, 1981; MacDonald et al., 1983). One mechanism is mediated by free fatty acids found in the neutral lipids of pulmonary surfactant

(Coonrod, 1986), whereas another mechanism is mediated by small peptides (described below). Some of the surfactant proteins (e.g. SP-A and SP-D) are also capable of other innate immune functions. SP-A acts as an opsinin to enhance nonspecific phagocytosis (Manz-Keinke et al., 1992; van Iwaarden et al., 1990). Interestingly, SP-A concentrations are lower in individuals with pneumonia (Baughman et al., 1993) and SP-A deficient animals are more susceptible to pneumonia (LeVine et al., 1997). SP-D is capable of binding to yeasts (Kuan et al., 1992; Schelenz et al., 1995), Gram-negative bacteria (Kuan et al., 1992), and lipopolysaccharide (Kuan et al., 1992). The interaction between SP-D and bacteria may a) enhance nonspecific binding of bacteria to alveolar macrophages, b) inhibit the binding of organism to respiratory epithelium, c) facilitate the physical clearing of agglutinated organisms by mucociliary clearance, or interfere with bacterial proliferation (Kuan et al., 1992). SP-D could also contribute to the inactivation or clearance of soluble lipopolysaccharide released at sites of colonization or infection (Kuan et al., 1992).

2.1.4. Alveolar macrophages

Alveolar macrophages are instrumental in maintaining normal lung structure and function through their capacity to scavenge particulates, remove macromolecular debris, kill microorganisms, function as an accessory cell in immune responses, recruit and activate other inflammatory cells, maintain and repair the lung parenchyma, and modulate normal lung physiology (Crystal, 1991). These cells serve this role by a variety of mechanisms including their ability to phagocytize, to express specific cell-surface receptors (Sibille and Reynolds, 1990), and to synthesize and release a broad group of mediators (Crystal, 1991; Sibille and Reynolds, 1990). Since alveolar macrophages are instrumental in pulmonary innate immunity, it is not surprising then that many respiratory viruses depress alveolar macrophage function and many products from *P. haemolytica* (e.g. leukotoxin, endotoxin, capsular polysaccharide, etc., *figure 2*) kill alveolar macrophages directly (Sutherland, 1985) or similarly depress macrophage function (Czuprynski et al., 1989).

2.1.5. Antimicrobial peptides

Resident and inflammatory cells, epithelial cells, and serous and mucous secretions in the respiratory tract contain a number of antimicrobial peptides for innate protection against bacterial infections. Resident mononuclear and inflammatory polymorphonuclear leukocytes on the mucosa of the respiratory tract contain α-defensins as part of the phagolysomal killing mechanisms (Ganz et al., 1990; Lehrer et al., 1983; Selsted et al., 1983). In an inflammatory lesion containing mononuclear and inflammatory polymorphonuclear leukocytes, α-defensins are released from both live and dead cells. To what extent these α-defensins are antimicrobial to bacteria in inflammatory exudate is not known. It is possible that α-defensins are inactivated by serous fluid (Panyutich and Ganz, 1991; Panyutich et al., 1994, 1995) and necrotic debris thus not being antimicrobial at all or they may contribute further to the lesion by inducing airway epithelial cell damage (Soong et al., 1997).

Cathelicidins, a group of diverse antimicrobial peptides identified in mammalian neutrophils, are found in sheep (Bagella et al., 1995; Mahoney et al., 1995). All cathelicidins are synthesized as inactive prepropeptides with highly conserved prepro segments (Zanetti et al., 1995). Sequential processing leads to the release of c-terminal active peptides thought to play an important role in host

