Thesis Ref. No. _____

EPIDEMIOLOGY OF OVINE PASTEUROLLOSIS IN LUME DISTRICT, EAST SHEWA ZONE OF OROMIYA REGION, ETHIOPIA

MSc Thesis

By

Sadia Hajie

Addis Ababa University, College of Veterinary Medicine and Agriculture, Department of Clinical Studies

June, 2015

Bishoftu, Ethiopia

EPIDEMIOLOGY OF OVINE PASTEUROLLOSIS IN LUME DISTRICT, EAST SHEWA ZONE OF OROMIYA REGION, ETHIOPIA

A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical Veterinary Epidemiology

By

Sadia Hajie

June, 2015

Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by:Sadia Hajie entitled "Epidemiology of Ovine pasteurollosis in East Shewa Zone of Oromia region, Ethiopia" and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Science in Tropical Veterinary Epidemiology.

Dr Tilayee Demisee	_____	_____
Chairman (title and name)	Signature	Date
Dr Bekele Megersa	_____	_____
External Examiner	Signature	Date
Dr Reta Duguma	_____	_____
Internal Examiner	Signature	Date
Dr Fufa Abbuna	_____	_____
Major Advisor	Signature	Date
Dr Barbara Wieland	_____	_____
Co- Advisor	Signature	Date
Dr Fufa Abbuna	_____	_____
Department chairperson	Signature	Date

STATEMENT OF AUTHOR

I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis submitted in partial fulfillment of the requirements for MSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Name: Sadia Hajie Signature: _____

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: June 15, 2015

ACKNOWLEDGEMENTS

I appreciate Dr Fufa Abbuna, my advisor, for his valuable advice and devotion of his time in correcting the manuscript of my Thesis and my special appreciation also goes to my co.advisor Dr. Barbara Wieland who had advised me on matters pertinent to the thesis/dissertation**.** .

My special gratitude also goes to International Livestock Research institude (ILRI) who provides me financial support and facilities to attend this program and all staff members of Sebeta National Veterinary Research instititute those in the microbiology laboratory .

Most profound gratitude goes to my husband Jemal Jibrilo for his entire share which cannot be listed out due to limitation of space and time and to my Son Fahmi Jemal for providing me a comfort zone in which I feel free and ease.

Finally I would like to thank my family, brothers and sisters, and all my relatives and friends. Thanks to Allah.

TABLE OF CONTENTS

TABLE OF CONTENT CONT…

TABLE OF CONTENT CONT…

LIST OF ABBREVATIONS

ADV	Adenoviruses
BHV	Bovine herpes Virus
BPI	Bovine para influenza
BRSV	Bovine respiratory syncytial virus
BoARD	Bureau of Agriculture and Rural Development
CA	Coagulation test
ELISA	Enzyme linked immuno sorbent assay
H	Histophilus
IHA	Indirect haemagglutination test
IHC	Immuno-histochemical
IL	Interleukin
LKT	Leukotoxin
LPS	Lipopolysaccharide
M	Mannheimia
P	Pasteurella
PCR	Polymerase Chain Reaction
PI	Parainfluenza
RSV	Respiratory syncytial virus
SPA	Sheep pulmonary adenocarcinoma
TNF	Tumour Necrosis Factor
URT	Upper respiratory tract

LIST OF TABLES

LIST OF APPENDIXS

ABSTRACT

The study of ovine pasteurellosis was conducted in Lume districts, East Shoa Zone of Oromia region, Ethiopia to determine the prevalence of M*annheimia haemolytica*, *Pasteurella trehalosi* and *Pasteurella multocida* from nasal swabs (384), abattoir specimens (145*),* and the serotype diversity among the species from sheep sera (150). A total of (115) isolates of *M. haemolytica*, *P. trehalosi* and *P. multocida* were isolated from nasal swabs of apparently health and clinically sick sheep and from pneumonic lungs. The *M. haemolytica, P. trehalosi and Pasteurella multocida* was isolated from the nasal swabs (11.2%), (7.6%) and (2.1), whereas *M. haemolytica* isolates from pneumonic lungs (11.7 %), *P. trehalosi (*10.3*)* whereas *Pasteurella multocida* was the lowest among species isolated (2.1 %). The overall isolation rate of *M. haemolytica* and *P. trehalosi* and *Pasteurella multocida* was 15.7%, 11.5% and 2.9 respectively. From 145 lung samples collected and cultured, Pasteurella was isolated successfully in 35 (24.1%) sheep.Out of 35, the percentage recovery rate of *M.haemolytica* 17(11.7%), *P.trehalose* 15(10.3%) and *P.multocida* 3(2.1) % from the lung lesion sample respectively. On the basis of these results *M. haemolytica* and *P.trehalose* was the most common cause of pasteurellosis in sheep at Lume district. A total of 150 sheep sera were examined for serotype specific antibodies using indirect haemagglutination test for *M. haemolytica, P.trehalose* and *P.multocida* serotypes. Variation in prevalence among the different serotypes was observed (P< 0.001). The IHA test revealed that serotype A1, A2,A7,T3,T10,and T15 were the dominant serotypes with 23.3%, 42.6%,32,51.3,29.3 and 30% positive by IHA whereas serotypes PAs (*P.multocida* biotype A) and T4 were the least positive with 14.6% and16% respectively.Generally Both bacterial and serological results of this study showed that the causal agents of pasteurellosis are prevalent in the area, and serotypes A1,A2, A7, T3,T10 and T15 were dominant over the other serotypes.

Keywords*:* *Mannheimia and pasteurella* spp, nasal swabs *Pasteurella*,isolation, serotypes,IndirectHaemagglutination(IHA),Lume(EastShewa).

