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A Review on Pneumonic Pasteurellosis in Small Ruminants

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Abstract

Small ruminant production plays a great role in the livelihood of smallholder farmers. But their production is constrained by pneumonic pasteurellosis and it is a high-priority issue at the national level. So, in this paper relevant aspects of pneumonic pasteurellosis in small ruminants are reviewed. The disease is most frequent as air is the main route of transmission. They are characterized by high fever, coughing, dyspnea, and muco-purulent nasal discharge that commonly develops in immunocompromised hosts. Stressors being psychological and/or physical are associated with poor management practices. It is a multifactorial disease. But clinical infections are mainly caused by *Mannheimia haemolytica*, *Bibersteinia trehalosi* and *Pasteurella multocida*. Eleven of the known 17 serotypes of *M. haemolytica* and *B. trehalosi* has so far been identified in Ethiopia. Virulence factors like cell capsule, fimbriae, and endotoxin play a great role in the disease development. The disease causes heavy losses that deserve control. However, the presence of multiple serotypes without cross-protection, and the development of drug resistance complicated its control. Moreover, the causative agents are normal commensal of upper respiratory tract which may cause infection in immunocompromised conditions. Therefore, proper management, sound diagnostic methods and the available serotypes should be considered in vaccine preparation.

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Introduction

Small ruminants play an important role in nutritional security of millions of rural people especially the landless small holder farmers in tropical countries (Daphal *et al*., 2018). Because they require low inputs such as small initial capital, fewer resources and maintenance cost (Jibat *et al.*, 2008).

And they play a great role in the contribution of meat, milk, and wool production, and have a potential to replicate and grow rapidly. The great Indian leader and freedom fighter M. K. Gandhi "father of the nation" designated goats as "poor man's cow," emphasizing the importance of small ruminants in poor countries (Chakraborty *et al*., 2014). Sheep and goats play a significant role in the nation's economy in the overall production system of large and small scale farmers. The meat and milk are major sources of protein while skins, live animals and carcasses account for a significant proportion of exports to generate income (Chakraborty *et al*., 2014; Demissie *et al*., 2014; Welay *et al*., 2018). Goat and sheep production supply more than 30% of all domestic meat consumption (Megra *et al*., 2016). The leather industry gets most raw materials in the form of skin from sheep and goats (Jibat *et al*., 2008).

Ethiopia has 31.30 million sheep and 32.74 million goats (CSA, 2018). Efficient utilization of small ruminants in Ethiopia and their contribution to the national economy is limited due to a combination of health problems, poor management systems and malnutrition. Those problems lead to poor reproductive performance of sheep and goats (Disassa *et al*., 2013; Alemneh and Tewodros, 2016; Welay *et al*., 2018). Among the wide range of diseases that affect sheep and goats, pneumonia, a respiratory disease arising from an inflammatory response of the lung parenchyma, is the major disease limiting the development of animal production in the tropics (Mekibib *et al*., 2019). It is observed as a major problem commonly encountered in flocks, affecting groups or individuals of all ages and types of sheep and goats (Daphal *et al*., 2018). Regardless of etiology, infectious respiratory diseases of sheep and goats contribute to 5.6 percent of the total diseases of small ruminants globally (Hindson and Winter, 2002; Chakraborty *et al*., 2014).

Bacterial pneumonia is the most common respiratory problem in small ruminants reported and frequently diagnosed in veterinary clinics in Ethiopia (Clothier, 2010; Hailu *et al*., 2017; Mekibib *et al*., 2019). Respiratory diseases are most frequent as aerosol spread is the main means of transmission (Mekibib *et al*., 2019). They are the main cause of economic losses to producers of ruminants and they represents 8% of the total production costs including medical expenses, poor food conversion, increased production costs, and decreased food availability for humans (Rico *et al*., 2017). Of all the respiratory diseases of small ruminants, pasteurollosis is a high-priority issue at the national level due to the significant economic losses it causes through mortality, morbidity, and the high cost of treatment (Megra *et al*., 2016; Sadia *et al*., 2016).

It is a common respiratory infection in Ethiopia, causing outbreaks of acute pneumonia in all ages of sheep and goats (Disassa *et al*., 2013). The productivity of sheep is unsatisfactory large due to diseases, and pneumonic pasteurellosis is one of the most important infectious diseases of sheep and goats (Jasni *et al*., 1991; Abdullah *et al*., 2015; Berhe *et al*., 2017; Legesse *et al*., 2018). The disease is caused by *Pasteurella* species that are often encountered in small ruminants as major pathogens (Daphal *et al*., 2018). Some of the specific causative agents of pneumonic pasteurellosis are *M. haemolytica*, *P. multocida* and *Bibersteinia (B.) trehalosi* which were more frequently isolated from pneumonic animals than from animals without pneumonia (Afata, 2018).