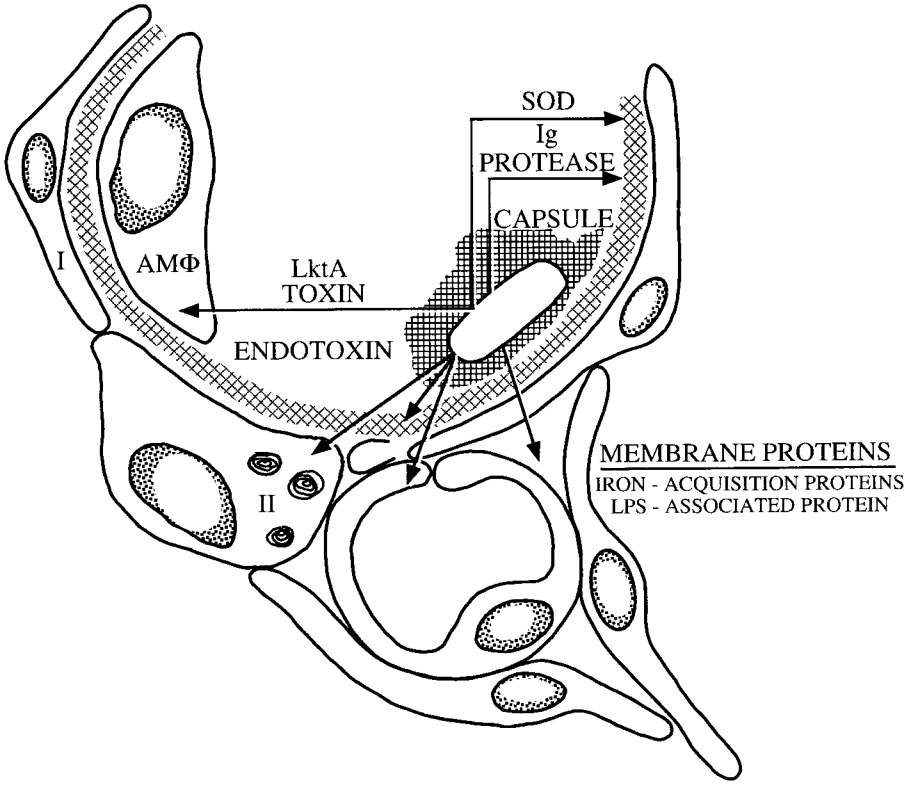


Figure 2. A schematic showing the interaction among *Pasteurella haemolytica* capsular polysaccharides, toxins (LktA and endotoxin), enzymes and outer membrane proteins with epithelial lining fluid, surfactant and cells in the alveolus. Once on the alveolar surface, capsular polysaccharide may facilitate colonization by a lectin adhesion-receptor binding mechanism through surfactant protein SP-A (Brogden et al., 1989). Capsular polysaccharide may also impair phagocytosis and killing by alveolar macrophages (Czuprynski et al., 1991a). LktA is secreted that is cytocidal for ovine alveolar macrophages (Sutherland et al., 1983; Sutherland, 1985). Endotoxin is also produced that kills or injures epithelial cells, endothelial cells (Breider et al., 1990), alveolar macrophages, and complexes with pulmonary surfactant (Brogden et al., 1986) destroying surface tension (Brogden, 1991). Enzymes superoxide dismutase (Rowe et al., 1997) and immunoglobulin protease (Lee and Shewen, 1996) may inactivate oxygen radicals generated on the tissue surface and immunoglobulins in the epithelial lining fluid, respectively. High affinity iron uptake (Ogunnariwo and Schryvers, 1990) may also occur from pro-oxidant iron that may be present (Gutteridge et al., 1996). The overall role of enzymes such as O-sialoglycoprotein endopeptidase and neuraminidase as well membrane proteins such as lipopolysaccharide-associated protein (Brogden et al., 1995) and other surface antigens (Donachie et al., 1984) in the pathogenesis of pneumonic pasteurellosis has not been firmly established.

defense both as antimicrobial agents and as modulators of inflammation (Boman, 1995). Six genes encoding sheep cathelicidins have been reported in the Genbank database including two encoding pro/arg rich peptides, two encoding alpha-helical amphipathic peptides, and two encoding identical dodecapeptides. To what extent

they contribute to the innate defense of the lung is not yet known.

Epithelial cells in the respiratory tract contain β-defensin intracellular peptides that contain 29–34 amino acids with six conserved cysteine residues for intramolecular disulfide bonding (Ganz et al., 1990). β-defensins are usually arginine-rich, cationic proteins with broad antimicrobial, antiviral and cytotoxic activity (Ganz et al., 1990). Some are chemotactic, opsonic, or may modulate hormonal responses (Ganz et al., 1990). Active peptides originate from post-transcriptionally modified charge-neutralized preprodefensins (Michaelson et al., 1992).

In cattle, two β-defensins, tracheal antimicrobial peptide (TAP), and lingual antimicrobial peptide (LAP), have been described in columnar cells of the pseudostratified epithelium throughout the conducting airway and tongue (Diamond et al., 1991, 1992; Schonwetter et al., 1995). TAP genes have also been identified in sheep (Iannuzzi et al., 1996). Like other β-defensins, TAP is 4 084 Da in size, cationic, and has in vitro antimicrobial activity against Gram-negative bacteria, Gram-positive bacteria and yeasts (Diamond et al., 1991). β-defensin mRNA is found to be widely expressed in numerous exposed epithelia and is at higher levels in tissues that are constantly exposed to and colonized by *P. haemolytica* (Stolzenberg et al., 1997). These results suggest that β-defensins are an integral component of the inflammatory response providing a rapidly mobilized local defense.

Usually α-defensins and β-defensins are not found in mucus and serous respiratory secretions. However, antimicrobial activity exists in bronchoalveolar lavage fluid. Sheep pulmonary surfactant is also bactericidal when bacteria and surfactant mixtures are incubated with normal serum (Brogden, 1992). This is due to small (721.6–823.8 Da), surfactant-associated anionic peptides (SAAP); H-GDDDDDD-OH, H-DDDDDDD-OH and H-GADDDDD-OH. These peptides are very similar to the charge-neutralizing propeptide of sheep trypsinogen (de Haen et al., 1975) which when synthesized also have antimicrobial activity (Brogden et al., 1997).