1. INTRODUCTION

Sub-Saharan Africa is endowed with a large population of sheep and goat. However, animal productivity per head is low. Ethiopia lies within the tropical latitude of Africa and has an extremely diverse topography, a wide range of climatic features and a multitude of agro-ecological zone which makes the country suitable for different agricultural production system. This in turn has contributed to the existence of a large diversity of farm animal genetic resource in the country. Sheep constitute the second major component of livestock in Ethiopia (Anon, 2004).

Despite the large livestock population of Ethiopia the economic benefits remain marginal due to prevailing diseases, poor nutrition, poor animal production systems, reproductive inefficiency, management constraint and general lack of Veterinary core (Moredun, 2009). One of the diseases that reduce small ruminant productivity and cause substantial losses through high morbidity and mortality not only in Ethiopia but also throughout the world is pasteurellosis (Kusiluka and Kambarage, 1996; Habashy *et al*., 2009).

Ovine pasteurellosis is disease mainly caused by *Mannhaemia haemolytica(Pastuerella hemolytica), Pastuerella multocida* and *Pastuerella trehalosi (Bibersteinia trehalosi) are* the three most commonly isolated bacterial agents from pneumonias that result in high rates of morbidity and mortality in sheep. *Pasteurella multocida* and *Mannheimia haemolytica* are causative agents of several economically significant veterinary diseases. Serious infectious diseases as fowl cholera, bovine hemorrhagic septicemia, and porcine atrophic rhinitis are caused by *P. multocida* whereas *M. haemolytica* is the causative agent of shipping fever or pneumonic pasteurellosis in sheep (Michael, 2008).

Pasteurella multocida was first found in 1878 in fowl cholera-infected birds. However, it was not isolated until 1880, by Louis Pasteur - the man whom *Pasteurella* is named in his honor (Katherine, 2008). Now strains of *P. multocida* are grouped serologically into 5 capsular types (A, B, D, E and F) and 16 somatic lipopolysaccharide-types (1–16). *P. multocida* strains have also been characterized by outer membrane protein (OMP)-type and 16S rRNA-type. 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 (Richard, 2009).

Mannheimia haemolytica has been the subject of extensive reclassification in the past: first called *Bacterium bipolare multocidum* by Theodore Kitt in 1885, it was renamed *Pasteurella haemolytica* in 1932 and classified into two biotypes (A and T) based on its ability to ferment the sugars arabinose and trehalose, respectively. These biotypes were 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 an indirect haemagglutination test (Biberstein and Gills, 2002). After years *pasteurella haemolytica* biotype A was allocated to a new genus and renamed *Mannheimia haemolytica* while the 4T serotypes named *Bibersteinia trehalosi.* Recently because of serotypes A11 differently classified as *M. glucosida, M. haemolytica* is considered having twelve serotypes (A1, A2, A5-A9, A12-14, A16 and A17) based on capsular antigen typing (Sarah, 2011). The genus *Mannheimia* now contains several species including *M. haemolytica, M. granulomatis, M. glucosida, M. ruminalis* and *M. varigena* (Mohamed and Abdelsalam, 2008).

In Ethiopia, a number of attempts have been made so far to identify the different serotypes of both *Mannheimia* and *Pasteurella* spp. in small ruminants. Recent work on pasteurellosis of small ruminants has demonstrated different species and serotypes of, *M. haemolytica* and *P. trehalosi* such as A1, A2, A5, A6, A7, A8, A9, A11, A12, A13, A14 and T3, T4, T10 and T15. Of which A1, A2, A8, A7, T3 and T4 are the dominant serotypes (Gelagay, 1996; Tesfaye, 1997; Aschalew, 1998; Mekonen, 2000; Mesele, 2005).

These species are commensally resident in the animal body as normal constituents of the nasopharyngeal micro flora and are all capable of causing infection when the body defense mechanisms are impaired. The condition usually appears when sheep are exposed to combinations of predisposing factors such as adverse physical condition, physiological stress, bacterial and viral infections (Anon, 2004).When the respiratory defenses of animals are weaken by predisposing factors *Pasteurella* species can colonize the lower respiratory tract in large

numbers and induce severe fibro purulent bronchopneumonia. It causes widespread financial losses because of death, reduced live weight, delayed marketing, treatment costs and unthriftness among survivors (Hawari *et* al., 2008).

The pathogenesis of pneumonic pasteurellosis remains a subject of considerable speculation and controversy due to the complex nature of the disease and the lack of consistency in experimental results (Sarah, 2011**).** Regardless of the economic importance of this pathogen, relatively little is understood about the precise mechanisms of pathogenesis, specifically how *M. haemolytica* adheres to the respiratory tract epithelium even if previous studies have demonstrated the ability of *M. haemolytica* to adhere to epithelial cells *in vitro* (Kisiela and Czuprynski, 2009*).*

The application of the advanced diagnostic techniques as electron microscope investigation or DNA analysis of the microorganisms has helped a great deal in the elucidation of the virulence factors of the organism and their role in pathogenesis, which helped in the development of potential candidate vaccines as well as new-targeted generations of antibiotics. Despite the application of advanced investigation and diagnostic techniques on both the organism and the affected animal species, pasteurellosis still continue to contribute to heavy losses in sheep production and remain a hazardous threat to human health worldwide (Ragy, 2005).

Small ruminant production in the study districts is playing an important role in generating cash income, manure, social value, meat, skin.The predominant practices are mixed management system, traditional housing and grazing of natural pasture.Several investigations have been conducted in different countries and regions on the species and its serotypes that cause Pasteurellosis in sheep. The different studies conducted in Ethiopia indicated that pasteurellosis is a major threat to sheep production. Some of these studies were those in Amhara Regional State particularly Debre Birhan (Gelagay *et al.,* 2004) and South Wollo (Belay, 2007) and Oromia Regional State particularly selected sites of Arsi Zone (Mekonnen, 2000) as well as those in Debre Birhan, Harshin and Jijiga (Deressa *et al*.) but there is no available study in East Shoa Zone of Oromia region. In Lume district, despite annual vaccination against pneumonic pasteurellosis with a monovalent vaccine (inactivated *P. multocida* biotype A), there are high mortality and morbidity following respiratory distress (OBOARDAR, 2012/2013). Pasteurollosis

is therefore 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. The main problem is absence of an extensive study on epidemiology of this disease as well as absence of a cost effective prevention and control methods which suits best for different phenotypes and serotypes of the agent.

