A REVIEW ON PNEUMONIC PASTEURELLOSIS (RESPIRATORY MANNHEIMIOSIS) WITH EMPHASIS ON PATHOGENESIS, VIRULENCE MECHANISMS AND PREDISPOSING FACTORS

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Summary


Pneumonic pasteurellosis is one of the most economically important infectious diseases of ruminants with a wide prevalence throughout the continents. The disease is characterized by an acute febrile course with severe fibrinous or fibrinopurulent bronchopneumonia, fibrinous pleurisy and septicaemia. Infected animals may die within a few days of the onset of clinical signs, but those which survive the acute attack may become chronically infected. Mannheimia haemolytica is well established to be the major aetiological agent of the disease although Pasteurella multocida has also been incriminated in many acute outbreaks. Both Mannheimia and Pasteurella species are commensally resident in the respiratory tract of healthy ruminants and are capable of causing infection in animals with compromised pulmonary defense system. Hence, the disease is essentially triggered by physical or physiological stress created by adverse environmental and climatic conditions such as extremely bad weather, poor management, overcrowding, transportation or previous infection with respiratory viruses, mycoplasma or some other pathogenic organisms. In the present review, relevant aspects of pneumonic pasteurellosis are described and discussed in cattle, sheep and goats with more emphasis on pathogenesis, virulence mechanisms and predisposing factors.

Key words: Mannheimia, mannheimiosis, Pasteurella, pasteurellosis, pneumonia, ruminants

INTRODUCTION

Respiratory tract infections are of a common occurrence in various species of domestic and farm animals. However, pneumonic pasteurellosis, also known as respiratory mannheimiosis, is the most common example with a wide prevalence in ruminant animals. The disease, in its typical clinical form, is highly infectious, often fatal and with very serious economic impact in animal industry. It is well established that pneumonic pasteurellosis is responsible for the largest cause of mortality in feedlot animals in which the disease accounts for approximately 30% of the total cattle deaths worldwide. The global economic impact of the disease is very well recognized and more than one billion dollars are annually lost in beef cattle industry in North America alone (Boudreaux, 2004). The catastrophic effect of the disease was also evident in sheep farming and remarkable economic losses were also attributed to massive fatalities in feedlot animals and acute field
outbreaks. In addition, substantial amount of money was further lost, almost every year, in improving farm management, animal husbandry and chemotherapeutic and vaccination programmes.

TAXONOMY AND PUBLIC HEALTH IMPLICATIONS

The term pasteurellosis was broadly used to designate a number of infections in domestic animals caused by Gram-negative non-motile facultative anaerobic rods or coccobacilli formerly grouped under the genus Pasteurella (after Louis Pasteur). For several decades, the genus Pasteurella was believed to be only one single genus with numerous species responsible for or associated with a wide range of systemic, pulmonary and septicaemic infections in various species of farm animals, particularly ruminants. However, with more recent advancements in molecular biology involving DNA hybridization studies and 16S rRNA sequencing, most of the formerly recognized species were found to share a number of common features and became the subject of intensive revision and reclassification. In this respect, Pasteurella haemolytica biotype A was allocated to a new genus and renamed Mannheimia. This new genus now contains several species including M. haemolytica, M. granulomatis, M. glucosida, M. ruminalis and M. varigena (Angen et al., 1999a). The name Mannheimia was given in tribute to the German scientist Walter Mannheim for his significant contributions in the recent taxonomy of the family Pasteurellaceae. On the other hand, Pasteurella haemolytica biotype T was first reclassified as P. trehalosi. However, this organism was recently revised and removed to a new separate genus by the name of Bibersteinia trehalosi (Blackall et al., 2007). In addition, avian Pasteurella species including P. gallinarum, P. paragallinarum and P. volantium were similarly removed to a new separate genus named as Avibacterium (Blackall et al., 2005).