When functioning properly, these peptides are thought to be capable of clearing infecting microorganisms and maintaining sterility in the tracheobronchoalveolar areas. It is possible that initial infections with viral or primary bacterial agents break down the antimicrobial barrier directly by injuring and destroying epithelial cells or indirectly by creating a microenvironment filled with serous fluid and necrotic cellular debris capable of inactivating antimicrobial activity. Little is known about the requirements necessary for peptide efficacy or the effects of metabolic, physiologic or genetic dysfunction on peptide antimicrobial activity. The following are a list of physical and physiological stresses, viral infections and bacterial infections that may compromise protective pulmonary innate immune mechanisms mentioned above. At any level, loss of innate protective function may release *P. haemolytica* from its usual commensal status to cause disease.

3. THE ROLE OF STRESS

Stress is an amorphous predisposing factor that often increases the susceptibility of animals and man to respiratory tract infections (Biondi and Zannino, 1997). However, the effect of psychological, physiological and physical environmental stress is difficult to assess (Swanson, 1995). In sheep and goats, a stressful situation or environment to one animal may be tolerated by another and generally is affected by age and breed. While stresses are difficult to measure, some indicators can be used. These include increased body temperature, heart rate, plasma cortisol,

glucose, free fatty acids, beta-hydroxybutyrate and urea; decreased body weight and hydration (Knowles et al., 1995).

Common physical environmental stresses, either alone or in any combination, predispose many sheep and goats to respiratory infections. These include heat, crowding, limited space, exposure to inclement weather, poor ventilation with high levels of moisture and barnyard gases, handling and transport (Knowles et al., 1995), castration and docking, weaning and change in feed, exhaustion and hunger during transportation (Cole, 1996), excessive dust in feedlots, high loads of parasites, and mixing of animals from different sources (Gilmour, 1980; Martin, 1996).

Experimentally, stress can be shown to lower innate resistance to *P. haemolytica* infection. For example, in calves, abrupt changes in temperature have been shown to increase the numbers of *P. haemolytica* in the nasopharynx (Jones, 1987), whereas extensive stressful exercise (Anderson et al., 1991) and cold temperatures (Diesel et al., 1991) have been shown to increase the susceptibility of animals to respiratory infection by *P. haemolytica*.

4. THE ROLE OF VIRUSES

Viruses affecting the respiratory tract of sheep and goats are widespread and have been reviewed. Viruses associated with acute infections are parainfluenza-3 (PI-3) virus (Sharp, 1990), respiratory syncytial virus (RSV) (Wellemans, 1990), adenoviruses (AdV) (Belák, 1990), sheeppox (Merza and Mushi, 1990b) and the closely related goatpox virus (Merza and Mushi, 1990a), herpesvirus (Rosadio et al., 1984), and reovirus (Darbyshire, 1990). Virus isolation, serologic epidemiology, and pathogenesis studies have established the role of PI-3 virus in respiratory tract disease of small ruminants. Respiratory syncytial virus has been isolated from both sheep (LeaMaster et al., 1984) and goats but clinical disease and virus distribution is less well defined. Mild rhinitis has been reported in sheep naturally infected with RSV (LeaMaster et al., 1984) and moderate clinical signs have been reported in goats. Adenoviruses have been isolated from healthy sheep and goats but more frequently the isolations are associated with some form of clinical disease. Currently there are six recognized ovine (OAdV) serotypes in sheep and several isolates of goat adenovirus (GAdV) that need to be serotyped (Gibbs et al., 1977; Lehmkuhl et al., 1997). In addition there have been two serotypes of bovine adenovirus (BAdV-2 and -7) isolated from sheep and one serotype of ovine (OAdV-5) (Nguyen et al., 1988) isolated from goats. The AdV are widely distributed in the sheep and goat population but the importance of this group of viruses in respiratory tract disease of small ruminants has yet to be established. Of the serotypes studied, BAdV-2, -7, OAdV-5 and -6 and a serologic variant of OAdV-6 (strain RTS-151) have been shown to produce both clinical disease and lesions in inoculated lambs (Cutlip et al., 1996). A recent isolate of GAdV produced clinical signs and lesions when inoculated into young goats (Lehmkuhl et al., 1997). Sheeppox and goatpox infections have shown to involve the respiratory tract but have limited distribution. There is little evidence to implicate either herpesvirus or reovirus as pathogens of the respiratory tract of small ruminants when compared to the effects produced by the other viruses.