Therefore, the objectives of this study are;

- To isolate and characterize most prevalent *Pasteurella* and *Mannheimia* species in sheep in Lume districts of East Shoa Zone of Oromia Region.

- To determine the possible major serotypes of *Pasteurella* involved in respiratory symptoms/infections of sheep in Lume districts of East Shoa zone of the Oromia region.

2. LITRATURE REVIEW

2.1 Pasteurella and Mannheimia

The *Mannheimia* and *Pasteurella* are small, non-motile, non-spore forming, gram- negative rods or coccobacilli and facultative anaerobic bacteria that belong to the family *Pasteurellaceae.* They are oxidase and catalase positive and reduce nitrates and attack carbohydrates fermentatively. Bipolarity of *Pasteurella* and *Mannheimia* can be seen in Giemsa-stained or Leishman-stained smears (Quinn *et. al*., 2002).

2.1.1 Taxonomy and classification

The *Mannheimia* and *Pasteurella* are grouped taxonomically in

Superkingdom	*Bacteria*
Phylum	*Proteobacteria*
Class	*Gammaproteobacteria*
Order	*Pasteurellales*
Family	*Pasteurellaceae*
Genera	*Mannheimia* and *Pasteurella (NCBI,* 2004*).*

Based on number of characteristics including pathogenicity, antigenic nature and biochemical activity, *Pasteurella haemolytica* can be differentiated into two biotypes, biotype A and T. Biotype A ferments arabinose and whereas biotype T ferments trehalose; however, based on molecular, biological techniques and analysis of phenotypic data biotype T was reclassified as *P. trehalosi* and biotype A as *M. haemolytica* and M. *glucosida* (Kilian and Fredericksen, 1981).

Based on quantitative evaluation of phenotypic and genomic characteristics (Angen and co-workers,1999) have classified trehalose-negative *[P]*. *haemolytica* complex into five new species 1. *M.haemolytica. 2. M. glucosida. 3. M. ruminalis*. 4. *M. granulomatis*
5.*M. varigena.* Based on extractable surface antigens, 17 serotypes of *M. haemolytica* and *P. trehalosi* are recognized. Serotype 3, 4, 10, and 15 are classified as *P. trehalosi.*

The remaining serotypes (1, 2, 5, 6, 7, 8, 9, 12, 13, 14, 16 and 17) are classified as *Mannheimia* except serotype 11, which varied from biotype A by being CAMP test negative and fermenting the cellobiose and salicin and is reclassified as *M. glucosida* (Kilian and Fredericksen, 1981). In addition to isolates that express these serotypes, approximately 10% of disease isolates recovered from cattle and sheep are untypeabale (Quine *et al*., 1986; Gilmour and Gilmour, 1989).

The *P. Multocida* serogroups have been identified based on difference in capsular polysaccharide and are designated A, B, D, E and F. They are further subdivided into 16 somatic types based on their serological differences of their cell wall lipopolysaccharides (Carter, 1984). It is known that besides the geographical distribution these serogroups are more or less specific with regard to the host and the disease induction (Quinn *et al.,* 1994; Boyce *et al.,* 2000).

2.1.2 Morphology and staining characteristics

Freshly isolated strains of *Pasteurella* and *Mannheimia* from the carcasses of animal appear as short ovoid rods measuring 1μm in length and 0.5-0.8 μm in width and most of them are capsulated. After repeated culture the organism tends to form longer rods and become more pleomorphic. The organisms are Gram negative with a tendency to bipolar staining when stained with Methylene blue or Leishman‟s stain. Old culture usually revealed Gram-negative rods of various sizes. Cells are arranged in chains and filamentous forms are occasionally observed. The bipolarity feature is lost due to continuous culturing (Sharma and Adlakha, 1996; Carter, 1984).

2.1.3 Growth requirements

The organisms are aerobic or facultative anaerobic. The optimum temperature for growth is 37 0C at pH 7.2 to 7.4. Although non-enriched media support their growth, the *Pasteurella* and *Mannheimia* species grow best in the presence of serum or blood. Bovine blood is more suitable than the blood of sheep and horse for the demonstration of haemolysis (Carter, 1984; Biberstein and Hirsh, 1999; Quinn *et al.,* 2002). These microorganisms grow well in medium containing amino acids, a mixture of salts, vitamins, sugars like galactose and glucose (Smith and Phillips,

1990). *Mannheimia* spcies requires a higher concentration of iron for production of cytotoxin than is needed for growth (Gentry *et al*, 1987; Angen *et al*., 1999).

2.1.3 Cultural characteristics

The colonies produced by *Mannheimia haemolytica* and *P. trehalosi* are odorless, moist, smooth, grayish, translucent measuring approximately 1-3 mm in diameter on tryptose-serum agar or blood agar plates while the colonies of *P. multocida* are round, grayish, shiny and non-haemolytic. Some colonies of pathogenic strains of P*asteurella multocida* are mucoid due to the production of think hyaluronic acid capsules. The colonies have a subtle but characteristic odor (Merchant and Packer, 1983; Quinn, *et al*., 2002).