Before the establishment of this newly revised classification, Pasteurella haemolytica was known to comprise two biotypes: A and T, based on fermentation of arabinose and trehalose, respectively. Within these two biotypes, 17 serotypes were further identified on basis of soluble or extractable surface antigens by passive haemagglutination procedure or rapid plate agglutination test (Carter & Chengappa, 1991). Serotypes 1, 2, 5, 6, 7, 8, 9, 11, 12, 13, 14, 16 & 17 belong to biotype A which was reclassified as M. haemolytica. However, serotype A11 was later reclassified as M. glucosida (Younan & Fodor, 1995). The rest of serotypes (3, 4, 10 & 15) belong to biotype T, which was initially reclassified as P. trehalosi before being finally moved to a separate new genus as already mentioned. It is worth mentioning that M. haemolytica, P. multocida and P. trehalosi (Bibersteinia) constitute the most important members of the family Pasteurellaceae that pose serious hazards in livestock industry. These species are commensally resident in the animal body as normal constituents of the nasopharyngeal microflora and are all capable of causing infection when the body defense mechanisms are impaired. Their presence is mainly confined to ruminants with most adequately characterized strains originating from cattle, sheep and goats (Biberstein & Hirsh, 1999). Examples of the most commonly recognized diseases associated with M. haemolytica include shipping fever in cattle, primary and secondary pneumonia in cattle, sheep and goats, septicaemia and mas-
titis in sheep and a number of non-specific inflammatory lesions in various species of domestic animals (Quinn et al., 2002). *P. multocida* is, on the other hand, associated with haemorrhagic septicaemia in cattle and buffaloes and enzootic pneumonia complex in young ruminants (Jones et al., 1997). Other diseases such as fowl cholera and snuffles (an upper respiratory tract infection occasionally accompanied by pleurisy, pneumonia or fatal septicaemia in rabbits) are also caused by *P. multocida*. With regard to *P. trehalosi* (*Bibersteinia trehalosi*), this organism is frequently associated with acute systemic disease or septicaemia in young sheep (Dyson et al., 1981; Jones et al., 1997). Other *Pasteurella* species of pathogenic significance in domestic animals include *P. caballi* which causes pneumonia and peritonitis in horses, *P. canis*, *P. stomatis* and *P. dagmatis* and these are associated with pneumonia and oral infections in dogs and cats. In addition, there are many other *Pasteurella* and *Mannheimia* species which can cause occasional infections in domestic and laboratory animals such as *M. granulomatis*, the causative agent of fibrogranulomatous panniculitis (lechiguaná) in cattle (Riet-Correa et al., 1992) and *P. pneumotropica* that causes secondary pneumonia in rats, mice and rabbits (Biberstein & Hirsh, 1999; Quinn et al., 2002). Although, *Pasteurella* and *Mannheimia* have long been considered primarily animal pathogens but they were also reported to produce serious systemic and/or localized infections in human beings. For example, *P. multocida* was reported to cause acute or chronic pneumonic lesions in human patients (Beyt et al., 1979; Klein & Cunha, 1997; Marinella, 2004). Also, *P. dagmatis* was reported to cause fatal peritonitis and septicaemia in human patients (Ashley et al., 2004). However, the most common types of *Pasteurella* lesions produced in humans were mostly detected in the skin and soft tissues. In this respect, several species including *P. multocida*, *P. canis*, *P. stomatis* and *P. dagmatis* were occasionally recovered from abscesses and wound infections caused by animal bites or scratches (Holst et al., 1992; Holmes et al., 1999).

**AETIOLOGY OF THE DISEASE**

It is now evident that *M. haemolytica*, which was formerly known as *P. haemolytica*, is the main causative agent of the disease although a number of investigators still believe that *P. multocida* is also involved (Radostits et al., 2000; Lopez, 2001; Quinn et al., 2002). However, the pathogenic role of *P. multocida* was more evident in sheep in which it was responsible for many serious outbreaks (Unesh et al., 1994; Black et al., 1997). It is worth mentioning that *M. haemolytica* and *P. multocida* are commensally present as normal constituents of the nasal and pharyngeal microflora of healthy ruminants (Richard et al., 1989; Jansi et al., 1991; Shewen & Conlon, 1993). Both organisms were frequently isolated from the nasopharynx and trachea of sick animals and also from apparently healthy ones (Ojo, 1975; 1976; Schiefer et al., 1978; Al-Tarazi & Dangall, 1997; Biberstein & Hirsh, 1999). Earlier studies in cattle, however, demonstrated that the mean nasal colony count of *P. haemolytica* was much higher in sick animals than in healthy ones (Thomson et al., 1975). The significant rise in the nasal colony count of *P. haemolytica* in sick animals demonstrated the ability of the organism to proliferate at a remarkably greater extent that was sufficient to cause infection in susceptible animals. *P. haemolytica* has also
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been isolated in pure culture from pneumonic lungs of acute untreated cases of shipping fever in cattle (Yates, 1982; Fodor et al., 1984; Allan et al., 1985) and from different cases of enzootic pneumonia in sheep and goats (Gilmour, 1980; Bakke & Nostvold, 1982; Oros et al., 1997). The organism was also recovered from similar cases of acute fibrinous bronchopneumonia in goats (Gourlay & Barber, 1960; Mugera & Kramer, 1967; Fodor et al., 1984; 1989; Hassan, 1999). The principal serotype associated with the disease was A1 although further investigations have also indicated the significant role of serotype A6 (Odendaal & Henton, 1995; Donachie, 2000). It has also been observed that P. haemolytica biotype “A” serotype 1 predominated in bovine pneumonias while serotype 2 was mostly dominant in the ovine and caprine disease (Fodor et al., 1984; Morck et al., 1989; Hassan, 1999). Moreover, M. haemolytica serotype 7 was also reported to cause acute outbreaks in sheep (Odugbo et al., 2004a). Other serotypes of M. haemolytica such as A6, A9 and A11 were also proved highly pathogenic and capable of causing severe infection characterized by acute fibrinous pneumonia in sheep (Odugbo et al., 2004b).