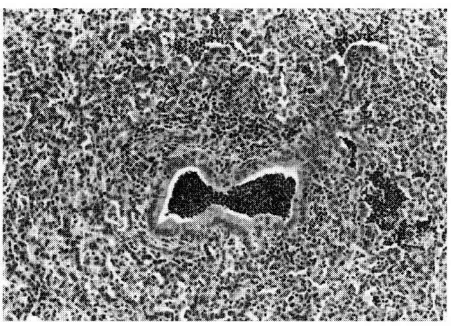
Respiratory viral infections increase the susceptibility of sheep and goats to secondary bacterial infection. Generally these infections affect mucociliary clearance mechanisms in removing organisms that reach the lower respiratory tract (Jakab, 1982). This hypothesis has been

the focus of much research in recent years. Generally, sheep are first infected with virus and then with *P. haemolytica* to assess if the resulting lesions are similar to those seen in natural infections from which these agents were originally isolated. PIV-3 (Davies et al., 1981a; Cutlip et al., 1993), BRSV (Sharma and Woldehiwet, 1990; Al-Darraji et al., 1982a, b, c), OAV-6 (Cutlip et al., 1996; Lehmkuhl et al., 1989) can all increase susceptibility of sheep to *P. haemolytica.*

Combined infection of lambs with OAV-6 followed by *P. haemolytica (figure 3)* induces lesions more severe than that seen with either agent alone (Cutlip et al., 1996). The combined infection causes fibropurulent pneumonia with edema, focal necrosis and pleuritis *(figure 3a–c),* resulting in early death of lambs or slow resolution of lesions (Cutlip et al., 1996). These findings were consistent with those of other studies and resemble lesions commonly seen in lambs dying of pneumonia in the field.

Combined infection of lambs with PI-3 and *P. haemolytica* also caused a severe acute fibrinopurulent bronchointerstitial pneumonitis with focal necrosis that closely resembled lesions seen in natural cases of acute enzootic pneumonia (Cutlip et al., 1993). However, chronic fibrinonecrotic changes with abscesses, characteristic of that seen in lambs of feedlot age, could not be induced. Similarly, lesions in lambs inoculated with RSV and *P. haemolytica* conformed in most gross and histopathologic features to those lesions in naturally occurring cases of ovine pneumonic pasteurellosis (Al-Darraji et al., 1982b). Lesions consisting of interstitial pneumonitis and severe exudative pneumonia with focal necrosis and hemorrhage were induced.

(a)



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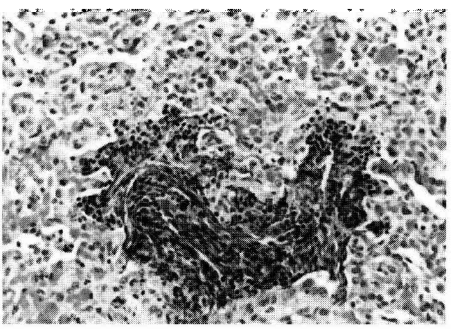


(c)

Figure 3. Lesions seen in lambs experimentally infected with ovine adenovirus type 6 and *Pasteurella haemolytica* A1. At 5 days after *P. haemolytica* exposure, consolidation in a large area of the anteroventral part of the lung can be seen (a). At 7 days after *P. haemolytica* exposure, severe hyperplasia of alveolar epithelium can be seen (b) and at 10 days after *P. haemolytica* exposure, severe streaming of neutrophils and alveolar macrophages engorged with protein in a terminal bronchiole can be seen (c).

Overall, viral infection is thought to create an ideal microenvironment consisting of necrotic cells and proteinaceous fluid in the lung favoring bacterial growth by interfering with the mucociliary clearance mechanism of the respiratory tract, and by depressing the capacity of resident lung macrophages to take up and kill bacteria (Jakab, 1982). With the addition of *P. haemolytica* as a secondary bacterial infection, the infection becomes severe with fatalities.

5. THE ROLE OF BACTERIA

Like viruses, some respiratory bacterial infections also increase the susceptibility of sheep and goats to secondary *P. haemolytica* A2 infection. For example, *Mycoplasma species* are common in the respiratory tract of sheep (Brogden et al., 1988; Thirkell et al., 1990) and may contribute to the pneumonia problem (Davies et al., 1981b; Donachie and Jones, 1982). The combination of *Mycoplasma ovipneumoniae* and *P. haemolytica* A2 induce a proliferative (atypical) pneumonia of lambs (Jones et al., 1982). Clinical signs of atypical pneumonia are frequently mild or inapparent, yet its effect on growth and production may be severe (Donachie and Jones, 1982). Lesions consist of peribronchiolar cuffing and interstitial change characterized by mononuclear cells causing thickening of alveolar septa. In some lambs, an exudate of macrophages and neutrophils are seen. While *M. ovipneumoniae* may facilitate the pulmonary establishment of *P. haemolytica*, with apparent exacerbation of the disease in atypical pneumonia, it was thought that there was a concomitant restriction of the invasiveness of *P. haemolytica* as compared to its involvement in other cases of pneumonic pasteurellosis (Jones et al., 1982).

