American-Eurasian Journal of Scientific Research 14 (4): 64-76, 2019
ISSN 1818-6785

DOI: 10.5829/idosi.aejsr.2019.64.76

Ovine Pneumonic Pasteurellosis: A Review

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Absract: Sheep constitutes a significant proportion of the Ethiopian livestock industry, with the total population estimated at 26.1 million. Raising of sheep ruminants had more comparative advantage than raising of other larger species. However, the productivity of sheep is unsatisfactory largely due to diseases and poor animal management practices. Pasteurellosis is one of the important infectious diseases of sheep responsible for the low productivity and the associated economic loss resulting from death, reduced live weight, delayed marketing, treatment cost and unthriftness among survivors. Bacterial pneumonia is most often caused by *Mannheimia haemolytica* and *Pasteurella multocida* which are more frequently associated with the outbreak of acute pneumonia and death of sheep in all age groups. Clinically, it is an acute infectious disease that develops when the immune system of the animal is compromised by stress factors such as crowding, transportation, draughtand inclement weather. *M. haemolytica* and *P. multocida* constitute the most important members of the family *Pasteurellacae* commonly occurring as commensal microflora of the naso-pharyngeal of ruminants that are involved in causing respiratory infections in ruminants when body defense mechanisms are impaired. In Ethiopia, a monovalent vaccine (Inactivated *P. multocida* biotype A) produced at the National Veterinary Institute (Ethiopia). But, studies in central highlands of Ethiopia indicate *M. haemolytica* serotype A2 and A7 were reported to occur at high frequency. This may suggest the need for the development of a multivalent vaccine using the most prevalent serotypes.

Key words: Ethiopia · *Mannheimia haemolytica* · *Pasteurella multocida* · Molecular characterization · sheep

INTRODUCTION

Sheep constitutes a significant proportion of the Ethiopian livestock industry, with the total population estimated at 26.1 million [1]. Raising of sheep ruminants had more comparative advantage than raising of other larger species. Owing to their high fertility/fecundity and short generation interval making them a source of income in relatively short period as well as an investment venture. However, the productivity of sheep is unsatisfactory largely due to diseases and poor animal management practices. Diseases and poor management practices are known to be the major factors that largely contribute to the low productivity of small ruminants [2].

Pasteurellosis is one of the important infectious diseases of sheep responsible for the low productivity and the associated economic loss resulting from death, reduced live weight, delayed marketing, treatment cost and unthriftness among survivors [3]. Pneumonic pasteurellosis is extremely common in sheep and can be responsible for enormous financial losses worldwide. The condition usually appears when sheep are exposed to combinations of predisposing factors such as adverse physical condition, physiological stress, bacterial and viral infections. As the exact nature of these combinations is unknown, much remain to be understood about why the disease occurs in the way it does [4].

Bacterial pneumonia is most often caused by *Mannheimia haemolytica* and *Pasteurella multocida* which are more frequently associated with the outbreak of acute pneumonia and death of sheep in all age groups [5]. Clinically, it is an acute infectious disease that develops when the immune system of the animal is compromised by stress factors such as crowding, transportation, draught and inclement weather [6].

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M. haemolytica, formerly known as *Pasteurella haemolytica* and *P. multocida* constitute the most important members of the family *Pasteurellacae* commonly occurs as commensal microflora of the naso-pharyngeal of ruminants that are involved in causing respiratory infections in ruminants when body defense mechanisms are impaired [7]. Both are known to be bacterial pathogens associated with severe respiratory diseases of sheep and cattle [8]. *P. multocida*, alone or in association with other pathogens can cause shipping fever in cattle and sheep, which may also be caused by *Mannheimia haemolytica*, in the absence of *P. multocida*. However, *Mannheimia haemolytica*, is the bacterium most frequently isolated from cases of shipping fever in sheep and goats worldwide [8, 9] and is the most important bacterial pathogen considered as the cause of ovine pasteurellosis [10, 11].

M. haemolytica exists in two biotypes, A and T, which are further divided into serotypes based on their surface antigen. Type A comprises A1, A2, A5, A6, A7, A8, A9, A10, A11, A12, A13, A14and A16 while type T comprises T3, T4, T10and T15. Biotype A is particularly associated with pneumonic pasteurellosis in sheep, whereas biotype T causes systematic pasteurellosis in lambs. *Mannheimia haemolytica* is one of the infectious agents most frequently associated with pathologic damage of ovine respiratory tract that causes fibrinous and necrotizing lobar pneumonia and pleuropneumonia [12].