The involvement of P. haemolytica (M. haemolytica) as a causative agent of pneumonic pasteurellosis has long been demonstrated by experimental inoculation of the organism in susceptible animals. In this respect, earlier experiments by Carter (1956) produced variable pneumonic lesions by intravenous, intranasal or intratracheal inoculation of P. haemolytica in cattle. Consistent lesions of acute fibrinous bronchopneumonia were later induced by intratracheal inoculation of large doses of P. haemolytica in four-month old calves (Friend et al., 1977). The gross and microscopic lesions of the affected lungs in experimentally infected calves were similar to those observed in natural field outbreaks. Clinical evidence of pneumonic pasteurellosis was further obtained in calves and adult cattle by intranasal or intratracheal inoculation of P. haemolytica in combination with cold stress (Gibbs et al., 1984; Slocombe et al., 1984). However, clinical signs and pathological lesions of acute pneumonic pasteurellosis were also induced by endobronchial inoculation of the organism in two-week old calves without stress or any other predisposing factor (Vestweber et al., 1990). Similar experiments have also demonstrated the positive role of M. (P) haemolytica as a causative agent of pneumonic pasteurellosis in sheep in which young lambs were more susceptible than adult sheep to type A strain of P. haemolytica (Smith, 1960). Pneumonic lesions and generalized infections were also induced by intratracheal inoculation of relatively larger doses of P. haemolytica in older lambs and adult sheep (Smith, 1964; Al-Darraji et al., 1982; Cutlip et al., 1996). In other experiments, the intratracheal inoculation of sheep with P. haemolytica serotype A2 of ovine origin resulted in severe clinical signs of acute bronchopneumonia and death within 72 hours after challenge (Foreyt & Silflow, 1996). More recent studies have further indicated that intratracheal inoculation of M. haemolytica serotypes A1, A2, A6, A7, A9, A11 and some other untypable strains produced severe clinical signs and pathological lesions of acute fibrinous bronchopneumonia in experimentally infected sheep (Odugbo et al., 2004b). It was also observed in the previously described studies, that the pneumonic lesions produced by M. (P) haemolytica in the experimentally infected animals were closely similar to
those observed in natural cases of the disease. Experimental evidence has also confirmed the role of \( M. (P) \) haemolytica as a major aetiological agent of pneumonic pasteurellosis in goats and the clinical and pathological manifestations of the disease were not apparently different from those observed in sheep (Ngatia et al., 1986; Zamri et al., 1991; Mohamed, 2002). In addition, goats were further employed as model animals for experimental studies of pneumonic pasteurellosis using \( P. multocida \) harvested from pneumonic lungs of goats, rabbits and sheep (Zamri et al., 1996). The resultant infections were acute, subacute or chronic and the gross and histopathological lesions produced by \( P. multocida \) in the experimentally infected goats were similar to those observed with \( P. haemolytica \).

**CLINICAL FEATURES**

Pneumonic pasteurellosis is a disease that mainly occurs in animals with compromised pulmonary defense mechanism. In cattle, spontaneous infections frequently occur following previous exposure to a stressing experience such as transportation or shipping and hence the name “shipping fever” was derived. Sheep and goats are fairly susceptible and could also contract the disease if they were similarly exposed to physical stress or unfavourable environmental conditions. Pneumonic pasteurellosis in cattle is generally recognized as an acute febrile respiratory disease with fulminating fibrinous or fibrinopurulent bronchopneumonia and fibrinous pleurisy. Observable clinical signs of acute respiratory distress usually develop within 10 to 14 days in adult animals after being exposed to stress but a much earlier onset is more typical (Radostits et al., 2000). Nevertheless, infected animals in severe cases may die as a result of toxaemia even before the development of significant pulmonary lesions. In this case sudden death may be the first sign of acute outbreaks particularly in young calves. After the onset of respiratory disturbances, infected animals appear extremely dull with reduced appetite and remarkable depression. They soon develop high fever, anorexia and rapid shallow respiration accompanied with profuse mucopurulent nasal and ocular discharges. Later on, productive cough, which is accentuated by physical effort or movement, usually develop in most of the infected animals. Marked dyspnoea with an expiratory grunt may be observed in very advanced stages of the disease (Dungworth, 1993; Lopez, 2001). In acute outbreaks, the clinical course of the disease is relatively short (2–3 days) terminating in death or recovery in either treated or non-treated animals. However, a number of sick animals that survive the acute phase may become chronically infected. The severity of the disease is rather variable under field conditions and serious economic losses would ultimately result from massive fatalities in acute outbreaks or from poor productivity in chronically infected animals. The clinical course of the acute disease in sheep and goats is very much similar to that observed in cattle ending in death within 12 to 24 hours in severe cases or recovery within a few days (Gilmour, 1980; Brogden et al., 1998). Infected sheep and goats also develop high fever with clinical evidence of severe respiratory involvement manifested by dyspnoea, froth at the mouth, cough and nasal discharges. Young animals are more susceptible than adults and they develop more severe infection in which sudden death may occur with or without any previous warning clinical signs.
PATHOGENESIS AND VIRULENCE FACTORS

The pathogenesis of pneumonic pasteurellosis remained a subject of considerable speculation and controversy due to the complex nature of the disease and the lack of consistency of the results obtained by experimental approach. In earlier literature, Yates (1982) reviewed several findings of field workers and researchers who demonstrated that *P. haemolytica* cannot act alone as the causative pathogen of the disease in the absence of a well-defined predisposing factor. Failure of induction of the disease by direct inoculation of the organism in healthy animals was attributed to the rapid clearance of the bacteria by pulmonary defense mechanisms. On the other hand, clinical signs of acute pneumonic pasteurellosis were successfully induced by intratracheal or endobronchial inoculation of a pure culture of *P. haemolytica* in cattle (Vestweber *et al*., 1990), sheep (Foreyt & Silflow, 1996) and goats (Ngatia *et al*., 1986) without the involvement of any predisposing factor. Many other authors also believe that pneumonic pasteurellosis is a secondary bacterial complication of a previous viral infection of the respiratory system (Carter, 1973; Jakab, 1982; Cutlip *et al*., 1993; 1996). However, the sequential development of the pulmonary lesions is highly mediated by complex interactions between the naturally existing causative organism in the upper respiratory tract, the immunological status of the animal and the role of predisposing factors in the initiation of infection. The majority of *M. (P) haemolytica* infections are mostly endogenous, caused by the normally resident bacteria on the upper respiratory tract, although exogenous infections can also occur by direct contact with sick animals or through infected aerosols. In either situation, the disease is essentially triggered by sudden exposure to a stressful condition or by initial infection with certain respiratory viruses, mycoplasma or bacteria. Stress and/or viral infection would eventually impair the local pulmonary defense mechanisms by causing deleterious effects on the ciliating cells and mucous coating of the trachea, bronchi and bronchioles. The causative bacteria from the nasopharynx will then reach the ventral bronchi, bronchioles and alveoli by gravitational drainage along the tracheal floor and thereby become deeply introduced into the lung tissue. Endotoxins produced by rapid growth and multiplication of the bacteria in infected lobules will cause extensive intravascular thrombosis of pulmonary veins, capillaries and lymphatics. These vascular disturbances eventually result in focal ischaemic necrosis of the pulmonary parenchyma accompanied by severe inflammatory reaction dominated by fibrinous exudate (Slocombe *et al*., 1985; Jones *et al*., 1997; Lopez, 2001). Formation of antigen-antibody complexes may also contribute to the vascular permeability and chemotaxis of neutrophils with the subsequent release of lysozyme (Kim, 1977). The severity of lesions, however, depends on the rate and extent of bacterial proliferation and the amount of endotoxin released, which in turn depends on the virulence of the bacterial strain and the degree to which the defenses of the host are impaired (Hilwig *et al*., 1985; Dungworth, 1993).