Bordetella parapertussis has also been isolated from ovine lungs (Porter et al., 1994) and thought to be involved in pneumonia (Chen et al., 1989). Most likely, *B. parapertussis* predisposes lambs to secondary *P. haemolytica* pneumonia. Destruction of ciliated cells and the presence of alveolar edema fluid and alveolar exudate may be contributing factors that lower the bactericidal mechanisms of the lung. For example, pneumonia can be induced in mice with cell-free extracts of *B. parapertussis* (Chen et al., 1990). These extracts induced marked infiltration of neutrophils and macrophages into the alveolar septa, bronchiolar and alveolar spaces, and hyperplasia of peribronchiolar and perivascular lymphoid tissue. Ultrastructurally, damage to ciliated cells, type 1 pneumocytes and alveolar macrophages was seen. Secondary *P. haemolytica* A2 bronchopneumonia can be induced in mice (Jian et al., 1991; Porter et al., 1995b) and lambs (Porter et al., 1995a) following *B. parapertussis* infection. Successive exposure in vivo to both organisms induces a syngeristic effect that results in reduction of the phagocytic capacity of alveolar macrophages and killing of *P. haemolytica* (Hodgson et al., 1996).

Other bacteria that are isolated with *P. haemolytica* in pneumonic infections include *P. multocida* (Ngatia et al., 1986) *Staphylococcus species*, *Streptococcus species*, *Escherichia coli* (Ngatia et al., 1986), Chlamydia (Ngatia et al., 1986) and *Haemophilus species*.

6. OTHER COMPLICATIONS

Occasionally, other conditions increase the susceptibility of sheep and goats to secondary *P. haemolytica* infection. These include airway obstruction (Pfeffer, 1988), inhalation of foreign objects, and parasitic infections.

7. *P. HAEMOLYTICA* ON THE ALVEOLAR SURFACE

Regardless of predisposing conditions, *P. haemolytica* is considered to be responsible for most secondary fibropurulent pleuropneumonias. The species is a normal commensal of the upper respiratory tract in both sheep and goats (Gilmour et al., 1974; Ngatia et al., 1985) and contains 13 capsular serotypes; 1, 2, 5–9, 11–14, 16 and 17 (Biberstein et al., 1960; Fodor and Varga, 1988; Younan and Fodor, 1995). Serotypes 3, 4, 10 and 15 have been reclassified as *Pasteurella trehalosi* (Sneath and Stevens, 1990). The principle disease caused by *P. haemolytica* is enzootic pneumonia which affects all ages of sheep in both intensively and extensively managed flocks (Gilmour, 1980). Lambs under 2 months of age may develop a more generalized, septicemic form of the disease (Gilmour, 1980). Serotypes 1 (Fodor et al., 1984; Prince et al., 1985) and 2 (Ball et al., 1993; Prince et al., 1985) are commonly isolated from sheep followed then by serotypes 6, 7 and 9 (Ball et al., 1993; Fodor et al., 1984; Prince et al., 1985). Serotype 2 is frequently isolated from goats (Fodor et al., 1984).

7.1. Histopathology of infections

Lesions of spontaneous and experimental pneumonic pasteurellosis in sheep and goats can be distinguished from lesions induced by other Gram-negative bacteria by the deposition of fibrin in the lungs and on the thoracic pleura. Excess serous fluid is often present in the pleural and peritoneal cavities. Lesions within the lungs consist of areas of consolidation with one to multiple foci of necrosis surrounded by hemorrhage (Al-Darraji et al., 1982b; Cutlip et al., 1996; Lehmkuhl et al., 1989; Ngatia et al., 1986). Slight hydropericardium can sometimes be seen.

Microscopically, changes consist of pneumonitis with multifocal areas of acute fibrinopurulent bronchopneumonia, coagulative necrosis and fibrinous pleuritis. Necrotic centers in groups of alveoli are outlined with congested capillaries and filled with fibrin, proteinaceous material, bacterial colonies, erythrocytes, neutrophils and macrophages. The central area is usually surrounded by a zone of spindle shaped, basophilic cells oriented to form whorls and parallel bundles of cells that give the appearance of streaming *(figure 3c)*. Near the lesion margin, exudate containing neutrophils and macrophages form an abrupt transition to normal lung with no fibrous tissue capsule. Within the zonal lesions, there may be hyperplastic pneumocytes and fibrinopurulent bronchiolitis. Zonal lesions encroach on interlobular septa and pleura: both thickened by fibrin and neutrophils and contain blood and lymphatic vessel thrombi.