Since pneumonic pasteurellosis is one of the serious problems of small ruminants, effective control and prevention of the disease is mandatory. The traditional therapy based on the extensive use of antibiotics, including mass medication of animals, has caused an increase in the incidence of multi-drug resistant *M. haemolytica* strains in many parts of the world [6, 13]. Hence, an alternative prophylactic strategy through vaccination is more desirable.

In Ethiopia, a monovalent vaccine (Inactivated *P. multocida* biotype A) produced at the National Veterinary Institute (Ethiopia) is being used for vaccination against ovine pasteurellosis although studies in central highlands of Ethiopia indicate *M. haemolytica* serotype A2 and A7 were reported to occur at high frequency [14, 15]. Although there is no published report on the efficacy of the currently used vaccine under field conditions, customer complaints on the high rates of mortality and morbidity following respiratory distress in different parts of the country have been documented despite the annual vaccination.

Several studies have been conducted in Ethiopia to determine the extent of the problem and the relative distribution of the different biotypes and serotypes of *M. Haemolytica*. The studies indicated that pasteurellosis is a major threat to sheep production and most serotypes of *M. haemolytica* biotype A are involved in pneumonic pasteurellosis with serotype A2 being the most prevalent [14, 16, 17]. Therefore, the aim of this paper is to review ovine pneumonic pasteurellosis caused by *M. haemolytica* and *P. multocida*.

Ovine Pasteurellosis: Pasteurellosis is a devastating condition affecting sheep of all ages. The predominant organism that causes disease in sheep in tropical climate is *M. haemolytica* and *P. multocida* [18]. It is one of the most common causes of mortality in all age's group of sheep and most often associated with stress [19]. The disease is of considerable economic significance and is responsible for a high mortality rate and substantial treatment costs [20].

The causative organism resides in the nacho-pharynx and tonsils of apparently healthy sheep. Predisposing factors include climatic changes and stressful conditions like transport, dipping and shearing. The prevalence of the disease can be reduced by proper management and by vaccination [21].

Etiology: *Mannheimia haemolytica* and *Pasteurella multocida* are involved mostly as etiological agents of the disease, which are commensally resident in the upper respiratory tract of healthy ruminants [22]. *Mannheimia haemolytica and Pasteurella multocida* are aerobic, non-motile, non-spore-forming, bipolar, gram-negative rods, associated with pneumonia and septicemia in all ruminants [23].

***Pasteurella multocida*:** *Pasteurella multocida*, as one of the main etiological agents of pneumonic pasteurellosis, leads to the production of a number of proteins and polysaccharides, which are thought to contribute to its virulence [24]. *Pasteurella multocida* is an important animal pathogen of the family Pasteurellaceae that pose serious hazard in livestock industry. *P. multocida* is a commensal of upper respiratory tract of cattle, sheep and goat [25]. The pathogenic role of *P. multocida*is more evident in sheep in which it is responsible for many serious of outbreaks [26]. Distinct serotype associations with specific host species are noted and some serotypes have also distinct disease syndrome. Thus, pneumonic pasteurellosis in sheep caused mainly by *P. multocida* are

serotype group A and D, while hemorrhagic septicemia in cattle and buffalo are caused by *P. multocida* serotypes B: 2 or E: 2 [21].

Pasteurella multocida is a small, gram-negative, non-motile, non–spore-forming coccobacillus with bipolar staining features. The bacteria typically appear as single bacilli on Gram stain; however, pairs and short chains can also be seen [27]. It grows on most laboratory media with the exception of bile containing media such as MacConkey agar [28]. It is oxidase and catalase positive and can ferment various carbohydrates. *Pasteurella multocida* has 16 serotypes using lipopolysaccharide antigens as tested by a gel diffusion precipitation test, although it has five serogroups (A, B, D, E, F) using capsular antigens as tested by a passive hemagglutination test [29, 30].

There are many virulence factors contributing to the pathogenesis of *P. multocida*. Currently known virulence factors include genes involved in the formation of the capsule, lipopolysaccharide (LPS), fimbriae and adhesions, toxins, iron regulated and iron acquisition proteins, sialic acid metabolism, hyaluronidase and outer membrane proteins (OMPs) [31]. The two key surface components, capsule and LPS, form the main typing basis of *P. multocida* [32].

Pasteurella multocida strains have been characterized by outer membrane protein (OMP) typing and 16S rRNA-typing. 16S rRNA typing revealed that the majority of clinical isolates belong to a single lineage containing seven 16S-types. However, a range of capsular types, OMP-types and host species were represented, indicating significant heterogeneity between closely related strains [33]. Currently three subspecies of *P. multocida* are recognized [34] and reported as; *P. multocida* subspecies multocida recovered from domestic animals, *P. multocida* subspecies septica from (Dog, cat and birds) and *P. multocida* subspecies gallicida from birds.

