Ruminant Pneumonic Pasteurellosis: Review on Epidemiology, Pathogenesis and Virulence Mechanism

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Abstract: 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 septicemia. Pasteurella haemolytica is well established to be the major etiological agent of the disease although Pasteurella multocida has also been involved in many acute outbreaks. Which are commensally resident in the upper 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. The ability of pathogenic bacteria to cause infection is also greatly influenced by certain endogenous factors like endotoxin, leukotoxin, fimbriae and cell capsule, which can enhance the pathogenicity of the organism and facilitate rapid invasion and destruction of target tissues of the susceptible host. The disease causes major economic loss in the feedlot industry and young growing animals are more susceptible. The pathogenesis of the disease is dependent on the complex interaction between the predisposing factors, immunological status of the animal and the causative agent. The status of ruminant pneumonic pasteurellosis in Ethiopia is found to be high and eleven of the 17 known serotypes of M.haemolytica, M.glucosida and B.trehalosi has been isolated and identified in ovine.

Key words: Ethiopia • Mannheimia • Mannheimiosis • Pasteurella • Pasteurellosis • Pneumonia • Ruminants

INTRODUCTION

Respiratory tract infections are 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. Mannheimia haemolytica, Bibersteinia trehallosi and Pasteurella multocida were involved as an etiological agent of the disease, which are commensally resident in the upper respiratory tract of healthy ruminants and are capable of causing infection in animals with compromised pulmonary defense system [1]. 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 [2].

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 [3]. The catastrophic effect of the disease was also evident in sheep and goat farming and remarkable economic losses were also attributed to massive fatalities in feedlot animals and acute field outbreaks. In addition, a substantial amount of money was further lost, almost every year, in improving farm management, animal husbandry and chemotherapeutic and vaccination programs, due to death and loss of production following illness [4].

Therefore, the objectives of this review paper are:

- To make a review on the epidemiology of pneumonic pasteurellosis in ruminants.
- To review on the pathogenesis and virulent mechanism of pneumonic pasteurellosis in ruminants.

**Literature Review on Ruminant Pneumonic Pasteurellosis:** Pneumonic pasteurellosis is a respiratory disease of animals of multifactorial etiology with *Mannheimia haemolytica*, *Bibersteinia trehalosi* and, less commonly *Pasteurella multocida* or *Haemophilus somnus* being some of the infectious agents involved. The disease is characterized by an acute febrile course with severe fibrinous or fibrinopurulent bronchopneumonia, fibrinous pleurisy and septicemia [5].

Although some species cause primary disease, many of the infections are secondary to other infections or result from various environmental and management stress. 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, feed and water shortage and distant transport or shipping [3, 6]. 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 [7, 8].

*Pasteurella* and *Mannhemia* species are generally extracellular organisms that elicit many humoral immune responses. It is associated with the assembly in to the feedlots of large groups of animals from diverse geographic, nutritional and genetic backgrounds. The disease is typically seen in feeder animals 7-10 days after assembly in a feedlot. Morbidity can approach 35% and case fatality ranges from 5-10% [9].

**Etiology of the Disease**

**Characteristics and Role the Etiological Agent:** *Pasteurella* and *Mannhemia* species measures 0.2µm - 2.0µm in length. *Pasteurella* species are characterized by bipolarity that is the staining of only the tips of cells is demonstrable with polychrome stain (example giemsa stain). The bacteria are Gram-negative, non-motile and non-spore forming, facultative anaerobic, small rods or coccobacilli [10].

It is now evident that *M. haemolytica*, which was formerly known as *P. haemolytica* biotype A, is the main causative agent of the disease although a number of investigators still believe that *P. multocida* is also involved [11]. However, the pathogenic role of *P. multocida* was more evident in sheep in which it was responsible for many serious outbreaks [12]. It is worth mentioning that *M. haemolytica* and *P. multocida* are commensally present as normal constituents of the nasal and pharyngeal micro flora of healthy ruminants and are all capable of causing infection when the body defense mechanisms are impaired [13]. Both organisms were frequently isolated from the nasopharynx and trachea of sick animals and also from apparently healthy ones [14].

*M. haemolytica* has also been isolated in pure culture from pneumonic lungs of acute untreated cases of shipping fever in cattle [15] and from different cases of enzootic pneumonia in sheep and goats [16]. The organism was also recovered from similar cases of acute fibrinous bronchopneumonia in goats [17].