It is also established that the ability of pathogenic bacteria to cause infection is greatly influenced by certain endogenous factors which can enhance the pathogenicity of the organism and facilitate rapid invasion and destruction of target tissues of the susceptible host. These factors are generally designated as virulence factors.
and constitute parts of the surface components of the bacterial cell and cellular products. Virulence factors are, in fact, capable of promoting adhesion, colonization and proliferation of the organism within the animal tissues. In other words, virulence factors are actively involved in conversion of the organism from commensal into pathogen (Confer et al., 1990; Gonzales & Maheswaran, 1993; Quinn et al., 2002). The role of virulence factors in the pathogenicity of M. (P) haemolytica have been extensively investigated (Gonzales & Maheswaran, 1993; Zecchinon et al., 2005) and the following paragraphs provide a brief account on this respect.

Cell capsule

The cell capsule constitutes an important virulence factor which plays vital roles in the pathogenicity of pathogenic bacteria and establishment of infection. The virulence mechanism of the cell capsule is mostly attributed to its ability to protect the invading organism against cellular and humoral defense mechanisms of the host. The capsular materials of M. haemolytica and other Pasteurella species were identified as polysaccharide basic structures produced during the logarithmic phase of growth of the bacteria. Each serotype of M. haemolytica produces a characteristic polysaccharide capsule in order to avoid phagocytosis by macrophages and polymorphonuclear leukocytes and to protect the organism against complement-mediated destruction of the outer membrane in serum (Brogden et al., 1989; Czuprynski et al., 1989). The capsular material of M. haemolytica can also interact with the pulmonary surfactant and thereby facilitates the adhesion of the invading organism to the respiratory tract epithelium of susceptible animals (Brogden et al., 1989; Whiteley et al., 1990).

Fimbriae

Fimbriae are smaller appendages present in the surface of many Gram-negative bacteria. They are specific surface structures of the bacterial cell wall which permit or enhance adherence to and colonization of the target epithelium of the susceptible animals. Fimbriae are present in various strains of Pasteurella and Mannheimia species. Two types of fimbriae have been detected in serotype 1 of M. haemolytica (Potter et al., 1988; Morck et al., 1987; 1989). One of them is large and rigid, measuring 12 nm in width and the other is smaller, flexible and measures only 5 nm. The large rigid fimbriae are composed of 35 KDa subunits and proved to be highly immunogenic. The two types of fimbriae produced by M. haemolytica are both capable of enhancing mucosal attachment of the organism and colonization of the lower respiratory tract epithelium of cattle and sheep. Successful colonization will thus enable considerable increase in the number of bacteria seeded in the lung tissue beyond the level that normal lung capacity could efficiently resolve (Gonzales & Maheswaran, 1993).

Endotoxin

Similarly to all other Gram-negative bacteria, the cell wall of M. haemolytica contains a lipopolysaccharide (LPS) endotoxin. This endotoxin is one of the most important virulence factors involved in the pathogenesis of pneumonic pasteurellosis. It has been shown that serotypes 2 and 8 of M. haemolytica possess a rough LPS while the other 14 serotypes have characteristic smooth LPS (Lacroix et al., 1993). Experimental evidence indicated that M. haemolytica endotoxin is directly toxic to endothelial cells and capable of altering leukocyte functions and causing lysis of blood platelets (Breider et al., 1990).
However, the precise mechanism of endothelial cell injury by endotoxin has not yet been fully clarified but the toxic effect of endotoxin could be reduced by neutrophils. Early work with purified LPS injected either intravenously or intraarterially at sublethal concentrations in sheep resulted in increased pulmonary arterial pressure, decreased cardiac output and decreased pulmonary, venous and systemic blood pressure (Keiss et al., 1964). Further subsequent investigations in calves also revealed a number of physiological effects of the purified LPS similar to those produced by chemical mediators such as thromboxane A2, prostaglandins, serotonin, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) (Emau et al., 1987). All these previously mentioned mediators were known to be responsible for the clinical signs associated with endotoxic shock.