***Mannheimia haemolytica*:** *Mannheimia haemolytica* is an important animal pathogen associated with pneumonic pasteurellosis. *Mannheimia haemolytica* (Formerly named *Pasteurella haemolytica*) is commonly isolated from the lungs of cattle with pneumonia [35].

Mannheimia haemolytica, which is a normal flora of the upper respiratory tract, may play a secondary role after the primary initiating agent suppressed the host defense mechanism and favors the multiplication of *Pasteurella* species leading to bronchopneumonia in purely pneumonic animal. *Mannheimia haemolytica* is the major cause of pasteurellosis among the three species even in Ethiopia as reported byAbera *et al.* [36]. *M. haemolytica* is the major causative agent involved in ovine pneumonic pasteurellosis as studied from nasal swab of pneumonic sheep from Haramaya Veterinary Clinic and counterpart of pneumonic lungs from the slaughtered non-pneumonic sheep [8].

Based on number of characteristics such as antigenic nature, pathogenicity and biochemical activity; *Pasteurella haemolytica* can be grouped in to two biotypes (A and T). Biotype A ferments arabinose, whereas biotype T ferments treehouse. Similarly, based on genomic characteristics, biological features and analysis of phenotypic content biotype T is reclassified (Named) as *P. trehalosi* and biotype A as *M. haemolytica*. These biotypes are further subdivided into 13 A serotypes (A1, A2, A5, A6, A7, A8, A9, A11, A12, A13, A14, A16 and A17) and 4T capsular serotypes (Serotypes 3, 4, 10 and 15), based on results from passive hemagglutination test. After years *P. haemolytica* biotype A is allocated to new genus and renamed as *M. haemolytica* while the T serotype as *B. trehalosi* [37].

The T serotypes are T3, T4, T10, T15 and serotype A except the 11A which is considered as *M. glucosida*, all A12 serotypes are (A1, A2, A5-A9, A12-14, A16 and A17) based on capsular antigen typing [38] categorized as *M. haemolytica*. 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. Moreover, *M. haemolytica* serotype 7 was also reported to cause acute outbreaks in sheep. 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 [39].

Predisposing Factors of Ovine Pasteurellosis

Environmental Factors: *Mannheimia haemolytica* and *P. multocida* occur as commensals in the upper respiratory and alimentary tracts of their various hosts. Although varieties of some species cause primary disease, many of the infections are secondary to other infections or result from various environmental stresses [28, 40]. The effects of different environmental stressors are believed to be important components of risk factors for pasteurellosis in many domestic ruminants. Although the effects of stressors are difficult to measure, some

indicators including increased body temperature, heart rate, respiratory rate and plasma cortisol have been correlated with disease. The disease appears to occur most often in animals that have undergone recent stresses such as transportation, weaning, or commingling with animals from unrelated farms [41].

Physiological responses to stress (Collectively called stress) include suppression of the immune system; consequently, prolonged stress may increase susceptibility to pathogens and to morbidity and mortality [42]. Environmental stresses most commonly associated with pneumonic pasteurellosis in livestock include heat, cold, wind, chill, humid, crowding, mixing with new animals; poor ventilation barn, handling, transport and deprivation from feed and water. Other predisposing factors, such as lack of sufficient energy or protein, inadequate colostrum consumption, specific vitamins, or certain minerals, also may compromise immunity further [43].

Agent Factors: Bacterial species incriminated in causing pneumonic pasteurellosis are generally extracellular parasites that elicit mainly a humoral immune response. Several virulence factors have been identified both for *P. multocida* and *M. haemolytica* and these virulence factors influence the outcome of bacteria-host interactions [28]. Among the major virulence components of *M. haemolytica* and *P. multocida* are the polysaccharide capsule, OMP, LPS, fimbriae, adhesins, extracellular enzymes and other factors that are still to be investigated and elucidated [44].

Pneumonic pasteurellosis has large part of the 12A serotypes of *M. haemolytica* described; A1 and A2 are established worldwide. Both A1 and A2 possess the ability to colonize the upper respiratory tract of cattle and sheep, they are however often species specific. Serotype A1 causes pasteurellosis in cattle and has been the subject of extensive study, while serotype A2 causes disease in sheep and is less-well characterized [38].

Host Factors: Disease progression is thought to depend on a complex interaction of host factors including species, age, breed and immune status and strain-dependent virulence factors of the agent such as production of toxins, adhesins and mechanisms for acquiring nutrients from the host [33]. Bronchopneumonia caused by *P. multocida* or *M. haemolytica* has a cranioventral lung distribution and affects all ages of animals worldwide. It can be particularly devastating in young animals. It is a common cause of morbidity and mortality in young, especially in those that have not received adequate colostrum or in which passive colostral immunity is waning [45]. Affected animals often die if not treated. The reasons for increased susceptibility to *M. haemolytica* infection in stressed animals are primarily attributed to the breakdown of innate pulmonary immune barriers by stress [43, 46].