The principal serotype associated with the disease was *M. haemolytica* serotype A1 although further investigations have also indicated the significant role of serotype A6 [18]. It has also been observed that *M. haemolytica* Serotype 1 predominated in bovine pneumonias while serotype 2 was mostly dominant in the ovine and caprine disease [17]. Moreover, *M. haemolytica* Serotype 7 was also reported to cause acute outbreaks in sheep [5]. 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 [19].

The involvement of *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 [20] produced variable pneumonic lesions by intravenous, intranasal or intratracheal inoculation of *M. haemolytica* in cattle. 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 [21]. Similar experiments have also demonstrated the positive role of *M. 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* [11]. Experimental evidence has also confirmed the role of *M. haemolytica* as a major etiological agent of pneumonic pasteurellosis in goats and the clinical and pathological manifestations of the disease were not apparently different from those observed in sheep [22].

**Taxonomy:** The term pasteurellosis was used to designate infections in domestic animals caused by Gram-negative bacteria formerly grouped under the genus Pasteurela with in the family pasteurellaceae. Based on number of characteristics including pathogenesis, antigenic nature and biochemical activities, *Pasteurella haemolytica* can be classified in to two biotypes, biotype A and biotype T. Biotype A ferments arabinose while biotype T ferments trehalose [23]. However, with more recent advancements in molecular biology involving DNA hybridization studies and 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* [24]. On the other hand, *Pasteurella haemolytica* biotype T was first reclassified as *B. trehalosi*. However, this organism was recently revised and removed to a new separate category by the name of *Bibersteinia trehalosi* [25].

Based on extractable surface antigens, 17 serotypes of *M. haemolytica* and *B. trehalosi* are recognized. Serotype 3, 4, 10 and 15 are classified as *B. trehalosi*. The remaining serotypes (serotype 1, 2, 5, 6, 7, 8, 9, 12, 13, 14, 16) are classified as mannheimia except serotype 11, which varied from biotype A by fermentation of cellobiose and salicin and is reclassified as *M. glucosida*. Serotypes of *P. multocida* have been identified based on difference in capsular polysaccharides and are designated as A, B, D, E and F [23].

It is worth mentioning that *M. haemolytica*, *P. multocida* and *B. trehalosi* constitute the most important members of the family Pasteurellaceae that pose serious hazards in livestock industry. Their presence is mainly confined to ruminants with most adequately characterized strains originating from cattle, sheep and goats [14].

**Pathogenesis**

**Geographical Distribution and Occurrence:** Distribution and occurrence of pneumonic pasteurellosis in ruminants is widespread and occur in tropical and subtropical climates as well as in the temperate countries [7]. Outbreaks, however, occur sporadically and unpredictable [3].

The prevalence of pneumonic pasteurellosis with in a flock or herd varies year to year. This may be due to rising and falling of immunity to the predisposing viral infections and to Pasteurella and mannheimia species itself [7]. Prevalence is reported to be variable based upon both geography and season. For example, in USA death losses due to mannheimiosis were observed to have increased between 1994 and 1999 with an average incidence rate of 1.42% [26]. *M. haemolytica* being commensal in the nasopharynx of ruminants, is an opportunistic pathogen which has frequently been isolated from 95% of tonsils and 65% of nasopharyngeal swabs of healthy animals in which approximately 65% of the tonsilar and 6% of nasopharyngeal isolates were biotype T, but most isolates from nasopharynx were biotype A [27].

**Morbidity and Mortality:** The morbidity may reach 35%, the case-fatality rate my range from 5-10% and the population mortality rate may vary from 0.75-1%. However, the morbidity and mortality rate may not be reliable because of wide variation in the method to calculate disease incidence and prevalence [1].

In the months of February and March 2002, an outbreak of a contagious acute respiratory disease of sheep and goats occurred in Milae District of Afar Region, Ethiopia. In which more than 30,000 animals were at risk. Out of a total of 722 sheep and 750 goats from four flocks, the morbidity rate was 57% and 53% and the mortality rate was 22% and 32% in sheep and goats, respectively. The case fatality rate had reached 38% in the sheep population and 59% in the goat population [28].

The peak incidence of the disease occurs within first three weeks after arrival of the calves, lamps and kids in the feedlot and also on the pasture due to extreme stress.
In order to make valid assessment of morbidity and mortality rates, it is imperative that case definitions are stated clearly, the population at risk is precisely measured and the period of observation is stated or incorporated into the rate [29].