Pneumonic pasteurellosis is one of the serious problems in small ruminants and it has a significant economic impact. Continuous commitment for effective control and prevention of the disease is mandatory to increase their contribution to the development of the country (Kehrenberg *et al*., 2001; Asresie and Zemedu, 2015; Legesse *et al*., 2018). However, control of pneumonic pasteurellosis is a difficult task because antimicrobial agents which were the most powerful tools to control such infections become unsuccessful due to incidence of drug resistant (Kehrenberg *et al*., 2001; Legesse *et al*., 2018). It requires also understanding of its epidemiology, for identifying the specific agents circulating in the country for successful vaccination (Afata, 2018). Vaccination is an important control method of pasteurellosis (OIE, 2012). But, development of an effective vaccine has been hindered due to multiplicity of serotypes and lack of cross-protection (Davies *et al*., 2001; Ayalew *et al*., 2006). Most ruminant pasteurellosis cases are caused by *M. haemolytica* and vaccines produced by the National Veterinary Institute (NVI) against the disease for annual vaccination is from *P. multocida* serotype A and B for small and large ruminant pasteurellosis respectively (Ayelet *et al*., 2004; Belege *et al*., 2017). So, due to use of inappropriate vaccines, presence of variation in vaccine and field strain, the outcome of vaccination programs are usually unsuccessful (Catley *et al*., 2009). Therefore, the objective of this paper is to review relevant aspects of pneumonic Pasteurellosis in Small Ruminants in Ethiopia.

Pneumonic pasteurellosis in small ruminants

Respiratory infections are common in various species of domestic animals. However, pneumonic pasteurellosis, also known as respiratory mannheimiosis, is most common among the respiratory tract infections 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 (Dereje *et al*., 2014; Rawat *et al*., 2019).

Pneumonia refers to the inflammations of the pulmonary parenchyma as well as associated with inflammation of bronchioles and pleurisy (Nejiban and Al-Amery, 2018). Respiratory diseases of small ruminants are multifactorial (Chakraborty *et al*., 2014). But members of the family Pasteurellaceae, are considered as a major respiratory pathogens in sheep and goats industry. They are all capable of causing infection when the body defense mechanisms are impaired (Mohamed and

Abdelsalam, 2008; Daphal *et al*., 2018). Pneumonic pasteurellosis is an acute infectious disease that causes wide spread financial losses because of death, reduced live weight, delayed marketing, treatment costs and unthriftness among survivors (Hawari *et al*., 2008).

In Ethiopia, pneumonic pasteurellosis has been a topic of frustration to veterinary practitioners and a topic of liability to ruminant producers (Afata, 2018). They are associated with poor management practices and occur as a consequence of severe stresses like transportation stresses, viral infections, over-crowded pens, poor housing conditions, sudden environmental changes, and other stressful conditions increase goat and sheep susceptibility to infections (Abdullah *et al*., 2015). Under stress, immunocompromised, pregnant, lactating, and older animals easily fall prey to respiratory habitats such as *M. haemolytica* and other species (Chakraborty *et al*., 2014).

Etiology of pneumonic pasteurellosis

There are multiple agents causing pneumonia in sheep and goats, such as bacterial, viral, and parasitic agents involved with other stressors. From these, bacterial agents drawn attention due to variable clinical manifestations, severity of diseases, and the re-emergence of strains resistant to many chemotherapeutic agents (Nejiban and Al-Amery, 2018). *Pasteurella* species are a major pathogens ofsmall ruminants (Daphal *et al*., 2018). Clinical infections caused by *Pasteurella* and *Mannheimia* species in domestic animals are mainly caused by three species notably *M. haemolytica*, *B. trehalosi* and *P. multocida* (Quinn *et al*., 2002; Belege *et al*., 2017; Legesse *et al*., 2018).

They are common commensal of the tonsils and nasopharyngeal microflora of healthy sheep and goats. They are small (0.2 x 1-2 µm) non-motile, non-sporing, fermentative, Gram negative rod and cocco-bacilli usually being pleomorphic that causes cranioventral bronchopneumonia affecting sheep and goats of all ages worldwide (Dousse *et al*., 2008; Abdullah *et al*., 2015; Legesse *et al*., 2018). They are oxidase-positive, and most species are catalase-positive. Although non-enriched media will support their growth, these organisms grow best on media supplemented with blood or serum (Quinn *et al*., 2002).