**Leukotoxin**

It has also been shown that *M. haemolytica* produces a soluble heat labile exotoxin known as leukotoxin because of its high specificity for leukocytes of ruminants (Shewen & Wilkie, 1983; Chang et al., 1986). The leukotoxin is a 102 kDa protein secreted at the logarithmic phase of growth of the bacteria and also considered as a main weapon or virulence factor for *M. haemolytica* (Zecchinon et al., 2005). In fact, the leukotoxin is pore-forming cytolysin which can produce several biological effects on leukocytes and blood platelets of ruminants. The most susceptible cells are bovine macrophages, neutrophils from most ruminant species, lymphocytes and cultured lymphoma cells. At low concentration, leukotoxin impairs phagocytosis and lymphocyte proliferation while at higher concentration it has a cytotoxic effect resulting in cell death due to lysis (Clinkenbeard et al., 1989; Clinkenbeard & Upton, 1991; Majury & Shewen, 1991). The lysis of cells is attributed to the formation of transmembrane pores in the target cell, and thereby allowing the movement of potassium, sodium and calcium ions through transmembrane gradients (Clinkenbeard & Upton, 1991). Leukotoxin also causes stimulation of polymorphonuclear leukocytes and activation of macrophages with consequent release of proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-8 (IL-8), leukotrienes and tumour necrosis factor (TNF). This action would further lead to the release of H$_2$O$_2$ which, in turn, is converted into hydroxyl radicals by alveolar endothelial cells. The free hydroxyl radicals cause considerable damage and necrosis of the pulmonary alveolar epithelium resulting in accumulation of oedema fluid and fibrin inside alveoli and interstitial spaces (Biberstein & Hirsh, 1999; Lopez, 2001). The leukotoxin and enzymes released following cytolysis are both chemotactic for various types of inflammatory cells causing more damage to the lung tissue due to increased cell recruitment into the area (Zecchinon et al., 2005). Furthermore, *M. haemolytica* leukotoxin was found to induce *in vitro* morphological alterations in calves’ neutrophils similar to those produced by the viable infective organism during the natural course of the disease in the living animal (Clinkenbeard et al., 1989; Clarke et al., 1998). The effect of leukotoxin and O-sialoglycoproteins produced by *P. haemolytica* A1 on bovine platelets activation was also investigated by Nyarko et al. (1998) and their results revealed that bovine blood platelets adhesion was considerably enhanced by both bacterial products. The authors stated that these two
bacterial proteins could directly interact with bovine platelets to initiate platelet aggregation and fibrin formation in the alveolar tissue of the affected lungs.

Other virulence factors

In addition to the previously mentioned factors, the pathogenicity of *M. (P) haemolytica* was also found to be influenced by many other intrinsic components that may serve as virulence factors. Examples of these include an iron-regulated outer membrane protein, toxic outer membrane protein and some extracellular enzymes that are involved in the pathogenesis of the disease (Biberstein & Hirsh, 1999; Quinn *et al*., 2002). The critical need for iron as an absolute growth requirement for various types of microorganisms including pathogenic bacteria has long been recognized (Bullen *et al*., 1978). However, the amount of free iron in the living body which might be readily available for the invading bacteria is extremely small under normal circumstances (Weinberger, 1978; Bullen, 1981). Possession of a specialized iron acquisition system is therefore essential for the survival of pathogenic bacteria in their susceptible hosts and several strategies have been adopted in this respect. However, the most important one is the siderophore-mediated iron uptake by certain types of pathogenic bacteria (Otto *et al*., 1992). Siderophore production has not yet been demonstrated with *M. haemolytica* or any other *Pasteurella* species except for some avian strains of *P. multocida* (Hu *et al*., 1986). Nevertheless, all *Pasteurella* species are capable of binding transferrin-iron complexes by virtue of iron-regulated outer membrane proteins that are expressed under poor iron condition (Morck *et al*., 1991; Reissbrodt *et al*., 1994). Furthermore, *P. haemolytica* serotype 1 was also reported to produce several antigenic proteins in iron-restricted cultures including a transferrin-binding protein that is specific for bovine transferrin (Ogunnariwo & Schryvers, 1990). With regard to enzyme production, some *P. haemolytica* serotypes including serotype 1 and serotype 2 were found to produce certain extracellular enzymes that are involved in the pathogenesis of the disease. One of them is neuraminidase, a unique neutral protease which specifically hydrolyses O-sialoglycoproteins (Frank & Tabatabai; 1981; Abdullah *et al*., 1992). This enzyme is produced *in vivo* during active *P. haemolytica* A1 lobar infection in goats (Straus & Purdy, 1994). Although the precise role of neuraminidase is not fully understood, it was suggested to play an active role in the colonization of epithelial surfaces by removing terminal sialic acid residues from mucin, thereby modifying normal host innate immunity (Biberstein & Hirsh, 1999). In addition, *P. haemolytica* A1 was also found to produce an IgG1 specific protease which was regarded as another virulence mechanism contributing to the pathogenesis of the disease in cattle (Lee & Shewen, 1996).