Risk Factors

Animal Risk Factor: The disease occurs most commonly in young growing animals from six months to two years of age but all age groups are susceptible. Calves, lambs and kids those are non-immune to M. haemolytica are considered to be more susceptible to the disease than calves, lamps and kids that have serum neutralizing antibodies to the organism and its cytotoxin. Animals that are recovered from the experimental disease are resistant to naturally occurring disease. The disease occurs commonly in outbreaks 7-10 days after arrived in the feedlot following stressful transportation or on the range due to other stress factors. This forms a major part of the ‘shipping fever’ complex, which is a major hazard in the practice of rearing beef cattle on range country and then transporting them long distance to other centers for growing and finishing [12].

Environmental and Management Risk Factors: The mixing of cattle from different sources is an important risk factor. The mixing of animals from different sources markets was associated with an increased risk of fatal fibrous pneumonia in young moved to feed lots shortly after sale. The effect of transportation and assembling of young result an increased in the level of plasma fibrinogen, which is an indication of some stress. Deprivation of feed and water followed by confinement in unfamiliar surroundings also results an increased fibrinogen. The response of the animals was dependent on the previous environmental management applied to them before assembly and transportation. Confinement in drafty or humid and poorly ventilated barns, exposure to inclement weather, transport and deprivation from water is commonly followed by outbreaks of diseases [11].

Pathogen Risk Factor: The frequency of isolation of Pasteurella and Mannheimia species from the nasal passage of normal healthy unstressed animal is low but increase as the animal are moved to the market and then to feedlots. The main virulence factors that have been identified in the strains of M. haemolytica include fimbriae, that may enhance colonization of URT and a capsule that inhibits complement mediated destruction of the organisms in serum [7]. Furthermore, the bacteria possess lipopolysaccharides which is toxic to endothelium and alters leukocyte function and a leukotoxin which is a pore forming cytolysin that affects leukocyte and platelets function where present at low concentration and cause cytolysis at high concentration [1].

Immune Mechanism: Calves, lambs and kids that have recovered from experimental disease are resistant to naturally occurring disease. Numerous M. haemolytica antigens may stimulate the immune response and animals become resistant to the disease. These antigens include capsular polysaccharides, Leukotoxin and surface antigens, including iron regulated proteins, serotype specific outer membrane protein and several other antigens that are less well defined [1, 14]. Aerosol exposure of animals to viable M. haemolytica elicits a protective immune response characterized by enhanced clearance of the organism from the lung and by protection against fibrous pneumonia, it is possible that the presence of pre-existing antibodies to leukotoxins in the lung may provide immunity by phagocytic leukocytes from the leukotoxin and by promoting phagocytosis and intracellular killing of the organism. Animals exposed to live organisms produce antibodies to both surface antigens and cytotoxins, whereas exposure to the killed vaccine results in the production of antibodies primarily to cell surface antigens [30].

Method of Transmission: Transmission of pasteurellosis probably occurs by the inhalation of infected droplets coughed up or exhaled by the infected animal, which may be clinical case or recovered carriers in which the infection persists in the upper respiratory tract. M. haemolytica and P. multocida that mediate contagion is an important factor in the spread of the disease [1].

Pathogenesis and Virulence Factor: The pathogenesis of pneumonic pasteurellosis remained a subject of considerable 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 [31] reviewed several findings of field workers and researchers who demonstrated that M. 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 [32].
On the other hand, clinical signs of acute pneumonic pasteurellosis were successfully induced by intratracheal or endobronchial inoculation of a pure culture of *M. haemolytica* in cattle [33], sheep [34] and goats [35] 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. However, the sequential development of the pulmonary lesions is highly mediated by complex interactions between the naturally existing causative organisms in the upper respiratory tract, the immunological status of the animal and the role of predisposing factors in the initiation of infection [20]. The majority of *M. 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 (example para-influenza-3 virus), mycoplasma or other 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 [1]. 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. 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 [40]. The capsular material of *M. haemolytica* can also interact with the pulmonary surfactant and there by facilitates the adhesion of the invading organism to the respiratory tract epithelium of susceptible animals [41]. 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 ischemic necrosis of the pulmonary parenchyma accompanied by severe inflammatory reaction dominated by fibrinous exudates [29, 36, 37]. Formation of antigen-antibody complexes may also contribute to the vascular permeability and chemotaxis of neutrophils with the subsequent release of lysozyme. The severity of lesions, however, depends on the rate and extent of bacterial strain and the degree to which the defenses of the host are impaired [30, 38]. 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*[42]. 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 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 [39].
**Endotoxin:** Similarly, to all other Gram-negative bacteria, the cell wall of *M. haemolytica* contains a 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 serotypes have characteristic smooth LPS [43]. 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 [44].