Concurrent Infection: *P. multocida* and *M. haemolytica* are commensals of the upper respiratory tract that can cause pneumonia either alone or in conjunction with other organisms. Primary infections with respiratory pathogens such as parainfluenza type 3, adenovirus, respiratory syncytial virus, *Bordetellaparapertussis*, or in particular *Mycoplasma ovipneumoniae* appear to predispose to secondary infection with *Pasteurella* and *Mannheimia*. The combined infection with certain respiratory viruses is commonly found to increase the susceptibility of sheep to secondary bacterial pneumonia's [47].

The most important viruses associated with acute respiratory tract infections in sheep include Para-influenza virus type 3, Respiratory syncytial virus, Ovine herpes virus type 1 and 2, Pest des petits ruminants and Ovine adenovirus. Chronic viral respiratory disease caused by Maedi-visna and ovine pulmonary adenocarcinoma. Respiratory viral infections affect mucociliary clearance mechanisms in lungs for removing the pathogens that reach the lower respiratory tract and thus increase the susceptibility of sheep to secondary bacterial infections. The respiratory viral agents create a favorable environment in the lungs supporting the bacterial growth by interfering with the mucociliary clearance mechanism of the respiratory tract and by down regulating the phagocytosis by the pulmonary macrophages as cited by Rawat *et al*. [5].

Parasitic pneumonia caused lung worms like *Dictyocaulusfilaria*, *Protospronylusrufenscens* and *Mycotic pneumonia* caused by aspergillus species and foreign bodies in the upper respiratory tract, aspiration pneumonia also differential diagnosis [48].

The deleterious effect of certain *Mycoplasma* species on the respiratory system of ruminant animal has long been recognized. The most important examples of these pathogens include *M. ovipneumoniae* [49]. Most of these *Mycoplasma* species are known to contribute to the development of severe pneumonic lesions either alone or in association with pneumonic pasteurellosis of animals [50]. The synergistic role of some other bacterial

organisms in this connection was also evident. Furthermore, a number of other unrelated conditions such as twin pregnancy, selenium deficiency, mycotoxins and 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 [18].

Mode of Transmission: Transmission of agents of pneumonic pasteurellosis probably occurs by inhalation of infected droplet, coughed up or exhaled from infected animals which may be clinical case or recovered carriers in which the infection persist in the upper respiratory tract [51]. *Pasteurella multocida*and *Mannheimia haemolytica* are highly susceptible to environmental influences and it is unlikely that mediated contagion is an important factor in the spread of the disease. When conditions are optimal, particularly when sheep are closely confined in inadequately ventilated trains or held for long periods in holding pens and feed lots, the disease may spread very quickly and affect high proportion of the herd within short hours. Animals at pasture are able to move freely and the rate of spread may be slower [22].

Pathogenesis: Stress appears to be an important factor that allows *Pasteurella*, *Mannheimia*, *Mycoplasma ovipneumoniae*, other bacteriaand viruses to multiply and impair the normal physical defense mechanisms, facilitating invasion of lung tissue and development of pneumonia. In sheep, alveolar macrophage function is impaired after viral pneumonia which results in decreased clearance of inhaled bacterial pathogens, allowing them to become established [52].

The virulence of *M. haemolytica* and *P. multocida* is mediated by the action of several factors, including endotoxin, leukotoxin and capsular polysaccharide, that afford the bacteria advantages over host immunity. The leukotoxin is particularly important in the pathogenesis, because it is specifically toxic to ruminant leukocytes, resulting in fibrin deposition in lungs and on pleural surfaces. The lipopolysaccharide endotoxin contributes to adverse reactions in the lungs and also leads to systemic circulatory failure and shock. The capsular polysaccharide prevents the phagocytosis of the bacteria and assists in attachment to the alveolar epithelial surface [53].

Pathogen-host interactions result in tissue damage, especially because of massive influx of neutrophils. As these neutrophils are lysed, enzymes are released that cause more lung tissue damage. Survival of the acute phase of pneumonic pasteurellosis depends on the extent of lung involvement and damage in the lower respiratory tract. Sheep that recover may have chronic respiratory problems, including reduced lung capacity and weight gain efficiency if ≥ 20% of the lung was damaged [52].