They were formerly grouped under the genus *Pasteurella* (after Louis Pasteur). However, with more recent progress in molecular biology involving DNA hybridization and the sequencing of 16s rRNA most of the formerly recognized species were subjected for intensive revision and reclassification. In this respect, *P.haemolytica*, biotype A was allocated to a new genus and renamed *Mannheimia*. On the other hand, *P.haemolytica* biotype T was first reclassified as *P.trehalosi* (*Bibersteina*) (Dereje *et al.*, 2014). All serotypes of *M. hemolytica* and *P. multocida* can be involved in pneumonic pasteurellosis (Mitku *et al*., 2017).

Pasteurella multocida

P.multocida is a bacterium that can be part of the normal upper respiratory tract flora of many animal species (Dabo *et al*., 2007). *P. multocida* is an opportunistic pathogen. It is one of the most important respiratory pathogens of domestic ruminants. It can cause mild chronic upper respiratory tract inflammation, serious outbreaks of acute pneumonia or septicaemia (Einarsdottir *et al*., 2016; Rawat *et al*., 2019).

There are five serogroups of *P. multocida* (A, B, D, E and F) using capsular antigens as tested by passive haemagglutination tests (Berhe *et al*., 2017). The organisms are further subdivided into about 16 somatic types (1-16) on the basis of serological differences in cell wall lipopolysaccharides (Quinn *et al*., 2002). Out of five capsular serogroups of *P. multocida*, A and D are usually associated with Pasteurellosis (Daphal *et al*., 2018). In addition, untypable strains have been described, which can beacapsular and about 10% of isolates are untypable from ruminants. The capsule serves as to protect the bacteria from desiccation, phagocytosis and bactericidal complement activity, and capsular bacteria are more virulent than acapsular strains (Kirkan and Kaya, 2005; Einarsdottir *et al*., 2016).

P.multocida and *M.haemolytica* are most often associated with bacterial pneumonia which causes the outbreak of acute pneumonia and death of goats in all age groups (Rawat *et al*., 2019). *P. multocida* is also associated with enzootic pneumonia complex in young ruminants (Mohamed and Abdelsalam, 2008). It grows on most laboratory media except on bile containing media such as MacConkey agar (Hawari *et al*., 2008). Colonies of *P. multocida* are greyish in colour, round in shape, shiny and non-haemolytic. Colonies of some pathogenic strains are mucoid due to the production of thick hyaluronic acid capsules. The colonies have a subtle but characteristic sweetish odour (Quinn *et al*., 2002).

Mannheimia haemolytica

M.haemolytica has undergone significant reclassification in the past: first called *Bacterium bipolaremultocidum* by Theodore Kitt in 1885, and it was renamed *P.haemolytica* in 1932. Then, it classified into two biotypes (A and T) based on its ability to ferment the sugars arabinose and trehalose, respectively (Haig, 2011). These biotypes further divide into serotypes based on their surface antigen (Ayelet *et al*., 2004). *M. haemolytica* is composed of a collection of 17 serotypes based on capsular antigen typing. The seventeen *M. haemolytica* serotypes were reorganized into *B.trehalosi* containing four serotypes (T3, T4, T10, T15), *M. haemolytica* containing 12 serotypes (A1, A2, A5–A9, A12–A14, A16, A17) and *M. glucosida* with one serotype (A11) (Berhe *et al*., 2017). Serotypes of A1 and A2 of *M. haemolytica* are predominant out of twelve serotypes. Serotype 11 (A11), which varied from biotype A by fermentation of cellobiose and salicin sugars was recently reclassified as *M. glucosidal* (Daphal *et al*., 2018).

The commonly recognized diseases associated with *M.haemolytica* are pneumonia or pleuropneumonia in ruminants of all ages, septicaemia in suckling lambs, mastitis in ewes, and arthritis, meningitis and middle-ear infections in sheep and a number of non-specific inflammatory lesions in various species of domestic animals (Quinn *et al*., 2002; Kirkan and Kaya, 2005). *M. haemolytica* and *P. trehalosi* colonies are haemolytic and odourless. They grow as pin-point, red colonies on MacConkey agar but most pathogenic *Pasteurella* species do not grow on MacConkey agar (Quinn *et al*., 2002).

Biberstinia trehalosi

B.trehalosi is complicated by the constantly evolving nomenclature of the organism as it undergoes increasing differentiation and extensively reorganized from other members of the Pasteurellaceae family. It was part of the *P.haemolytica* complex, which consists of biotypes A and T. The organisms once assigned to the T biotype were named *P.trehalosi*. However, there is clear evidence that the species is not closely affiliated with *P.multocida*, the type species of the genus *Pasteurella* (Blackall *et al*., 2007).