The *Mannheimia haemolytica* and *P. trehalosi* grow on blood agar in the form of smaller colonies with slight thickening in the center. On horse, sheep or rabbit-blood agar the colonies are circular surrounded by a narrow zone of β - haemolysis, but on blood agar plates made from young lambs gives rise to double-zone of β- haemolysis- an inner complete and an outer wide partial, which increases in size at room temperature. They produce a diffusible substance that enhances the hemolytic effect of *staphylococcus aureus* β-toxin. *Mannheimia haemolytica* and *P. trehalosi* is very distinct from *Pasteurella multocida* by their growth on MacConkey agar as pink to red colonies (Smith and Phillips, 1990; Quinn *et al*., 2002).

2.1.4 Biochemical characteristics

Although *Pasteurella* and *Mannheimia* spp. have limited fermentative ability of carbohydrates, they utilize number of carbohydrates with acid production but not gas (Table. 1). In triple sugar iron (TSI) agar slopes, yellow (acid) slant, yellow (acid) butt without H2S production are typical reaction for *Pasteurella* and *Mannheimia* spp. (Quinn *et al*., 2002*).*

Table 1. Comparison showing the fermentative activity between *Pasteurella* and *Mannheimia* species

Feature	*P. multocida*	*M. haemolytica*	*M. trehalosi*	*P.pneumotropica*
Hemolysis on bloodagar	-	+	+	-
Growth on McConkey agar	-	+	+	v
Distinctive odor from colonies	+	-	-	-
Indole production	+	-	-	+
Catalase activity	+	+	-	+
Urease activity	-	-	-	+
Gas from carbohydrates	-	-	-	+
Ornithine decarboxylase activity	+	-	-	+
Acid production				
Lactose	-	+	-	V
Sucrose	+	+	+	+
D-Trehalose	V	-	+	+
L-Arabinose	V	-	-	-
Maltose	V	+	+	V
D-xylose	-	+	-	V

Source: Quinn *et al* (2002) + = Positive; - =Negative; V = Variable

2.1.5 Chemical and physical properties

None of the *Pasteurella* is resistant to adverse agents, and can easily be killed with common chemical and physical agents. Exposure of suspension to disinfectant such as to 0.5 % phenol for 15 minutes, to heat at 55 oC, to ultraviolet light and colonies on solid media to sun light are lethal, and are susceptible to commonly used antibiotics ,(Quinn *et al*., 1994).

2.2 Epidemiology

2.2.1 Occurrence and Distribution of Pasteurella Species

Pneumonic Pasteurellosis is one of the most economically important infectious diseases of small ruminants with a high prevalence occurs throughout the world (Prabhakar *et al*., 2012). It was first described in Iceland and subsequently has been reported in many countries such as Australia, Britain, Ethiopia, Norway, South Africa, Somalia and USA (Habashy *et al*., 2009**).** The prevalence of Pasteurellosis with in flocks varies from year to year and from countries to countries. The disease also seen in all age groups in every season of the year (Amin, 1998).

The epidemiologies of pneumonic pasteurellosis causing bacteria are carried in the URT of healthy animal but the bacterium is not easily detectable. In case of diseased or stressed animal the bacteria can shed and isolated in a greater frequency (Kusiluka and Kambarage, 1996). *M. haemolytica* is the causative agent of several economically significant veterinary diseases occurring in ruminants and, much more rarely, in other animal species (Amin, 1998).

In sheep and goats, it is also usually endemic with occasional sporadic outbreaks, involving animals of all age groups. Mortality varied from 5 to 30%, while the morbidity from 10 to 60% (Amin, 1998). Outbreak of pneumonic pasteurollosis is often associated with changes in the environment and occur in s3r5e zpring and summer, but can occur sporadically at any time of the year (Jasni *et al*., 1990).

There were several investigations on isolation and identification of these causative agents of pasteurellosis in sheep and goats in different areas as indicated in the following tables.

Table 2. Prevalence of *Pasteurella* Species in different geographical locations.

Country	Locality	Host species	MH	PM	PT	Authors
Ethiopia	Debre Brehan	Sheep	25.1	20	9.3	Biruk *et al*. (2013)
	Gimba	Sheep	25.2	2.8	4.2	Biruk *et al*. (2013)
	Asella	Sheep	26.4	2.3	7.4	Biruk *et al*. (2013)
	Afar (Mille)	Sheep	-	-	13	Shiferaw *at al*. (2006)
		Goats	-	-	26	Shiferaw *at al*. (2006)
Egypt		Sheep	79.4	-	20.6	Kaoud *et al.* (2010)
		Goats	90.7	-	90.4	Kaoud *et al.* (2010)
Sudan		Goats	90.6	0.98	9.4	Elsheikh and Hassan (2012)
Jordan		Sheep	4.1	37.8	-	Hawari *et al*. (2008)
		Goats	25	10	-	Hawari *et al*. (2008)

-, indicates not present. MH = *M. haemolytica*, PM = *M. haemolytica* and PT = *P. trehalosi*

2.2.2 Hosts range and susceptibility

M.haemolytica has wide host range and has been isolated from Sheep, goat, pig, poultry; cattle, camel, horse, donkey,Mule,Bighorn sheep, dog and cat are hosts of the disease (Blood, 1994; De Alwis, 1992).There are also wild animals like elephant, monkey, deer, rabbit, bison, elk, moose, mountain goats, deer and rodents associated with pneumonic lesion and septicemia.

Mannheimia haemolytica occurs in all ages of sheep and goats but lambs and kids during the first few months of life and dams at lambing are more susceptible. Generally, *M. haemolytica* infections are the most prominent and highly pathogenic microorganism that causes very severe respiratory diseases of ruminants (Smith and Phillips, 1990). *Mannheimia glucosida* is mostly isolated from nasal cavity of sheep and causes pneumonia and different disease condition. *Mannheimia ruminalis* is isolated from rumen of cattle and sheep and its association with disease

has not been reported (Bisgaard *et al*., 1986). *Mannheimia granulomatis* is mostly isolated from rabbits and hares and rarely from cattle and associated with pneumonia and skin granulomas in cattle. Whereas *M. verigena* is isolated from cattle and pigs, causes pneumonia, sepsis and other diseases (Angen *et al., 1*999). Septicemic pasteurellosis occurs more commonly in conjunction with pneumonic form of the disease in young lambs (Gilmour and Gilmour, 1989; Radostits *et al.*, 1994).