Clinical Findings: Acute respiratory disease caused by *M haemolytica* is uncommon in adult sheep, unless there is a predisposing problem such as ovine pulmonary adenocarcinoma or other viral infection. Clinical signs include acute onset depression, lethargyand inappetance and are consistent with profound endotoxemia. Sudden death may occur without clinical signs having been observed. Affected sheep are typically separated from the remainder of the flock and are easily caught and restrained. On approach, they may show an increased respiratory rate with an abdominal component. Affected sheep are typically febrile (>40.5°C [104.9°F]). The mucous membranes are congested and there may be evidence of dehydration with sunken eyes and extended skin tent duration. Auscultation often does not reveal significant changes other than an increased respiratory rate. Rumen contractions are reduced or absent. There may be evidence of diarrhea. Frothy fluid may be noted around the mouth during the terminal stages [52].

Diagnosis

Clinical Signs: Pneumonic pasteurellosis is an important disease caused by *Pasteurella multocida* and *Mannheimia haemolytica*in sheep. *M. haemolytica* causes two main diseases in sheep and cattle: pneumonic pasteurellosis and systemic pasteurellosis (Enzootic septicemia). A wide variety of clinical signs, ranging from sudden death to occasional coughing, may occur in sheep affected with pneumonic pasteurellosis and it is frequently fatal [54].

Acute pneumonia caused by *M. haemolytica* is uncommon in adult sheep, unless there is a predisposing problem such as ovine pulmonary adenocarcinoma or other viral infection. Affected sheep often appear depressed, lethargy with a nasal discharge, exhibit inappetance and weight loss, have high temperatures (40.4°C-42°C) and are consistent with profound endotoxemia [22]. Most cases occur during the first two weeks after transportation and the course of disease can be rapid with death occurring before the above clinical signs of disease are observed.

Postmortem Lesions: There is marked pulmonary consolidation, usually involving at least the antero-ventral part of the lungs. The lung is firm and the cut surface usually reveals an irregular, variegated pattern of red, white and gray tissue due to hemorrhage and necrosis. Occasionally sequestrate of necrotic lung tissue are found. *Pasteurella multocida* causes fibrino-purulent bronchopneumonia without the multifocal coagulation hemolytic necrosis that characteristics of fibrinous lobar pneumonia associated with *M. haemolytica*. The basic postmortem lesions are acute fibrin hemorrhagic pneumonia with pleurisy adhesion [5].

The most obvious changes in affected animals are the edema, widely distributed hemorrhages and general hyperemia. In most cases, there is an edematous swelling of the head, neck and brisket region. Incision of the swellings reveals a clear or straw-colored serous fluid. The edema is also found in the musculature and the sub serous petechial hemorrhages, which are found throughout the animal, are particularly characteristic. Blood-tinged fluid is often found in the pericardial sac and in the thoracic and abdominal cavities. Petechial hemorrhages are particularly prominent in the pharyngeal and cervical lymph node [55].

Histologically the characteristic lung lesion is acute inflammation and emboli in small arterioles and capillaries [55]. Generally the diagnosis depends on the history of age, recent movement, weaning or housing, isolation and identification of the causative agent [56].

Isolation and Characterization of the Agents

Bacterial Culture and Species Identification: In spite of the molecular advances, morphology and phenotyping are routinely used for primary identification of Pasteurellaceae from clinical pneumonic samples or postmortem lesions. All of the *Pasteurella* species can be isolated by culturing appropriate clinical specimens on blood agar. *Pasteurella multocida* will grow at 37°C on blood or chocolate agar. Colonies are smooth, gray and non-hemolytic after 24 hours of incubation. Colonies have a characteristic chemical odor ("Mousy" odor) on protein containing media (Tryptone broth) due to large amounts of indole produced from the amino acid and it does not grow on enteric selective media like MacConkey agar [27].

Mannheimia haemolytica is odorless (Indole negative), hemolytic, but grow on MacConkey agar unlike *P. multocida*. They 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 aerobic or facultative anaerobic with fastidious growth requirements [26]. They are oxidase and catalase positives, reduces NO_3 to NO_2 and urease negative. 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) [57]. 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 [44].

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 [57]. They can also ferment large number of carbohydrates in anaerobic conditions. Most of them produce acid from common sugars except lactose [58]. All strains of *M. haemolytica* ferment Mannitol, Glucose, Maltose, Sorbitol and sucrose without gas production. Indole, Urease, Methyle blue and VP reaction are negative. Catalase and oxidase reaction are positive. Typically they don't to ferment trehalose but ferment L-arabinose [59].