PATHOLOGY OF THE DISEASE

The pathological alterations associated with pneumonic pasteurellosis have been extensively investigated particularly in cattle (Thomson, 1974; Friend *et al*., 1977; Rehmtulla & Thomson, 1981; Gibbs *et al*., 1984; Slocombe *et al*., 1984; Dungworth, 1993; Jones *et al*., 1997; Lopez, 2001). The gross lesions in affected lungs were generally described as a prototype of fibrinous (lobar) bronchopneumonia with prominent fibrinous pleurisy and pleural effusions. The inflammatory process is well dominated by fibrinous
exudation in the pulmonary alveoli accompanied by interstitial oedema and congestion, imparting a marbled appearance of the cut surface of the affected parts of the lung tissue. Lesions are always bilateral with cranioventral distribution, usually below a horizontal line through the tracheal bifurcation. The apical and cardiac lobes are the mostly affected parts, but in severe cases, infection may be more extensive involving substantial portions of the diaphragmatic lobe. The diseased portions of the lung become remarkably consolidated or hepatized, dark red in colour and covered with tangled masses of fibrinous strands. The interlobular septa are often distended with yellow gelatinous oedema fluid or fibrin and the pleura are noticeably thickened and dull with focal adhesions to the thoracic wall. Multiple areas of coagulative necrosis are consistently observed within the pnemonic portions of lung parenchyma and they appear irregular in shape and sharply demarcated with thick white boarders and deep red central zone. The mediastinal and bronchial lymph nodes are frequently congested, oedematous and/or haemorrhagic and the trachea and major bronchi are occasionally flooded with conspicuous amounts of frothy fluid. The histological features of the affected lungs are dominated by diffuse capillary congestion, interstitial and alveolar oedema together with vascular thrombosis of capillaries, small blood vessels and pulmonary lymphatics. Alveolar wall necrosis is commonly seen in many affected lobules with the presence of variable amounts of fibrinous exudate and inflammatory cells inside the alveoli. The necrotic areas are usually surrounded by a rim of elongated cells, often referred to as “swirling macrophages” or “oat cells” which are now thought to be degenerated neutrophils mixed with alveolar macrophages (Dungworth, 1993). Bacterial colonies are frequently detected in the vicinity of the necrotic areas of the lung tissue. Fibrinocellular exudate is frequently observed inside the bronchi and bronchioles with accumulation of dark swirling macrophages inside alveoli and alveolar ducts. P. haemolytica antigen has been detected in the necrotic alveolar walls and also in fibrin, serous exudate and degenerating leukocytes by the use of immunoperoxidase technique (Haritani et al., 1987). In addition, fibrinous pleurisy is also indicated by massive deposition of fibrinous exudate and diffuse infiltration of leukocytes in the plural surfaces. It is worth mentioning that the gross and histopathological lesions in M. haemolytica-infected lungs of cattle were apparently similar to those produced by P. multocida. However, some authors suggested that the most striking differences between them were the lack of focal necrosis of the lung tissue and the presence of large numbers of neutrophils in cases of P. multocida pneumonia (Schiefer et al., 1978; Ames et al., 1985; Haritani et al., 1987). Other differential features between M. haemolytica and P. multocida pneumonias were also described by Dungworth (1993) who stated that M. haemolytica tends to cause fulminating fibrinous lobar pneumonia while P. multocida usually causes fibrinopurulent bronchopneumonia.

The pathological findings of natural and experimental infections of pnemonic pasteurellosis in sheep were basically similar to those reported in cattle although a number of different features were occasionally spotted (Brogden et al., 1998; Lopez, 2001). The disease in sheep may either be acute with haemorrhagic or fibrinonecrotic lobar pneumonia and serofibrinous pleurisy or chronic with fibri-
nopolurulent bronchopneumonia leading to abscessation and fibrinous adhesions with the thoracic wall. In addition, excessive amounts of serous or serofibrinous fluid were frequently observed in the pericardial, pleural and peritoneal cavities (Dungworth, 1993). The pathological findings in the pneumonic lungs of spontaneous or experimental M. (P) haemolytica infections in goats were also similar to those observed in sheep and cattle (Hayashidani et al., 1988; Zamri et al., 1991; Dungworth, 1993; Brogden et al., 1998; Lopez, 2001). The most consistent findings in goats were also dominated by extensive bilateral cranioventral consolidation (hepatization) of the apical and cardiac lobes with fibrinous strands covering most of the affected parts of the lung. Fibrinous pleurisy with or without adhesions was observed in most cases, while some other cases showed focal abscesses from which pure cultures of M. (P) haemolytica were isolated (Hassan, 1999; Mohamed, 2002).

Extensive and wide spread pulmonary vascular thrombosis is well observed as a consistent finding in natural and experimental infections with M. (P) haemolytica in cattle, sheep and goats. However, the thrombi are usually limited to small blood vessels, capillaries and lymphatics of pneumonic lungs, but large vessels are not apparently affected. An unusual incidence of thrombotic occlusion of a large pulmonary vein of an adult Angora goat with an acute fibrinous bronchopneumonia due to M. (P) haemolytica infection was further documented by Scholes & Kelly (1997). The occurrence of such thrombi in large pulmonary blood vessels may well reflect the highly potent procoagulant effect of M. haemolytica endotoxin on the vascular endothelium of the lung of infected animals.

PREDIPOSING FACTORS

The most important predisposing factors involved in the incidence of pneumonic pasteurellosis in cattle, sheep and goats could be summarized as follows.