7.2. Extracellular *P. haemolytica* products and their role

A general schematic showing the interaction among *P. haemolytica* capsular polysaccharide, toxins (leukotoxin LktA and endotoxin), enzymes and outer membrane proteins with epithelial lining fluid, surfactant and cells in the alveolus is shown in *figure 2*. *Pasteurella haemolytica* contains enzymes which may allow it to proliferate and colonize on respiratory surfaces. These include O-sialoglycoprotein endopeptidase (Abdullah et al., 1992; Otulakowski et al., 1983) and neuraminidase (Muller and Mannheim, 1995; Straus et al., 1993a, b; Straus and Purdy, 1994; Tabatabai, 1995). The gene for sialoglycoprotease *(gcp)* has been cloned and sequenced and codes for a protein of 35.2 kDa (Abdullah et al., 1991). All A biotypes have the *gcp* gene and sialoglycoprotease activity with the exception of serotype A11 (Lee et al., 1994). Similarly,

all A biotypes produce a neuraminidase of 150–200 kDa with the exception of serotype 11 (Straus et al., 1993a). Although these enzymes are implicated as virulence factors, their overall role in the pathogenesis of pneumonic pasteurellosis has not been firmly established.

Pasteurella haemolytica isolates (Lo et al., 1987), including those from sheep (Sutherland et al., 1983), produce an RTX LktA that is closely related to pore-forming toxins from a variety of Gram-negative bacteria (Welch et al., 1995). LktA is a large (estimated 105 kDa molecular mass) calcium-dependent, pore-forming protein toxin thought to play a central role in the disease process (Lainson et al., 1996). LktA is cytotoxic to ruminant leukocytes (Clinkenbeard et al., 1989; Sutherland, 1985) and platelets (Clinkenbeard and Upton, 1991). Neutrophils and mast cells exposed to LktA release oxygen-free radicals (Maheswaran et al., 1992), proteolytic enzymes (Czuprynski et al., 1991b), eicosanoids (Clinkenbeard et al., 1994) and histamine (Adusu et al., 1994). Lkt is produced as a zymogen, the product of the *lkt*A gene. It is then post-translationally cleaved by the *lktC* gene product to the active 105 kDa toxin. There is some variation in the amount, size, and activity of Lkt protein produced by different strains (Saadati et al., 1997).

Purified Lkt, induces lesions in the lungs of ruminants consisting of consolidated areas with edema of interlobular septa and hemorrhage (Whiteley et al., 1991). Microscopically, interlobular septa, pleura and peribronchial interstitium are expanded because of edema and fibrin deposition. There is thrombosis of lymph vessels, hemorrhage, and moderate to marked infiltration of intact and degenerated neutrophils and macrophages (Whiteley et al., 1991).

7.3. Cell-associated *P. haemolytica* products and their role

Pasteurella haemolytica also contains cell-associated products capable of inducing tissue damage and include capsular polysaccharide (Adlam et al., 1984; 1986; Brogden et al., 1989; Whiteley et al., 1990); lipopolysaccharide (Brogden et al., 1995; Brogden et al., 1984; Lacroix et al., 1993); and membrane proteins such as lipopolysaccharide-associated protein (Brogden et al., 1995) and other surface antigens (Donachie et al., 1984), proteins involved in iron acquisition (Ogunnariwo and Schryvers, 1990), and periplasmic superoxide dismutase (Rowe et al., 1997).

7.3.1. Capsular polysaccharide

Capsular polysaccharides vary in composition among *P. haemolytica* serotypes (Adlam et al., 1984, 1986, 1987). Purified CP (serotype 1) alone induces multiple foci of discoloration (Brogden et al., 1989). Microscopically, alveoli and interlobular septa are filled with edema fluid and terminal airways and alveoli contain a moderate number of neutrophils *(figure 4b).*

Pasteurella haemolytica A1 capsular polysaccharide may be involved in an adhesin–receptor interaction with surfactant allowing the organism to attach to the alveolar epithelial cell surface. These cells contain carbohydrate on the surface which binds a lectin (SP-A) in pulmonary surfactant. SP-A is a calcium-dependent mannose binding lectin that may bind the →3)-β-N-acetylamino mannuronic- (1→4)-β-N-acetylmannosamine- (1→ structure of the capsular polysaccharide. However this has not yet been shown. More importantly, preincubation of alveolar macrophages with A1 capsular polysaccharide impairs phagocytosis and killing of *P. haemolytica* (Czuprynski et al., 1991a). Capsular polysaccharide A2 may allow *P.*

haemolytica to mimic host membranes rich in sialic acid residues and thereby effectively go undetected by host defenses (Adlam et al., 1987). These findings become particularly important since capsular polysaccharide can be found in excess throughout pulmonary lesions (Whiteley et al., 1990).