Pasteurella Multocida serogroups A and D are worldwide spread serogroups which can be found in a wide range of domestic animals (e.g. from fowl to calves, pigs, sheep, goats, and rabbits) in which they cause various infections (Quinn *et al*., 1994). Serogroups B and E have been found predominantly in tropic areas where they induce hemorrhagic septicemia in cattle and wild ruminants. The serogroup F has been known as a causative agent of fowl cholera but in recent time, it has also been found in some mammalian species in different parts of the world. Three biotypes or subspecies of *P.multocida* are recognized, namely *P. multocida* subspecies *multocida* recovered from domestic animals, *P. multocida* subspecies *septica* recovered from various sources of including dogs, cats birds and man and *P. multocida* subspecies *gallicida* recovered from birds (Quinn *et al.*, 1994). Clinical infections caused by *Pasteurella* and *Mannheimia* species in domestic animals are mainly attributable to *P. multocida, M. haemolytica* and *P. trehalosi* (Table.3).

Table 3. The Principal hosts and disease conditions caused by the pathogenic *Pasteurella* and *Mannheimia* species in domestic animals

Host species	Disease	Organisms
Cattle	Bovine pneumonic pasteurellosis Septicaemic disease	*P. haemolytica* A 1,*P .multocida* A *P .multocida* serotype B:3 and 4
Sheep and Goats	Pneumonic pasteurellosis, Septicaemic pasteurellosis	*P. haemolytica* A *P. haemolytica* T
Pigs	Hemorrhagic septicemia Atrophic rhinitis Pneumonia	*P multocida* serotype B:2 Toxigenic strains of *P multocida* type D, occasionally, type A *P multocida* type A
Poultry/turkeys	Fowl cholera	*P multocida* type A Common serotypes are A:1, A:3, A:4 .

Source: De Alwis (1992)

2.2.3 Source of infection and mode of Transmission

The sequential development of Pasteurollosis in sheep is *M. haemolytica* and *P. trehalosi*, while *P. multocida* (biovars A and D) is occasionally associated with the disease. *Pasteurella* and *Mannheimia* species are common commensals on mucus membranes of most animal species in all climatic zones. Most ruminants are asymptomatic carriers of *M. haemolytica* or *P. trehalosi* and they frequently carry strains of *P. multocida* as well (Carter, 1984; Biberstein and Thomson, 1996).

The disease is highly mediated by complex interactions between the naturally existing causative organism in the upper respiratory tract, the immunological status of the animal and the role of predisposing factors in the initiation of infection. The spread of the disease from infected animal to healthy animals is by direct contact, by inhalation and consumption of contaminated feed (Brogden *et al*., 1998).

The majority of *M. haemolytica* infections are mostly endogenous, the organism, which is normally commensals of the upper respiratory tract, may invade the tissue of immune suppressed animals, although exogenous infections can also occur by direct contact with sick animals or through infected aerosols. Lambs acquire the infection soon after birth, probably transmitted by close contact with their dams (Amin, 1998).

2.3 Associated Risk Factors

2.3.1 Predisposing Factors

Predisposing factors of pneumonic pasteurollosis fall into two main categories: first, management and environment factors like transportation, over crowd, climate changes and stress situations such as bad housing condition are the main predisposing factors and second are infectious agents that have been incriminated epidemiologically in association with outbreaks of pneumonic pasteurellosis (Ackermann and Brogden, 2000).

Secondary bacterial infection can be a major complication of acute respiratory viral diseases, such as PPR, PI 3, ADV and RSV 2-5 (Aydın, 1997). These viruses dramatically increase the susceptibility of sheep and goats to secondary *M. haemolytica* infection. Primary infection of the lower respiratory tract, with *Mycoplasma ovipneumoniae* and *Bordetella parapertussis* can also increase the susceptibility of sheep and goats to secondary *M. haemolytica* infection (Brogden *et al*., 1998; Sherrill, 2012).

It is possible that initial infections with viral or primary bacterial agents break-down the antimicrobial barrier consisting of beta defensins and anionic peptides found in epithelial cells, resident and inflammatory cells, and serous and mucous secretions of the respiratory tract. Loss of barrier integrity may release *M. haemolytica* from its usual commensal status (Brogden *et al*., 1998; Mohamed and Abdelsalam, 2008; Sherrill, 2012).

2.3.2 *Pathogenesis and Virulence Factor*

The primary development of Ovine pasteurollosis highly mediated by complex interactions between the naturally existing causative organism in the upper respiratory tract, the

immunological status of the animal and the role of predisposing factors in the initiation of infection. In either situation, the disease is essentially triggered by sudden exposure to a stressful condition or by initial infection with certain respiratory viruses or bactria like *Mycoplasma* (De Alwis, 1992; Sherrill, 2012).

In healthy animals, the mucociliary ladder, the cellular and humoral defense mechanisms of the respiratory tract serve in clearing the *Pasteurella*. Stress factors cause immuno-suppression through the release of steroids from the adrenal cortex inhibiting the leukocyte production that lead to marked increase in circulating leukocytes and significant reduction of the leukocyte numbers in tissues. The suppression of respiratory defense mechanism leads to multiplication of resident *M. haemoiytica* and *P. multocida* in the upper respiratory tract, transformation of the organism to become the pathogenic strain, invade the lung tissue and initiate severe fibrinous pneumonic lesions (Amin1998; Sherrill, 2012).