Stress

Stress is an intrinsic condition that was consistently reported to increase the susceptibility to various types of infectious diseases in man and animals (Stephens, 1980; Biondi & Zannino, 1997). However, the effect of stress is more evident with respiratory tract infections in which pneumonic pasteurellosis may well provide the most appropriate example in veterinary medicine. Stress may either be psychological as induced by fear, restraint, handling or physical, resulting from hunger, thirst, fatigue or thermal extremes (Grandin, 1997). Stress cannot be measured grossly in an individual animal, yet, a number of clinical and biochemical parameters such as elevated body temperature, increased heart rate, decreased body weight and increased levels of plasma cortisol, glucose, free fatty acids, urea and betahydroxybutyrate were generally regarded as useful indicators (Knowles et al., 1995; Morton et al., 1995; Warriss et al., 1995). The reaction of animals to stress is rather variable even within individual animals of the same species. The role of stress in the natural incidence of pneumonic pasteurellosis was clearly evident by the fact that the disease onset is mainly associated with sudden exposure to stressful situations created by adverse physical, environmental or climatic conditions. The most common examples of these include extremely hot or cold weather with high levels of humidity, overcrowding in a limited space, poor ventilation, bad management, rough handling and distant transport or shipping (Thomson et al.,
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1975; Slocombe et al., 1984; Radostits et al., 2000). In fact, transport was the most commonly recognized predisposing factor associated with field outbreaks in cattle in which the name “shipping fever” was derived. Other stressful situations such as excessive dust in feedlots, high load of internal or external parasites and mixing of animals from different sources were also encountered (Gilmour, 1980; Martin, 1996). Predisposing factors may either act alone or in combination and their significant role in the establishment of infection has repeatedly been demonstrated by experimental means in cattle (Gibbs et al., 1984; Slocombe et al., 1984), sheep (Gilmour, 1980; Knowles et al., 1995; Martin, 1996) and goats (Ojo, 1976; Zamri et al., 1991; Mohamed, 2002). Stress can also be induced artificially by administration of certain drugs and chemical compounds (e.g. dexamethazone) and the effect of the chemically-induced stress on the susceptibility to pneumonic pasteurellosis was found to be similar to that obtained in natural circumstances (Zamri et al., 1991; Mohamed, 2002; Malazdrewich et al., 2004). The reason for the increased susceptibility to M. (P) haemolytica infection in stressed animals was primarily attributed to the breakdown of the innate pulmonary immune barriers by stressors (Martin, 1996; Brogden et al., 1998). Stress and viral infections were also reported to increase the level of mammalian tissue fibronectin and thereby promote the growth of Gram-negative over Gram-positive bacterial populations (Woods, 1987).

Respiratory viruses

Previous or combined infection with certain respiratory viruses was commonly found to increase the susceptibility of farm animals to secondary bacterial pneumonias (Carter, 1973; Al-Darraji et al., 1982; Cutlip et al., 1993; 1996; Brogden et al., 1998; Hodgson et al., 2005). Examples of the most important viruses associated with acute respiratory tract infections in farm animals include parainfluenza-3 virus (PI-3), bovine herpesvirus type 1 (BHV-1), respiratory syncytial virus (RSV), adenoviruses (ADV), and reovirus (Rosadio et al., 1984; Dinter & Morein, 1990). The majority of these viruses were reported to increase the susceptibility to secondary M. (P) haemolytica infection in susceptible animals. For example, initial infection with infectious bovine rhinotracheitis virus (BHV-1) followed by P. haemolytica, in calves, resulted in very severe febrile disease with clinical signs and pulmonary lesions indicative of pneumatic pasteurellosis (Jericho & Langford, 1978). An acute fatal respiratory disease was also induced in cattle by previous infection with BHV-1 challenged with M. haemolytica (Hodgson et al., 2005). The combined infection with PI-3 virus and P. haemolytica in lambs was also found to cause severe fibrinopurulent bronchointerstitial pneumonia with focal necrosis that closely resembled lesions seen in natural cases of acute enzootic pneumonia (Cutlip et al., 1993). The simultaneous inoculation of lambs with RSV and P. haemolytica similarly resulted in the development of massive pulmonary lesions closely resembling those observed in naturally occurring cases of ovine pneumatic pasteurellosis (Al-Darraji et al., 1982). Moreover, infection with ovine adenovirus-6 followed by P. haemolytica induced more severe lesions in lambs than those produced by either agent alone (Cutlip et al., 1996). The authors observed that the combined inoculation of lambs with the virus and bacteria caused fibrinopurulent pneumonia with oedema, focal necrosis
and pleuritis. In their experiments, the combined viral-bacterial infection either resulted in early death of experimental animals or in slow resolution of lesions in a manner resembling field cases of enzootic pneumonia. All of these previously mentioned findings clearly demonstrated that the initial viral infection would eventually increase the susceptibility of animals to subsequent bacterial infection. Although the precise role of respiratory viruses in enhancing secondary bacterial infections is not clearly understood, a variety of mechanisms had been proposed in this respect. For example, viral infections are well established to produce adverse affects on the mucociliary clearance mechanism involved in the removal of pathogenic organisms that reach the lower respiratory tract (Jakab, 1982). Virus-induced injury to the respiratory epithelium was also believed to enhance bacterial attachment and subsequent colonization of target tissues. Respiratory viruses were also reported to impair the phagocytic function of pulmonary alveolar macrophages. Hence, infected macrophages lose their phagocytic ability and fail to release chemotactic factors for other cells. More recent studies, however, indicated that respiratory viral infection enhanced toll-like receptors (TLR) expression and increased proinflammatory responses which contribute to the severity of *M. haemolytica* infection (Hodgson et al., 2005). The TLRs have been suggested to play a critical role in detecting bacterial infection and inducing proinflammatory responses (Aderem & Ulevitch, 2000).