Table 1: Summary of Culture characteristic and Biochemical tests used as reference for isolation and identification of Pasteurellacae organisms

Features	*M. haemolytica*	*P. multocida*
Haemolysis	+	-
MacConkey	+	No growth
TSI	+	+
Catalase	+	+
Indole (SIM)	-	+
Odor	-	+
Arabinose	+	-
Trehalose	-	-
Maltose	V	+
Oxidase	+	+
Glucose	+	+
Sucrose	+	+
Lactose	+	v
H_2S production	-	-
Motility(SIM)	-	-
Urease	-	-

+ = indicates positive reaction, - = indicates negative reaction V= variable
Source: [26]

Serological Technique: Serotype differentiation is based on sugar composition of the capsule as well as the composition of LPS component of the cell membrane. Serotyping of *M. haemolytica* is based on extractable surface antigens. Serogroups of *P. multocida* is identified based on difference in capsular polysaccharide which is

further subdivided in to somatic types based on serological differences of their lipopolysaccharide. Many methods of OMP extractions were reported for use as antigen in serological detection and surveillance of infection as well as in preparation of vaccines [60]. Conventionally, the identification of the specific serotype is carried out using one or more serological methods. Most of these tests are used for capsular typing [61].

Indirect Hemagglutination Test (IHA): Is a sensitive, reliable and economical serodiagnostic method. It is extensively used for serotyping and detection of antibodies against *Pasteurella multocida* and *Mannheimia haemolytica* species [62]. It is used for capsular typing using sheep red blood cells coated with bacterial extracts. The test can be carried out in tubes or platesand is performed in two rows [61]. Capsular antigen is extracted from 18-24 hours culture of bacteria of known serotypes in tryptose soya broth which is inactivated in a water bath at 60°C for 1 hour. The culture is centrifuged at 2000 rpm for 10 minutes and resuspeneded with equal volume of PBS. Then centrifuged at 5500 (rpm) for 15 minutes. Finally the clear supernatant is collected into sterile test tubes, used as capsular extract antigen.

On the other hand fresh sheep blood is collected in Alsever's solution at proportion of 3:5 and the suspension is centrifuged at 2000 rpm for 10 minutes, washed twice with phosphate buffer saline (PBS) solution (3 times a total wash). For sensitization of the sheep red blood cells 100 µl of packed sheep red blood cell is added to 10 ml of capsular extract antigen and then 50 µl of glutaraldehyde is added and homogenized with gentle shaking and incubated for 1hour at 37°C. After incubation the suspension is centrifuged and washed twice with PBS solution. Finally the pellet is adjusted with PBS solution to give a 1% suspension of RBC. In V-bottomed micro-plates 90µl of PBS solution is added to first row (A) wells and 50µl to the rest wells of 96 well microliter plate. Ten µl of test sera is added to the first row, mix thoroughly and serially diluted by pipetting 50µl up to row H. Fifty micro liters of sensitized RBC is added to each well and incubated for one hour at 37 °C. Result is recorded based on complete or more than 50% agglutination seen in each well. The titer showing 1/40 dilution and above is taken as positive [14, 63].

Rapid slide/plate agglutination test: A single colony is mixed with a drop of saline on a slide, a drop of specific antiserum is added and the slide is warmed gently. A coarse, floccular agglutination appears within 30 seconds. Old cultures may give a fine, granular agglutination that takes longer to appear [60]. This is a specific, rapid plate agglutination procedure for serotyping *M. haemolytica*. The procedure did not require special antigen preparation and yielded essentially the same results as the indirect haemagglutination procedure. Simply a drop of antiserum (Approximately 10µl) is placed on a clean glass surfaceand then a small amount of *M. haemolytica* colony from blood agar is picked up on an inoculating needle and mixed with the serum. A strong positive reaction in the form of clumping and clearing occurs as the mixture is stirred with the needle. Negative reactions remain turbid [28].

Agar Gel Immunodiffusion Test: Are used for what is described as capsular as well as somatic typing depending on the antigens and antisera used. Somatic typing by agar gel immune diffusion tests uses heat-treated cell extracts. *M. haemolytica* can be distinguished by their Lipopolysaccharide, SDS-PAGE profile [64]. Examination of OMP preparations by SDS-PAGE showed major differences between strains of *M. haemolytica* isolated from the same or different host species. Yet the individual serotypes are not accurately identifiable by this method due to the great similarity in their protein band resolution. The isolation of outer membranes and inner membranes of serotypes of *M. haemolytica* Al allowed for identification of their major proteins. Therefore this phenomenon has been used to differentiate between the isolates [44]. The double-diffusion technique is employed. Wells are punched in the solid agar in a circular pattern with one center well surrounded by six peripheral wells [61].

Molecular Identification: Conventional polymerase chain reaction (PCR): Molecular methods of bacterial identification have been proved valuable to overcoming some limitations of the conventional biochemical and serological methods and better sensitivity and rapidity [65]. Molecular identification is further advance accuracy of characterization in pure and/or mixed cultures, speed of detection, determination of taxonomic position and indulgent of intraspecies genetic relationships. Conventional polymerase chain reaction (PCR) methods of detection of agent in clinical specimen appear increasingly preferred. PCR is used to detect or sense a sequence of DNA unique to *Pasteurella* and *Mannheimia* species [17].