Stress and/or viral infection would eventually impair the local pulmonary defense mechanisms by causing deleterious effects on the cilliating cells and mucous coating of the trachea, bronchi and bronchioles. The causative bacteria from the nasopharynx will then reach the ventral bronchi, bronchioles and alveoli by gravitational drainage along the tracheal floor and thereby become deeply introduced into the lung tissue (Mohamed and Abdelsalam, 2008).
Pathogenic bacteria produce virulence factors that enhance their ability to escape host defense mechanism and increase the ability of the organisms to colonize and invade deeper tissues. Members of *M.haemolytica* and Pasteurella species produce a number of substances that are associated with the pathogencity of the microorganisms. Among the most important are the capsule and polysaccharides ,iron regulated outer membrane proteins(IROMP),toxic outer membranes proteins,adhesions,leukotoxins and enzymes (hylanidase and neuraminidase) that may involve in disease production (Birbertein and Hirsh,1999**)**

2.3.2.1 Capsule and capsular polysaccharides

The capsule play important role of which are the interface with phagocytosis and the protection of outer membrane from the deposition of membrane attach complexs by activation of complement system (Birbertein and Hirsh, 1999**).**Capsular polysaccharides also act as a lecithin to facilitate attachment of the bacteria to lining on the lung alveoli. Capsular polysaccharides of

the organism increase virulence of the organism by decreasing serum agglutination complement mediated serum killing and neutrophil phagocytosis and also function to mask cell surface component and prevent immune recognition (Laurence *et al*., 1990).

2.3.2.2 Lipo polysaccharides

Lps and Lekotoxin act in synergy to cause tissue damage and inflammation.LPS enhance the cytolytic activity and also enhance lekotoxin depend IL-8 and TNF-alpha expression in ruminant macrophage and also directly toxic to endothelial cells (Birbertein and Hirsh, 1999).

2.3.2.3 Iron regulated outer membrane protein

M.haemolytica produces a number of iron regulated outer membrane protein (IROMP) these include the transferin binding proteins Tbp1 nad Tbp2.These IROMP are thought to be key in iron acquisition, since the organism does not produce siderphores. *M.haemolytica* grown in the lungs of pneumonia over express the IROMP in support of their role in iron acquisition during the infection process (Highlander, 2001).

2.3.2.4 Neuraminidase

Neuraminidase produced by *M.haemolytica* and *Pasteurella* species and play role in colonization of mucosal surface. It decreases the gel forming capacity and viscosity of ruminant mucus which may lead to decreased clearance efficiency of mucociliary approach and permit bacteria to penetrate the mucus blanket and approach the epithelium (Laurence *et al*., 1990).Neuraminidase may also increase bacterial adherence by cleavage of sialic acid residues on the cell surface. The cleavage of sialic acid from cell surface will also decrease the net negative charge on the epithelial cell and allow closer approximation of the negatively charged bacterial cell surface to epithelium cell membrane and permit to increase absorption on the surface of cytotoxic factor (Laurence *et al*., 1990).

2.3.2.5 Leukotoxin

M.haemolytica species secrete a 101-105KDa leukotoxin. It is a toxin belongs to the family of toxin (repeats in toxin) because of the common feature of repeats of glycine rich sequences within the protein. In natural environment the leukotoxin exists in multimeric states with molecular weight of 150-300KDa or greater. Lekotoxin produces a lot of biological effects at

higher concentration to toxin create pore in the cell membranes that lead to swelling and lyses. At sublytic concentration the toxin activates neutrophils inducing inflammatory cytotoxic production invokes cytoskeleton changes and causes apotosis and the down regulation of MHC2 proteins on the surface of macrophages affecting their ability to present antigen (Birbertein and Hirsh, 1999).

The virulent determinants of *M. haemolytica* and *P.multocida* exert their influence not only to produce lesions which include alveolar edema, exudative inflammatory reactions and inter-alveolar hemorrhages, but also to maintain the presence of the organism in the respiratory tract by preventing phagocytosis and increasing resistance to complement and bactericidal effects of the host defense mechanism (Amin, 1998). Survival of the acute phase of pneumonic pasteurellosis is dependent on the extent of lung involvement and damage in the lower respiratory tract. Sheep and goats that recover may have chronic respiratory problems, including reduced lung capacity and weight gain efficiency if over 20% of the lung was damaged (Sherrill, 2012**).**

2.3.3 Pasteurollosis in sheep

The most common type of pasteurollosis in sheep is caused by the bacterium *P.haemolytica (*currently *Mannheimia hemolytica)*, *P. multocida* and *P. trehalosi* (currently *Bibersteinia trehalosi*). The three bacteria most commonly cause pasteurellosis in small ruminants. The systemic form is caused by *P. trehalosi* and the pneumonic form is by *M.haemolytica* type A and *P.multocida* type A and D (Donachie, 2000; Mohamed and Abdelsalam, 2008).

2.3.4 Systemic pasteurellosis

2.3.4.1 Clinical symptoms

Most cases are acute or Peracute, resulting in death within 8-24 hr after the onset infection. Because the course is very short, clinical signs may be easily overlooked. Animals first show dullness, then reluctance to move, fever, salivation, and serous nasal discharge. Edematous swelling is frequently seen, beginning in the throat region and spreading to the parotid region,

neck and brisket. Mucous membranes are congested. There is respiratory distress, and usually the animal goes down and dies within hours. Occasional cases longer for several days. Recovery is rare with no evidence of chronic form (Kopecha, 1997).

2.3.4.2 Lesions

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 (Kopecha, 1997). Histologically, the characteristic lung lesion is acute inflammation and emboli in small arterioles and capillaries (Gilmour, 1993).