7.3.2. Lipopolysaccharide

Variations in LPS structure occur among different serotypes (Lacroix et al., 1993) and among different isolates of the same serotype including serotype A1 (Davies and Donachie, 1996; Rimsay et al., 1981). The structure generally consists of lipid A, core polysaccharide of Hex_3Hep_5 2-keto-3-deoxyoctulosonic acid and Hex_2Hep_5 2-keto-3-deoxyoctulosonic acid, and a somatic polysaccharide of trisaccharide repeating units containing two D-galactose residues and one N-acetyl-D-galactosamine residue (Lacroix et al., 1993). Lipopolysaccharides, in general, induce adverse reactions in the lung and circulatory system often leading to metabolic failure and lethal shock. The ability of lipopolysaccharide to effect these changes is often related to its structure. Variations in *P. haemolytica* LPS structure (e.g. smooth versus rough) induce different pathophysiologic effects (Emau et al., 1986) and toxicity (Rimsay et al., 1981). Smooth *P. haemolytica* serotype A1 LPS induces greater increases in arachidonic acid and its metabolites (Emau et al., 1986) yet is not cytotoxic for bovine leukocytes (Confer and Simons, 1986) or ovine macrophages (Sutherland and Redmond, 1986). Rough LPS, however, is cytotoxic for ovine alveolar macrophages.

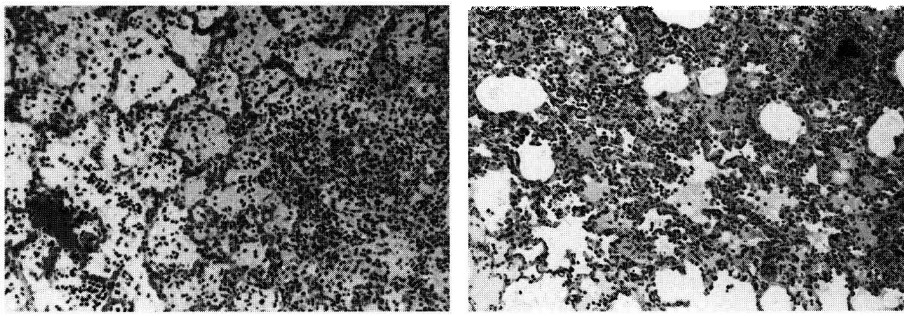
Lipopolysaccharide is generally cell-associated but during bacterial infections, it is released (as endotoxin) and can complex with pulmonary surfactant altering surfactant density and surface tension (Brogden et al., 1986). Any increase in surface tension due to an altered surfactant, abnormal quantity of surfactant, or abnormal concentration of surfactant constituents will have a deleterious effect on lung function. These include decreases in total lung capacity, static compliance, diffusing capacity and arterial PO_2. Also, anatomic changes, such as pulmonary edema, hemorrhage and atelectasis can result (Brogden, 1991).

Lipopolysaccharide is among the most inflammatory of *P. haemolytica* cell-associated products (Brogden et al., 1995; Whiteley et al., 1991). Lesions induced in the lungs of sheep with A1 rough LPS consist of large areas of hyperemia and edema that include the deposition area and parts of adjacent lobes (Brogden et al., 1984). Areas of hemorrhage and fibrous adhesions of the affected lobe to the thoracic wall can be seen. Microscopically, most alveoli and terminal airways are filled with proteinaceous fluid, fibrin, erythrocytes and neutrophils *(figure 4a)*. There are focal areas of cellular necrosis that leave only the structural framework intact. Both lymphatic and blood vessels are usually occluded with fibrin and neutrophils, and the pleura is covered by a layer of fibrinopurulent exudate.

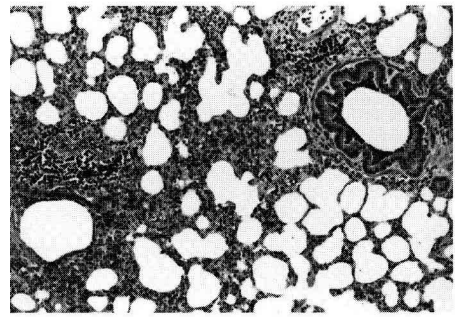
7.3.3. Membrane proteins and enzymes

Lipopolysaccharide-associated protein is probably also released with LPS during pulmonary infection. As a component of endotoxin, any biological activity would be masked by the potent inflammatory capability of the LPS moiety (Brogden et al., 1984). However, gross lesions are minimal and microscopic lesions *(figure 4c)* consist of accumulations of neutrophils in the alveoli, lesser numbers of macrophages, and abundant serofibrinous fluid (Brogden et al., 1995).