Multiplex Polymerase Chain Reaction (mPCR): Assay containing all two or more pairs of primers were specifically amplified serotype specific genetic targets for different *M. haemolytica* target serotypes was indicated

by the amplicon sizes in the gel images and testing done for several organism in the same assays. As an extension to the practical use of the PCR, 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 [65].

Multiplex PCR has been applied for the detection of PHSSA and Rpt2 genes of *M. haemolytica* using a primer pairs: Forward 5'-TTC ACA TCT TCA TCC TC-3', Reverse 5'-TTT TCA TCC TCTT CG TC-3' and Forward 5'-GTT TGT AAG ATATCC CAT TT-3' Reverse 5'-CGT TTT CCA CTT GCG TGA-3', respectively [65].

Ribotyping: is a molecular technique (Molecular typing) for bacterial identification that uses information from rRNA-based phylogenetic analyses [66]. It is rapid and specific method widely used in clinical diagnostics and analysis of microbial communities. All bacteria have ribosomal genes, but the exact sequence is unique to each species, serving as a genetic fingerprint. Therefore sequencing the particular 16S rDNA gene and comparing it to a data base would yield identification of the particular species [67].

Ribotyping involves the digestion of bacterial genomic DNA with specific restriction enzymes. Each restriction enzyme cuts DNA at a specific nucleotide sequence, resulting in fragments of different lengths. Recently, rRNA gene restriction analysis (Ribotyping) of strains of *M. haemolytica* has confirmed the distribution of specific clones (an intraspecific clonal distribution) in bovine or captive bighorn sheep herds [68].

Random amplified polymorphic DNA (RAPD): analysis has been applied for the distinction of strains belonging to the same species. Amplified polymorphic DNA (RAPD) analysis is one of the methods used to characterize and differentiate *Mannheimia* and *Pasteurella* isolates [69]. It is a fast, sensitive method for the epidemiological studies and PCR based method of genetic typing based on genomic polymorphisms. It involves use of single arbitrary primer in a PCR reaction, resulting in amplification of many discrete DNA. Random Amplified Polymorphic DNA has been used to investigate the genetic differences among *P. multocida* and *M. haemolytica* isolates from cattle and sheep [70].

Prevention and Control Approaches of Pneumonic Pasteurellosis: Pneumonic pasteurellosis is an acute infectious disease that causes wide financial losses due to death, reduced live weight, delayed marketing, treatment costs and unthriftness among survivors [28]. These bacteria are part of the normal microflora in the upper respiratory tract making the disease difficult to prevent [59]. On the other hand [13] reported the possibilities to prevent and control infections due to *Pasteurella* and *Mannheimia* species in animals.

Management: The most effective preventive method is good management and avoidance of stress. Because of common occurrence of the disease at the time of shipment from the range to the feedlot, much attention has been given to reduce the incidence of disease at this time. The sheep should be transported from the farm of origin directly to the fattening unit. The transport distance should be as short as possible and the animal should be handled in calm and considerate manners at all stages of transport. No single management practice has been effective in controlling this disease complex. Management practices which reduce stress, as well as early diagnosis and antibiotic treatment, are the key approaches of controlling disease within farm, especially during the first two to three weeks after arrival [71].

Treatment: Antimicrobials are still the tools of choice for prevention and control of infections due to *Pasteurella* and *Mannheimia*. However, indiscreet 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 animals production [13].

Antibiotic susceptibility tests are important, because resistance to antibiotics is frequent in *P. multocida* and *M. haemolytica*, although it is less common in other Pasteurellaceae [72]. *Pasteurella* are generally susceptible to antibiotics like, penicillin and tetracycline. However, the organism has been found resistant to a variety of antibiotics such as: Ciprofloxacin, Chlortetracycline, Cotrimoxazole, Furazolidone, Lincomycin, Ampicillin, Augmentin, Kanamycin, Apramycin and Cefatoxime. Penicillin and Tetracycline are considered as antibiotics of choice and cephalosporin are acceptable alternative for combating the infection [30].

Most sheep are showing some improvement within one to three days of initiating treatment. Antibiotics most commonly used are oxytetracycline at rate 20mg/kg, long acting and 10mg/kg daily for 3 days short acting; Tilmicosin is effective in reducing the population of *M. haemolytica* that colonizing the nasal cavities of calves with respiratory disease [65]. Treatment is frequently unrewarding unless it is begun very early in the disease process because of rapid progression of lung damage and endotoxin release. Parenteral fluids and anti-inflammatory agents are important adjuncts to

antibiotic therapy. Although septicemic pasteurellosis has favorable antimicrobial susceptibility, response to therapy is often disappointing. Administering prophylactic antibiotics to at-risk lambs may be beneficial [73].