**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 [45, 46]. The leukotoxin is considered as a main weapon or virulence factor for *M. haemolytica* [27]. In fact, the leukotoxin is preforming 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 [47]. 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 [48].

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 H₂O₂, 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 [14, 29]. 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 [27].

**Other Virulence Factors:** In addition to the previously mentioned factors, the pathogenicity of *M. 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 [11, 14]. The critical need for iron as an absolute growth requirement for various types of microorganisms including pathogenic bacteria has long been recognized. 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 [49].

**Status of Ruminant Pneumonic Pasteurellosis in Ethiopia:** In Ethiopia the prevalence of pneumonic pasteurellosis in ruminants is found to be high and eleven of the 17 known serotypes of *M. haemolytica*, *M. glucosida* and *B. trehalosi* has so far been isolated and identified in ovine in central, northeastern and southeastern high lands of the country [50-53] as indicated in (Table 1). In Milae Districts of Afar region from an outbreak in 2000 in sheep and goat *M. Haemolytica biotype T* was isolated from nasal swabs and lung and pleural fluid. In a study undertaken in calves with clinical signs of respiratory disease in the same area *M. haemolytica* and *P. multocida* isolates were obtained from nasal and transtracheal swabs [28]. *M. haemolytica* serotype A1 and A2 are the most common in the country. However, no remarkable studies have been done to know the prevalence and the actual organisms involved in pneumonic pasteurellosis of cattle [54].

Pneumonic pasteurellosis is common in which studies on the prevalence have revealed a frequent occurrence in the highlands and also in the lowland hot and humid areas with high morbidity and mortality. Losses due to death were also noticed in ruminants confined in quarantine station for export and on the farm [55].

Recent studies indicated that most cases of ruminant pasteurellosis are caused by *M. haemolytica* and vaccine produced by the NVI against the disease is from *P. multocida* serotype A and B which does not correspond to the real causative agent. This may be one possible explanation for high mortality observed from respiratory distress in North Showa (Ethiopia) despite the annual vaccination using monovalent vaccine [56]. The presence of multiple serotypes of *M. haemolytica* as well as *B. trehalosi* without cross protection becomes a challenge for the development of vaccine that is effective worldwide [57].
Table 1: Prevalence of ovine pasteurellosis, common isolates and identified serotypes in Ethiopia

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<th>Study site</th>
<th>Seroprevalence (%)</th>
<th>Bacteriological prevalence</th>
<th>Identified serotype</th>
<th>Source</th>
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*most common serotypes serologically
†most common isolates from pneumonic lung

CONCLUSIONS AND RECOMMENDATIONS

It is obvious 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 (mainly M. haemolytica) which is commensally resident in the upper 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. Pneumonic pasteurellosis is responsible for the largest cause of mortality in feedlot animals. The disease wide spread and occurs mainly in tropical and subtropical climates, in which morbidity may reach 35% and case fatality may range from 5-10%. The majority of M. haemolytica infections are mostly endogenous, although exogenous infections can also occur. The progression of the disease is enhanced by certain endogenous factors which can enhance the pathogenicity of the organism and facilitate rapid invasion, proliferation and destruction of target tissues of the susceptible host. In Ethiopia the prevalence of pneumonic pasteurellosis in ruminants is found to be high, in which most serotypes has been isolated and identified in ovine in different highlands of the country.

Therefore, based on the above conclusions the following points were recommended:

- Proper loading of trucks for animal transportation.
- Rule to control overloading, speed and management during transportation
- Protect animals from exposure to adverse environmental and climatic condition which causes stress that is the main predisposing factors.

REFERENCES


56. Gelagay, 1996. Epidemiological and serological investigation on multifactorial respiratory Disease and vaccine trials on high lands of North Showa (Ethiopia).DVM thesis. Addis Ababa University Faculty of Veterinary Medicine, Debrezeit, Ethiopia.