Biberstеinia trehalosi is frequently associated with acute systemic pasteurellosis or septicaemia in lambs. They can be isolated from the nasopharynx and trachea of sick animals and also from apparently healthy ones (Kirkan and Kaya, 2005; Mohamed and Abdelsalam, 2008). *Biberstеinia* bacterial genus named after Ernst L. Biberstein, who did a large part of the early characterization of this organism including the creation of the serotyping scheme and some of the earliest DNA–DNA relatedness studies that indicated the unique nature of this taxon (Blackall *et al*., 2007).

Epidemiology and method of transmission

Distribution and occurrence of pneumonic pasteurellosis in ruminants is wide spread and occur in tropical and subtropical climates as well as in the temperate countries. Pneumonic pasteurellosis is common in highlands and also in lowland hot and humid areas with high morbidity and mortality (Belege *et al*., 2017). *P.multocida* has a wide host range whereas *M. haemolytica* is largely restricted to ruminants and *B.trehalosi* to sheep (Quinn *et al*., 2002). Many species of the Pasteurellaceae family inhabit the mucous membranes of alimentary, respiratory and genital tract of mammals, birds, and reptiles (Dousse *et al*., 2008).

Transmission of pasteurellosis 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 (Belege *et al*., 2017). Since they are opportunistic pathogens, they are normally commensals of the upper respiratory tract and may invade the tissues of immunosuppressed animals. Exogenous transmission occurs through aerosols (Quinn *et al*., 2002). *P.multocida* and *M.haemolytica* are highly susceptible to environmental influences. When conditions are optimal, particularly when animals are closely confined in inadequately ventilated areas or held for long periods in holding pens, the disease may spread very quickly and affect high proportion of the flock within short hours (Radostitis *et al*., 2007). Especially group rearing practices in sheep and goats and their tendency to huddle predispose them to infectious and contagious diseases (Chakraborty *et al*., 2014).

Pathogenesis and virulence factors

Nutritional deprivation encountered in many colonising bacteria. It may be due to a low nutrient environment or host restriction mechanisms. Therefore, the organism must overcome host-response, competition with resident bacterial flora and achieve attachment for their survival and to be pathogenic (Rowe *et al*., 2001). The

pathogenesis of pneumonic pasteurellosis remained a subject of controversy due to the complex nature of the disease and the lack of consistency in experimental results (Haig, 2011). 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 (Belege *et al*., 2017).

Small ruminants are fairly susceptible and contract pneumonic pasteurellosis due to exposure to stress factors or unfavorable environmental conditions (Rawat *et al*., 2019). Stress may be either psychological as induced by fear, restraint, rough handling or physical, resulting from sudden exposure to stressful situations created by adverse environmental or climatic conditions(Mohamed and Abdelsalam, 2008). 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. Other stressful situations such as excessive dust in feedlots, high load of internal or external parasites and mixing of animals from different sources can also be involved (Belege *et al*., 2017). The effect of stress is more evident with respiratory tract infections in which pneumonic pasteurellosisis the most appropriate example in veterinary medicine (Mohamed and Abdelsalam, 2008).

The presence of the causative agents of pneumonic pasteurellosis in the nasopharynx has been shown to coincide with occurrence of the disease. However, there is no evidence available to indicate whether colonisation leads to disease or whether disease extends colonisation throughout a flock (Rowe *et al*., 2001). The organisms, which are normally commensals of the upper respiratory tract or exogenous pathogens may invade the tissues of immunosuppressed animals (Quinn *et al*., 2002). 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 (Mohamed and Abdelsalam, 2008).

Virulence factors promote adhesion, colonization and proliferation of the organism within the animal tissues. They are actively involved in conversion of the organism from commensal in to pathogen (Quinn *et al.*, 2002; Belege *et al.*, 2017). Factors like cell capsule, fimbriae, endotoxin, and leukotoxin play a great role in the pathogenicity of pneumonic pasteurellosis in sheep and goats (Mohamed and Abdelsalam, 2008). Factors of importance in the development of disease by *P. multocida* include adhesion of the pasteurellae to the mucosa and the avoidance of phagocytosis. Fimbriae may enhance mucosal attachment and the capsule, particularly in type A strains, has a major antiphagocytic role. In septicaemic pasteurellosis, severe endotoxaemia and disseminated intravascular coagulation cause serious illness which can prove fatal (Quinn *et al*., 2002).

Important virulence factors of *M. haemolytica* and *B. trehalosi* are fimbriae which enhance colonization; a capsule that inhibits complement-mediated destruction of the organisms; endotoxin which can alter leukocyte functions and is directly toxic to endothelial cells; leukotoxin (LKT), a pore-forming cytolysin that affects leukocyte and platelet functions when present at low concentrations and causes cytolysis at high concentrations. The subsequent release of lysosomal enzymes and inflammatory mediators from damaged cells contribute to severe tissue damage. Others like lipopolysaccharide (LPS) and outer membrane proteins (OMPs) can also serve as a pathogenicity mechanism in the process of causing pneumonia in ruminants. So, these bacterial virulence factors have an important role during colonization and invasion of host tissues (Quinn *et al*., 2002; Rico *et al*., 2017). *M. haemolytica* serotype A2 was more robust in its ability to resist nutrient deprivation for long periods. These survival mechanisms may have important implications for pathogenesis (Rowe *et al*., 2001).