**Mycoplasma and other microorganisms**

The deleterious effect of certain *Mycoplasma* species on the respiratory system of ruminant animals has long been recognized. The most important examples of these pathogens include *Mycoplasma mycoides* subsp. *mycoides*, *M. mycoides* subsp. *capri*, *M. bovis*, *M. ovipneumoniae* and *M. dispar* (Jones et al., 1982, Brogden et al., 1988; Thirkell et al., 1990). Most of these *Mycoplasma* species are known to contribute to the development of severe pneumonic lesions either alone or in association with pneumonic pasteurellosis (Davies et al., 1981; Jones et al., 1997). The synergistic role of some other bacterial organisms in this connection was also evident. For example, *Bordetella parapertussis* was found to increase the susceptibility to secondary *P. haemolytica* A2 pneumonia in mice (Jian et al., 1991; Porter et al., 1995a) and also in sheep (Porter et al., 1995b). The organism alone was occasionally isolated from pneumonic lesions of ovine lungs (Chen et al., 1989; Porter et al., 1994). The combined infection with *P. haemolytica* A2 and *B. parapertussis* was further reported to induce a synergistic effect with remarkable reduction of phagocytic capacity of alveolar macrophages (Hodgson et al., 1996). A filamentous bacterium known as cilia-associated respiratory (CAR) bacillus was also found to predispose to *P. haemolytica* infection in susceptible animals. This filamentous organism was originally recognized in wild rats with acute respiratory tract infections (Van Zwieten et al., 1980; MacKenzie et al., 1981) but it was later recovered from the respiratory tract of goats (Fernandez et al., 1996). The CAR bacillus was further reported to be involved in the incidence of enzootic pneumonia in goats together with many other organisms including *P. haemolytica* (Oros et al., 1997). In addition, a number of many other bacteria species such as *P. multocida*, *Streptococcus* spp., *Staphylococcus* spp., *Arcanobacterium pyogenes*, *Escherichia coli*, *Histophilus* (*Haemophi-
(M) somni and *Chlamydia* spp. were frequently isolated from pneumonic lungs of cattle, sheep and goats in which the primary lesions were mainly attributed to *P. (M) haemolytica* infections (Ngatia *et al.*, 1986).

**Other factors**

In addition to the previously mentioned predisposing factors, a number of other unrelated conditions such as twin pregnancy, selenium deficiency, mycotoxins, inhalation of foreign material and obstruction of pulmonary airways were also reported to have a predisposing role in the incidence of pneumonic pasteurellosis in susceptible animals (Reffett *et al.*, 1985; Pfeffer, 1988). Experimental evidence also indicated that the susceptibility to *P. (M) haemolytica* and *P. multocida* infections was significantly increased in laboratory and farm animals by the repeated administration of injectable or dietary iron compounds (Al-Sultan & Aitken, 1984; Ali, 1999; Mohamed, 2002). The increased virulence of *Mannheimia* and other *Pasteurella* species by iron compounds was primarily attributed to the vital role of iron as a growth-promoting factor for unicellular microorganisms. Parasitism was also regarded as an important predisposing factor in connection with pneumonic pasteurellosis. In this respect, concurrent or previous infection with common gastrointestinal parasites such as *Haemonchus contortus* was reported to increase the susceptibility to pneumonic pasteurellosis in goats (Zamri *et al.*, 1994). The adverse effect of the nematode on animal susceptibility was attributed to its ability to induce significant immunosuppression allowing the development of the pneumonic lesions. Similar findings were further obtained with *Fasciola gigantica* in which the susceptibility to pneumonic pasteurellosis in Nubian goats was remarkably increased with concurrent infection with the liver fluke (Mohamed, 2002).

**CONCLUDING REMARKS**

It is obvious from the present review and previous literature that pneumonic pasteurellosis is a highly complex multifactorial disease of a worldwide prevalence and distribution in cattle, sheep and goats. The disease primarily results from interaction of stress, immunity and the causative bacteria (*M. haemolytica*) which is commensally resident in the respiratory tract of susceptible animals. The major factors leading to stress and compromised immunity are naturally created by adverse environmental and climatic conditions and also by previous or co-infection with certain respiratory viruses, mycoplasma or some other types of bacteria. However, the precise role of *M. haemolytica* as a primary pathogen still requires further elucidation in the presence of huge amounts of discrepancies and contradictory information in the recorded literature. Although, numerous reports clearly indicated that the alleged organism (*P./M. haemolytica*) is not capable of causing the disease if challenged alone in experimental animals (Yates, 1982; Gilmour, 1980; Shewen & Conlon, 1993; Mohamed, 2002), however, successful induction of the disease was also accomplished in several occasions by intranasal, intratracheal or endobronchial inoculation of pure culture of *P. haemolytica* (*M. haemolytica*) without the involvement of stress or any other predisposing factors (Carter, 1956; Smith, 1960; 1964; Vestweber *et al.*, 1990; Ole-Mapenay *et al.*, 1997). The problem is that most of the previously reported results were based on a currently
non-valid classification and understanding of the causative organism. What was formerly believed to be a single organism known as *Pasteurella haemolytica* is now proved to contain two separate genera—*Mannheimia* and *Bibersteinia*. Hence, it would not be accurate to assume that all past studies with *Pasteurella haemolytica* were actually working with *Mannheimia haemolytica*. Even more specific studies that have used serotyping need to be carefully evaluated, because serotyping cannot be used to confidently assign isolates to species of the genus *Mannheimia* (Angen et al., 1999b). The past literature of the whole subject, therefore, requires further revision and critical evaluation in order to improve our current knowledge and understanding of this highly economically important disease.

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