2.3.5 *Pneumonic pasteurellosis*

2.3.5.1 Clinical symptoms

Pneumonic pasteurellosis in sheep is stress induced respiratory disease. Outbreaks of acute pneumonic pasteurellosis often commence with sudden death before clinical signs are observed. As outbreak proceeds respiratory signs become more apparent particularly in older sheep rather than in lambs. Signs include dullness, anorexia, and fever as high as-42.6oC; increase heart rate, a substantial weight loose and acute bronchopneumonia, dyspnea. Increased lacrimation and a cough are often present. On auscultation, respiratory sounds are loud and prolonged. Affected sheep shows frothy saliva in the mouth, and a serious nasal discharge. In acute cases, death occurs in 1 to 3 days (Gilmour and Gilmour, 1989; Radostits *et al.*, 1994).

2.3.5.2 Lesions

Generally a case from pneumonic pasteurellosis may present petechial and ecchymotic hemorrhages throughout the body. However, the most predominant characteristic changes occur in the thoracic cavity (Gilmour and Gilmour, 1989). In sheep dying from peracute cases, there are green gelatinous exudates on the pericardium and large quantities of straw colored pleural exudates. The lungs are enlarged, heavy and appear red to dark purple in color with frothy fluid from the cut surface (Radostits *et al.*, 1994). In acute and subacute cases, consolidation involving large area of the lung, often in the apical and dorsal diaphragmatic lobes as well as regular areas of lung necrosis with softening and cavitations in some cases are evident.

The pleurae may be thickened and opaque or clearly edematous and adhesion may be present in lung lobes (Gilmour and Gilmour, 1989). In protracted infection of pneumonic pasteurellosis, normal lung tissues are often demonstrated from areas of dark red consolidation, particularly when cut, and may contain pus filled nodules. An organized pleurisy with or without adhesion to the lobes is also found. Histologically, characteristic cellular alteration including, alveolar necrosis with alveolar spaces filled with clusters of spindle shaped macrophages (Gilmour, 1993; Radostits *et al*., 199).

2.4 Status of Pasteurollosis in Ethiopia

In Ethiopia, a number of attempts have been made so far to identify the different serotypes of both *Mannheimia* and *Pasteurella* spp. in small ruminants. Recent work on pasteurellosis of small ruminants has demonstrated different species and serotypes of, *M. haemolytica* and *P. trehalosi* such as A1, A2, A5, A6, A7, A8, A9, A11, A12, A13, A14 and T3, T4, T10 and T15. Of which A1, A2, A8, A7, T3 and T4 are the dominant serotypes (Gelagay, 1996; Tesfaye, 1997; Aschalew, 1998; Mekonen, 2000; Mesele, 2005). The list of serotypes/ species of *Pasteurella* and *Mannheimia* and their distribution are given in table 4. Gelagay (1996) reported prevalence of *M. haemolytica* serotype A2 (36%) and A8 (35%) in North Shoa zone of Amhara region. Mesele (2005) reported 1.1%, 5.6% and 25% prevalence of *P. trehalosi*, *M. haemolytica* and *P. multocida*, respectively from sheep slaughtered at Debre Zeit ELFORA export abattoir. In Arsi,

Mekonen (2000) reported 85%, 12% and 3% prevalence of *M. haemolytica*, *P. trehalosi* and *P. multocida.In South Wollo,Belay (2007)* reported Serotype A2 (34.5 %), A5 (33.5 %), A11 (27.5%) and A6 (27%) were the dominant serotypes identified, while A9 (8.5 %), A13 (6.5 %) and A14 (5.5 %) were the least among the eleven serotypes examined respectively.

Table 4. Different isolates of Manhemia and Pasteurella species and serovars in small ruminants in Ethiopia;

Place	**Isolated species**	**Serovars**	**Authors**
Addis ababa abattoir	*M.haemolytica*	-	Eshetu,1991
N.Shoa	*M.haemolytica*	A1,A2,A5,A6,A7,A8,A11,A13 and A15	Gelagay,1996
Wollo	*M.haemolytica* *P.treholsi*	A1,A2,A5,A6,A7,A8,A11 and A12 T3,T4 and T10	Tasfaye,1997
Arsi	*M.haemolytica* *P.treholsi*	A1,A2,A5,A7,A8,A9,A11,A12 and 13 T15	Mokkonin,2000
Diredawa abattoir	*M.haemolytica* *P.treholsi*	- -	Tasfaye and Abebe,2003
South Wollo	*M.haemolytica*	A1, A2, A5, A6, A7, A9, A11, A12, A13 and A14	Belay, 2007

2.5 Diagnosis

2.5.1 Field diagnosis

Field diagnosis of pneumonic pasteurollosis is based on history, clinical signs and gross pathological lesion of affected body part. Climatic change, animal movement and dipping have been reported with associated out breaks of pneumonic pasteurollosis (Kopcha, 2012).

2.5.2 Laboratory diagnosis

Laboratory diagnosis of pneumonic pasteurollosis involves both isolation and identification of agent and serology for confirmatory diagnosis of disease. In acute cases cultures obtained from tracheal swabs or washes or from lung lesions will be diagnostic but in chronic case bacterial cultures may be less reward because *P.haemolytica* may have the initial causes but the results of cultures may reveal *Arcanobacterium pyogens* ,a common causative agent of lung abscess (Kopcha, 2012). Histopathological examination is also useful especially when other types of pneumonia (e.g retro virus) induced interstitial pneumonia in adult sheep or goat is suspected (Ames *et al*., 1985).

2.5.3 Serological methods

2.5.3.1 Rapid slide agglutination test

A colony of pasteurella or *Mannhemia* mixed with a specific antiserum and on slide gives follicular agglutination in 30 seconds (De Alwis, 1993).

2.5.3.2 Indirect heamaglutination test

The most widely used which detects serotypes specific antibody. It is directed against serotype specific capsular antigens. The technique has been used not only in the sero epidemiological investigation only but also used for detection of serum antibody response to divide serotype in vaccine evaluation. The convenience and sensitivity of hemaglutination encourage worker to make some modifications such as Micro plate techniques and use of Gluteralaldehide fixed erythrocytes. Gluteralaldehydes couples antigens to RBC after few minutes and is stable than native RBC and also used kept for some weeks at 40c (Sloxome, 1983).