Clinical signs

Pneumonic pasteurellosis is a disease that occurs mainly in animals with impaired lung defense mechanism. Sheep and goats contract the disease if they are exposed to physical stress or unfavourable environmental conditions (Mohamed and Abdelsalam, 2008). A wide variety of clinical signs, ranging from sudden death to occasional coughing, may occur in sheep affected with pneumonic pasteurellosis (Afata, 2018). Clinical manifestations 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. In acute outbreaks, the clinical course of the disease is relatively short (2 to 3 days) terminating in

death or recovery in either treated or non-treated animals (Mohamed and Abdelsalam, 2008).

Infected animals appear extremely dull with reduced appetite and remarkable depression, high fever, coughing, dyspnea, muco-purulent nasal discharge, and anorexia that commonly develops when the immune system of the animal is compromised by stress factors such as crowding, transportation, draught, and unfavorable weather (Legesse *et al*., 2018). Later, a productive cough, usually develops in most infected animals, accentuated by physical effort or movement. Marked dyspnoea with an expiratory grunt may be observed in advanced stages of the disease (Mohamed and Abdelsalam, 2008).

Pneumonic pasteurellosiscan cause an acute febrile course with severe fibrinous or fibrinopurulent bronchopneumonia, fibrinous pleurisy and septicaemia. Infected animals may die in a few days since a beginning of clinical signs, but those who survive an acute attack can become chronically infected. Infected sheep and goats develop high fever with clinical evidence of severe respiratory impairment manifested by dyspnoea, foam in the mouth, cough and runny nose. Young animals are more susceptible than adults and develop more severe infection in which sudden death may occur with or without any previous warning clinical signs (Mohamed and Abdelsalam, 2008). Postmortem findings include ventral consolidation in the cranial lobes of the lungs and fibrinous pleural and pericardial effusions (Quinn *et al*., 2002).

Economic significance of pneumonic pasteurellosis

Goats have a significant role in Ethiopian livestock economy due to their remarkable adaptability to adverse environments. Together with sheep, they supply more than 30% of all domestic meat consumption, and generate income from exports of live animal, meat and skin (Asresie and Zemedu, 2015). But, disease constraints like respiratory diseases contribute to the great financial losses and the socio-economic development of poor farmers (Dereje *et al*., 2014). Pasteurellosis is one of the most common disease of ruminants that causes high mortality and morbidity, treatment costs, reduced weight gain, delayed marketing and unthriftiness among survivors of the flock (Kumar *et al*., 2015). Diseases causing respiratory problems in sheep have been known of great economic impact in the central highlands of Ethiopia with frequent records of outbreaks and mortalities (Legesse *et al*., 2018). The morbidity of pneumonic pasteurellosis may reach 35%, and the case fatality rate my range from 5-10% in small ruminants (Belege *et al*., 2017).

The severity of the disease is 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 (Mohamed and Abdelsalam, 2008). Prevalence of diseases and the resulting high mortality and morbidity rates are the major problems in Ethiopia (Gizaw *et al*., 2010). It is devastating particularly in young animals. It is a common cause of high morbidity and mortality in kids, especially who have not received enough colostrum. The disease occur more often in animals that have experienced recent stress such as transportation, weaning, or commingling with animals from unrelated farms (Assefa and Kelkay, 2018).

Status of small ruminant pneumonic pasteurellosis in Ethiopia

Several studies have been conducted in Ethiopia to determine the extent of the problem and the relative distribution of different biotypes and serotypes of pasturellae species (Afata, 2018). The prevalence of pneumonic pasteurellosis in ruminants is found to be high and eleven of the known 17 serotypes of *M. haemolytica*, *M. glucosidal* and *B. trehalosi* has so far been isolated and identified in ovine in central, northeastern and southeastern high lands of Ethiopia (Belege *et al*., 2017), as indicated in Table 1 below. An outbreak of contagious acute respiratory disease of sheep and goats has occurred in Milae district of Afar region. 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. *M. haemolytica* biotype T was isolated from nasal swabs, lung and pleural fluid of sheep and goats. *M. haemolytica* serotype A1 and A2 are the most common in the country. The studies indicated that pneumonic pasteurellosis is a major threat in the highlands and in the lowland hot and humid areas with high death and illness to domestic ruminant production (Belege *et al*., 2017).