Vaccination: An alternative non-antibiotic prophylaxis strategy through vaccination is more desirable. This involves the use of specific *Mannheimia* and *Pasteurella* vaccines. Monovalent vaccine against *M. haemolytica* and *P. multocida* infection has already been in use. Vaccines against pneumonic pasteurellosis involved will help to reduce the severity of the disease, since it is the secondary bacterial phase of the disease that contributes to both its severity and fatality [12].

Humoral immunity plays an important role in protection against the disease. New generation of vaccines based on sound scientific principles and knowledge of the pathogenesis of *M. haemolytica* infection are now available and some of these have been shown to be effective under field conditions, following either one or two administrations [12]. Experimental study indicated that vaccine against *M. haemolytica* A1 provides little or no cross-protection against *M. haemolytica* A2. Vaccines for *M. haemolytica* A2 have been reported to be beneficial in reducing death losses and decreased weight gains from both septicemic and pneumonic forms of pasteurellosis [74].

Recent studies indicate the use of gamma-irradiated *M. haemolytica* vaccine showed better protective efficacy than the commonly used formalin killed vaccine in laboratory animals as well as in sheep and hence could be potential alternative method of vaccine production against ovine pasteurellosis. Exposure to optimum doses of gamma radiation destroys the DNA of the bacterium, making it unable to replicate and establish an infection although some residual metabolic activity may survive. Thus, the irradiated microorganism can still find its natural target in the host. Gamma irradiated vaccines appear to be more effective than formalin killed vaccines against disease and has the added advantage of a longer storage life than "Live" vaccines [75].

Distributions and Economic Significance of Ovine Pasteurellosis: The geographical distribution of *M. haemolytica* is worldwide. However, the microorganism is reported most frequently in Asia and Africa countries where sheep or goat breeding is widespread. In Europe, pasteurellosis or mannheimiosis is also widespread involves many countries where sheep and cattle are present, such as the Netherlands, Germany, Italy and France [76]. *Pasteurella multocida* has also a major impact on the livestock industry in countries of Southeast Asia especially in Bangladesh where a severe economic loss has been recorded and is ranked as one of the most important contagious disease of cattle and buffaloes [20]. Pneumonic pasteurellosis an acute infectious disease that causes widespread financial losses because of death, reduced live weight, delayed marketing, treatment costs and unthriftness among survivors [28]. In Africa, especially in Ethiopia, bronchopneumonia mainly attributed to *M. haemolytica*, causes both morbidity (18.6%) and mortality (10.6%) in sheep and goats [15].

Status of Ovine Pasteurellosis in Ethiopia: Several studies were conducted in Ethiopia to determine the extent of the problem and the relative distribution of different biotypes and serotypes of *Mannheimia* species. In those studies, there were indications to the prominence of *M. haemolytica* especially serotype A1 and A2 are the most common in the country obtained from nasal and transtracheal swab with morphology, phenotypical and conventional characterization. However, molecular advances need to know the prevalence and the tangible organisms elaborate bovine and ovine pneumonic pasteurellosis concerning serotype distribution and the etiological diversity of the agent in the country [51]. The studies indicated that pasteurellosis is a major threat in the highlands and also in the lowland hot and humid areas with high death and illness to domestic ruminant production and most serotypes of *M. haemolytica* biotype A are involved in pneumonic pasteurellosis with serotype A1 and A2 in cattle and sheep being the most prevalent respectively [14, 16, 17, 63].

CONCLUSION AND RECOMMENDATIONS

M. haemolytica and *P. multocida* are commonly associated with cases of pneumonia in sheep in Ethiopia although the remaining other pathogens responsible for majority of the cases are yet to be determined. Molecular characterization revealed the existence of three genotypes of *M. haemolytica* circulating in Ethiopia and presence of *P. multocida.* This suggested that further extensive work to determine all pathogens associated with sheep pneumonia and the strain distribution of *M. heamolytica* and *P. multocida* to understand its molecular epidemiology at national level and design cost effective prevention and control methods. Based on the above conclusion, the following recommendations were forwarded:

- Vaccination of sheep against bacterial pneumonicpasteurellosis should be taken it consideration.
- Further extensive review to determine all pathogens associated with sheep pneumonia and the strain distribution of *M. heamolytica* and *P. multocida* should be conducted in Ethiopia.
- Due to its effectiveness of multivalent vaccine of pneumonic pasteurellosis caused by *M. heamolytica* and *P. multocida* extensive study through the country should be performed to produce multivalent vaccine.

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