Superoxide dismutase, detected in serotypes A1 and A2, are metalloenzymes that catalyse the conversion of highly toxic



(a) (b)



(c)

Figure 4. Lesions induced in the dorsum of the caudal portion of the cranial lobe of the right lung of sheep after deposition by fiberoptic bronchoscopy of *Pasteurella haemolytica* A1 lipopolysaccharide, capsular polysaccharide and lipopolysaccharide-associated protein. (a) In lipopolysaccharide-induced lesions, there was fibrinopurulent inflammation with edema, hyperemia, hemorrhage and foci of necrosis of alveolar epithelium. Alveolar and terminal airways were filled with proteinaceous fluid, fibrin and neutrophils. (b) In capsular polysaccharide induced lesions, alveoli and interlobular septa were filled with edema fluid. Terminal airways and alveoli contain neutrophils and proteinaceous fluid and interalveolar septa contain mononuclear cells. (c) In lipopolysaccharide-associated protein-induced lesions, there was a monocytic and lymphocytic peribronchiolar response containing few neutrophils and numerous macrophages with abundant cytoplasm in alveolar lumens.

superoxide radicals to hydrogen peroxide and oxygen (Rowe et al., 1997). This enzyme may protect *P. haemolytica* from oxygen radicals generated at the tissue and mucous surface during colonization as well as during the respiratory burst of phagocytosis. Further work, however, is needed to clarify the role of this enzyme in *P. haemolytica* infections.

8. IMMUNOLOGICAL DAMAGE AND ITS ROLE

Immune complex formation has long been thought to play a role in the pathogenesis of *P. haemolytica* pneumonia (Kim, 1977; Tizard, 1977) but has received little attention. For example, killed whole cells of *P. haemolytica* are not generally efficacious as vaccines (Confer et al., 1988) and may actually induce hypersensitivity. This may lead, on subsequent infection, to lesions of increased severity (Friend et al., 1977; Wilkie et al., 1980). In a recent study, severe lesions were induced in blood vessels of the lungs by the intratracheal injection of *P. haemolytica* A1 LPS (50 μg) into rabbits previously immunized with *P. haemolytica* killed whole cells emulsified with Freund's incomplete adjuvant (Ramirez-Romero et al., 1997); these lesions included perivascular oedema and neu-

trophil infiltration of the subintima, with degeneration and necrosis of the media. Smaller vessels were occluded by neutrophils in various stages of degranulation and were more severe than lesions induced by the intratracheal injection of LPS (50 μg) alone. The lesions, which were similar to those seen in natural cases of *P. haemolytica* pneumonia, were characterized by a fibrinopurulent inflammatory process with extensive interstitial oedema, fibrinous exudate and neutrophils.

9. AREAS FOR FUTURE RESEARCH

9.1. Epidemiology

Undoubtedly, new etiologic agents (including viruses, bacteria and fungi) and new serotypes of conventional agents of respiratory infections will emerge as selective reductive pressure is applied to control current agents and serotypes. These new organisms may be found as new primary causes of respiratory tract disease or as new predisposing agents to conventional pneumonic pasteurellosis. The CAR or cilia-associated respiratory bacillus is a good example. This organism was originally described in rodents (MacKenzie et al., 1981; Van Zwieten et al., 1980) and has recently been found in the respiratory tract of goats (Fernandez et al., 1996). It is now associated with enzootic pneumonia of goats with other organisms like *P. haemolytica* (Oros et al., 1997). Adenovirus in goats (Lehmkuhl et al., 1997) is another good example. More new agents will be identified and their role in respiratory infections will have to be determined.

As world demand for lamb and sheep increases, stresses will be applied to animals as they are live shipped vast distances by land (Jarvis and Cockram, 1994) and sea (Black et al., 1997) usually in large numbers and in close quarters under adverse conditions. Conventional causes of respiratory infections change and new unexpected infections arise increasing morbidity (~25.0 %) and mortality (2.42 %) (Black et al., 1997). Predisposing viruses probably move very fast through stressed sheep in these conditions. Work is needed to assess the changing flora in these animals as well as devise strategies to control losses.

9.2. Host–parasite relationships

Pasteurella haemolytica is a commensal in the nasopharynx (Rowe et al., 1997). Only after predisposition does it become an opportunistic pathogen. Work is needed to identify and characterize the host mechanism capable of holding *P. haemolytica* in stasis. Identification of this host mechanism will provide tremendous insight on the pathogenesis of pneumonic pasteurellosis as well as lead to more effective control strategies. Perhaps it is related to the recently described antimicrobial peptide barrier throughout the respiratory tract (Stolzenberg et al., 1997). It is not known if this barrier can hold normal flora in a commensal rather than opportunistic state. It is feasible that viruses may injure epithelial cells that produce these peptides and induce qualitative changes in peptide structure or quantitative changes in peptide concentration increasing the susceptibility of the animal to *P. haemolytica*. As more information becomes available, perhaps peptide concentrations can be increased in vivo or can be isolated and given parentally for prophylactic or therapeutic treatment. Finally, work is needed to determine if some *P. haemolytica* immunogens can sensitize sheep and actually induce severe immunologic damage should the animals be exposed naturally to or infected with *P. haemolytica*.

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