Diagnostic techniques

Small ruminant based economy can be viable and sustainable through the use of techniques for early and accurate diagnosis. Potential losses can be minimized by

using sound and proper diagnostic approach (Chakraborty *et al*., 2014). Accurate diagnosis of pneumonia is difficult and usually involves history of exposure to stressors, physical examination and identification of the etiological agent, *Pasteurella* species (Kumar *et al*., 2015; Mekibib *et al*., 2019).

Suitable specimens for laboratory examination from live animals include tracheobronchial aspirates, nasal swabs or mastitic milk. Specimens should be cultured on blood agar and MacConkey agar (Quinn *et al*., 2002). Typing can be accomplished by different approaches. Broadly, phenotypic and genotypic based typing methods are available but any typing method must have high differentiation power. It has to clearly differentiate unrelated strains and to demonstrate the relationship of organisms isolated from individuals infected from the same source. And also it should have reproducibility, the ability of a technique to yield the same result when a particular strain is repeatedly tested (Olive and Bean, 1999). Conventional phenotyping methods are routinely used for primary identification of Pasteurellaceae from clinical pneumonic samples (Catry, 2005).The conventional method of identification of a suspected isolate as *P. multocida* or *M. haemolytica* involves subjecting the isolate to a range of biochemical tests (Miflin and Blackall, 2001).

Culture methods

All of the *Pasteurella* species can be isolated by culturing appropriate clinical specimens on blood agar and *Pasteurellae* and *Mannheimia* species can be distinguished by colonial and growth characteristics (Quinn *et al*., 2002; Afata, 2018). *P.multocida* colonies are round, greyish, shiny and non-haemolytic on blood agar. Colonies of some pathogenic strains are mucoid due to the production of thick capsules of hyaluronic acid. The colonies have a subtle but characteristic sweetish odour.

All *P. multocida* are gram-negative, coccobacillary and did not grow on MacConkey agar. Whereas; *M. haemolytica* and *P. trehalosi* colonies are haemolytic and able to grow on MacConkey agar and they are odourless (Quinn *et al*., 2002; Alemneh and Tewodros, 2016). Their growth on artificial media is enhanced by the addition of serum or blood on which they appear after 24 hours of incubation as round, smooth, greyish colonies of moderate size (1-2 mm in diameter) (Miflin and Blackall, 2001). Up on Gram's staining they are Gram negative, small in size, pleomorphic coccobacilli or short rod in shape and often exhibiting bi-polar staining (Catry, 2005).

Biochemical tests

Subjecting the isolate to a range of biochemical tests allow for the identification of a suspect isolate (Miflin and Blackall, 2001). *Pasteurellae* and *Mannheimia* species can be distinguished by biochemical reactions. Strains of *P. multocida* can be differentiated by serotyping and biotyping, whereas *M. haemolytica* and *B. trehalosi* strains are differentiated by serotyping (Quinn *et al*., 2002).

M.haemolytica are non-motile and non-spore forming, fermentative with few exceptions; ferment sugars like glucose, sucrose and maltose and, most of them produce acid from common sugar but not H_2S gas. They are facultative anaerobic with fastidious growth requirements (Quinn *et al*., 2002).They are positive for oxidase, catalase and lactose and negative for urease biochemical tests (Miflin and Blackall, 2001). *P. multocida* in the biochemical test are characterized by indole formation, catalase, oxidase and glucose positive, but lactose negative (Hawari *et al*., 2008). Whereas, *B. trehalosi*are oxidase and trehalose positive but catalasenegative (Blackall *et al*., 2007). Characteristics of those bacteria for their differentiation is presented in Table 2 below.

Serological methods

Serological tests are generally of little diagnostic value in the majority of the diseases caused by *Pasteurellae* and *Mannheimia* species (Quinn *et al*., 2002). The methods used for the detection of antibodies may include ELISA, complement fixation test (CF), and agglutination test. In addition to these assays, toxin-neutralization assays, leukotoxin-neutralization (LN) assays, can be used for certain bacteria that secrete toxins. Example: detection of antibodies to *M.haemolytica* leukotoxin. As with virus neutralization test for viral infections, an LN assay indicates a functional antibody that interferes with the toxin-induced cytolytic process (Fulton and Confer, 2012).

Molecular methods

Conventional isolation and phenotypic methods remain a gold standard in proper diagnosis, but recently molecular methods have proved beneficial to overcome some limitations of the conventional biochemical and

serological methods (Ahmed *et al*., 2017; Daphal *et al*., 2018). The limitations of phenotypically based typing methods led to the development of the microbial genotype based typing methods. This minimize problems with typeability, reproducibility and, it enable for establishment of large databases of characterized organisms (Olive and Bean, 1999).

Molecular methods improve sensitivity and rapidity; and for precise and reliable confirmation and characterization of organisms (Daphal *et al*., 2018). Several molecular based assays are used in veterinary diagnostic laboratories to detect pathogens. These technologies allow to rapidly identify bacteria without the requirements of additional time-consuming biochemical tests. But, nucleic acid-based assays are usually not broad spectrum as compared to culture of bacteria (Fulton and Confer, 2012).

The nomenclature or taxonomical position of most Pasteurellaceae is based the sequencing of 16S rRNA. But this require extended laboratory equipment and experienced man power. So currently most applied alternative approaches are species-specific polymerase chain reaction (PCR)(Olive and Bean, 1999; Aarts *et al*., 2001). PCR technology can be applied for rapid, sensitive and specific detection of Pasteurella species (Kumar *et al*., 2015). The first type of approach is species specific PCR that amplify unique DNA sequences. It has been successfully developed for the toxA gene, psl gene and KMT1 region in *P. multocida*, and are used predominantly in clinical specimens for diagnostic purposes (Catry, 2005).

Recently several of such regions were combined to develop a multiplex capsular PCR typing system which is able to discriminate the five capsular types of *P. multocida* (Townsend *et al*., 2001).

Conventional PCR detection method is mostly used molecular technique for typing which rely on electrophoretic separation of DNA fragments based on different molecular lengths. The result is represented by a pattern of bands on a gel (Olive and Bean, 1999).

PCR assays are important for amplification of specific capsular and virulence genes of *P. multocida* and *M. haemolytica*. It is a preferred method to conventional bacteriological methods for faster analysis of infectious diseases (Hawari *et al*., 2008; Kumar *et al*., 2015). A known genetic region is amplified in a thermocycler to produce an amplified segment of nucleic acid. Those products are then compared to known positive controls by gel electrophoresis or sequenced and compared to published sequence for the specific agent. These gel-based PCR assays are qualitative, indicating only presence or absence of visualized product of the amplification (Fulton and Confer, 2012).

Multiplex PCR test is an advancement of PCR testing which can detect multiple bacteria with one test. This considerably reduce costs to the veterinarian compared to the one PCR assay. Multiplex PCR (mPCR) tests have been attempted for veterinary medicine, mostly for research purposes including typing of *P. multocida* and other bacterial and viral pathogens of animals (Fulton and Confer, 2012). This assay containing many pairs of primers can specifically amplify serotype specific genetic targets for different pathogens and target serotypes can be indicated by the amplicon sizes in the gel electrophoresis images. This technique has the potential to produce considerable savings in time and effort with in the laboratory without compromising on the utility of the experiment (Kumar *et al*., 2015).

Development of a multiplex PCR assay for capsular serogroup identification was performed following sequence determination and analysis of *P. multocida* capsular sero-group-specific regions, and then a multiplex PCR assay was developed that contained *P. multocida*-specific primers. Primer sets specific for serogroups A, B, D, E, and F (Townsend *et al*., 2001).

Species specific primers are used for Pasteurellae species detection. Molecular detection of *P. multocida* is possible by detection of the gene target KMT1 in *P. multocida*; PHSSA and Rpt2 in *M. hemolytica*; and LktA in *B. trehalosi* (Davies *et al*., 2001; Townsend *et al*., 2001; Hanthorn *et al*., 2014; Kumar *et al*., 2015).

Prevention and control methods

Pneumonic pasteurellosis is one of the priority diseases that deserve control. However, control of pneumonic pasteurellosis is difficult task that requires integration of various techniques (Disassa *et al*., 2013). Antimicrobial drugs were the most powerful tools to control such infections. But, extensive use of antimicrobials caused an increase in the incidence of drug resistant which reduce the efficacy of the antimicrobial agents used to control *Pasteurella* and *Mannheimia* infections (Kehrenberg *et al*., 2001; Legesse *et al*., 2018).

Table.1 Prevalence of ovine pasteurellosis, common isolates and identified serotypes in Ethiopia

Study site	Seroprevalence (%)	Bacteriological prevalence in pneumonic lung (%)	Identified serotype
Methara	47.5	-	A1*, A2, A5, A6*, A7*, A8*, A9, A11, A13, A14, T3, T4, T10, T15
North showa	62.7	-	A1*, A2, A5, A6*, A7+, A8*, A9, A11, A13, A14, T3, T4, T10, T15
Wollo	83	60	A1*, A2, A5, A6*, A7*, A8*, A11, A12, T3, T4, T10, T15
Arsi	56	56	A1, A2*, A5, A7*, A8*, A9, A12, A13, T13*, T15

*= most common serotypes serologically, += most common isolates from pneumonic lung, A = *M. haemolytica* biotype A, T = *M. haemolytica* biotype T. Source: (Belege *et al*., 2017).

Table.2 Differentiation of the main pathogenic *Pasteurella* and *Mannheimia* species

Feature	*M. haemolytica*	*P. multocida*	*B. trehalosi*
Haemolysis on sheep blood agar	+	-	+
Growth on MacConkeyagar	+	-	+
Distrnctiveodour from colonies	-	+	-
Motility SIM	-	-	-
lndole production	-	+	-
Catalase activity	+	+	-
Oxidase	+	+	+
Urease activity	-	-	-
Ornithine decarboxylase activity	-	+	-
Oxidative fermentative	Fermentative	Fermentative	Fermentative
Acid production from:			
Lactose	+	-	-
Sucrose	+	+	+
D-trehalose	-	-	+
L-arabinose	+	-	-
Maltose	+	-	+
D-xylose	+	V	-

+ = most strains positive; - = most strains negative; V = variable reactions. Source: (Quinn *et al*., 2002).

The control of infections with *Pasteurella* and *Mannheimia* species are difficult for two reasons: in most of the cases, the *Pasteurella* and *Mannheimia* isolates are not the only causative agents involved and now a days they are becoming resistant to antimicrobial agents available. Because of the rapid spread of resistance, the antimicrobial sensitivity of the *Pasteurella* and *Mannheimia* isolates should be tested and a suitable antibiotic should be chosen on the basis of the *in vitro* sensitivity test (Kehrenberg *et al*., 2001). And also, these bacteria are part of the normal microbiota in the upper respiratory tract, making the disease difficult to prevent (Kehrenberg *et al*., 2001; Catry, 2005).

Proper management

The most effective preventive method is proper management and avoidance of stress. No single management practice has been effective in controlling the disease. Management practices which reduce stress,

as well as early diagnosis and antibiotic treatment, are the key approaches of controlling disease (Afata, 2018). They are mainly influenced by a wide variety of environmental and management risk factors. Thus, the reduction or even elimination of predisposing factors is of major importance (Kehrenberg *et al*., 2001).

Control and prevention of pneumonic pasteurellosis can be done through vaccination, antimicrobial treatment for infected animals and implementation of a biosecurity plan (Afata, 2018). However, farmers are not fully aware to take animals to veterinary vaccination and treatment center to the affected ones as they considered health management as too expensive and distance due to topographical problems to veterinary delivery services (Welay *et al*., 2018).

Chemotherapy

Antimicrobials are still the tool of choice for prevention and control of infections due to *Pasteurella* and *Mannheimia* (Kehrenberg *et al*., 2001). Antibiotics are widely used to treat infectious diseases in both humans and animals, but the emergence of antibiotic resistance in previously susceptible bacterial populations is a very serious threat and now a major public health issue (Aarts *et al*., 2001). Long-acting oxy tetracycline is usually effective for treatment and may be used prophylactically for in-contact sheep and goats (Quinn *et al*., 2002). However, imprudent use of antimicrobials bears a high risk of selecting resistant bacteria, promoting the spread of resistance genes located on plasmids and transposons, and consequently, reducing the efficacy of the antimicrobial agents currently available for the treatment of food-producing animals (Kehrenberg *et al*., 2001).

Vaccination

Vaccination is the best control method of the disease and it is an alternative, non-antibiotic prophylaxis strategy (Abdullah *et al*., 2015). Use of vaccine is the most economic and feasible control method for developing nations (Disassa *et al*., 2013; Mitku *et al*., 2017). 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 (Belege *et al*., 2017). Eleven of the 17 known serotypes of *M. haemolytica*, *M. glucosida* and *B. trehalosi* have so far been isolated and identified in Ethiopia. However, they were not considered with respect to vaccination and vaccine preparation (Sisay and Zerihun, 2003).

It is clear from this review and previous literatures that pneumonic pasteurellosis is a multifactorial disease and it is a common respiratory disease of all ages of sheep and goats in Ethiopia. It causes heavy losses and poses serious hazard in small ruminant production. Control and prevention of pneumonic pasteurellosis is mandatory to increase their contribution to the development of the country. Vaccination, antimicrobial therapy and proper management practices are important measures to reduce its negative impact in the society. But now a day's prevention of a disease through vaccination become difficult due to the detection and presence of multiple serotypes circulating in the country without cross-protection. And antimicrobial drugs which were the most powerful tools to control and prevent bacterial infections become unsuccessful due to development of resistance to antimicrobial agents available in the market. Therefore, proper management required to reduce psychological and physical stressors, sound diagnostic methods needed for proper antimicrobial therapy to reduce development of drug resistance, and the available circulating serotypes of the disease agent in the country should be considered in vaccine production.

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