2.5.3.3 Indirect enzyme linked immuno sorbent assay (ELISA)

ELISA has been found to superior to micro titer agglutination for quantifying antibodies response in animals against *Pasteurella* and *Manhemia* (OIE, 1992; Rimler, 1993).

2.5.4 Molecular Methods

2.5.4.1 Polymerase Chain Reaction

PCR methods have been used for serotyping of Pasteurella in sheep and goats. The PCR based techniques have provided the alternative methods of characterization to overcoming the limitations of phenotyping. The capability of PCR to detect genetic sequences from minute quantities of DNA is advantageous compared to serologic forms of detection for several reasons: cross-reactions between antigen and antibody are avoided, strains that have been previously characterized as untypeable due to auto agglutination may be typeable by PCR, amplification of DNA by PCR makes it extremely sensitive, and PCR can be performed directly on samples without a wait for culture of the bacteria. And its limitation is follow up confirmation tests are always carried out.

There are common PCR types used in *Pasteurella* Serotyping such as conventional PCR, multiplex PCR and Real time PCR (Terry *et al*., 1998; Ranjan *et al.*, 2011). The advantages of the PCR compared with other tests include better speed, sensitivity, specificity and simplicity. It does not require culture or laboratory animals and is, therefore, safer as a result of the avoidance of handling live bacteria (Gautam *et al.*, 2004).

2.5.4.2 Molecular finger printing

This technique is also known as ‘pulsed field gel electrophoresis’ and it is a method of finger printing with high specificity and precision. Finger printing techniques provides a unique 'Signature' of a bacterial strain and has been used for the identification of microorganisms in a broad microbiological context (Ranjan *et al.*, 2011).

2.6 Differential Diagnosis

The differentiation of pasteurellosis from other causes of respiratory disease is based on the high mortality and rapid progression to death and in pneumonic pasteurellosis is dark red/purple areas, firm to the touch, are evident mainly in the anterior and cardiac lobes of the lung (Sherrill, 2012). The respiratory diseases which are confused with pneumonic pasteurellosis such as bacterial

pneumonia caused by *Mycoplasma* species (*M. ovipneumoniae* and *M. agalactiae*), *Bordetella parapertussis,Chlamydophila abortus*/*Chlamydophila pecorum* and *Streptococcus zooepidemicus,* contagious caprine pleuro pneumonia, pulmonary caseous lymphadenitis, lung abscesses caused by *Staphylococcus aureus*, f*usobacterium necrophorum, Actinobacillus lignieresii*, tuberculosis and *Corynebacterium pseudotuberculosis* (Bell, 2008; FAO, 1999).\

The Viral pneumonia also indicated caused by *para-influenza virus type* 3, *ovine adenovirus*, *respiratory syncytial virus, reovirus* types-1, 2 and 3, *herpes viruses*, *bovine herpesvirus* types-1 and 5, *ovine herpesvirus* types-1 and 2, and *caprine herpesvirus* type-1, *peste des petits ruminants*.Chronic viral respiratory disease caused by *maedi-visna* and *ovine pulmonary adenocarcinom* (Sherrill, 2012). Parasitic pneumonia caused by lung worms like *Dictyocaulus filarial*, *Protostrongylus rufescens* and *Muellerius capillaries* (verminous pneumonia), mycotic pneumonia caused by *Aspergillus* species, upper respiratory tract disease caused by laryngeal chondritis, nasal myiasis, nasal foreign bodies, nasal tumors– adenocarcinomas and aspiration pneumonia also a differential diagnosis (Bell ,2008).

2.7 Treatment

Treatment of ovine pasteurollosis is the effective method of control of the disease. Early treatment is important for more complete recovery. Knowledge of the antibiotic sensitivity pattern of prevalent strains is also important. In individual outbreaks, the sensitivity pattern of isolates from nasal secretions will give early information, but this may not reflect the status of strains from the lungs (Sherrill, 2012).

Thus, antibiotic sensitivity determination of strains isolated from the lungs at necropsy should not be missed especially in flock outbreaks and when valuable animals are involved or in acute or chronic cases when initial therapeutic attempts have failed. Commonly recommended antibiotics include ceftiofur penicillin, ampicillin, amoxacillin, tetracycline, oxytetracycline, tylosin, and florfenicol (Kopcha, 2012; Sherrill, 2012).

2.8 Prevention and Control

2.8.1 Management

Good management is the key method for preventing, while ***early*** diagnosis and proper treatment are particularly critical in successful controlling of the disease, (Sherrill, 2012).stress factor such as inadequate ventilation, overcrowding, coming of animals from various farm (feed lot), poor nutrition, failure of passive transfer of antibodies, transportation and other stress have all been associated with pneumonia outbreaks due to *P.haemolytica*.

So control and prevention that incorporates by avoiding or minimizing such factors will reduce the risk factors especially during extreme weather condition reduce the outbreaks of the disease. Thus control strategy through management is best way in developing country (Gilmour and Gilmour, 1989).

2.8.2 Immunoprophylaxis

Vaccination is the preferred method of prevention for all forms of pasteurellosis. There has been considerable activity in the development of effective vaccine for the control of pasteurellosis for sheep. Two different approaches have been exploited: First is the use of *pasteurella* vaccine containing the serotypes most commonly seen in a given locality, the second other approach is to control by vaccination of lambs with parainflueza-3 virus vaccine (Radostits *et al*., 1994). Killed vaccines; from locally isolated *P. multocida* types A and D and *M. haemolytica* and *P. trehalosi* in oil adjuvant are widely used for prevention of pneumonic pasteurellosis of ruminants. Such vaccines were found to be effective in prevention of the natural disease caused by homologous strains . Currently in Ethiopia, inactivated monovalent killed *P. multocida* type A vaccine is being used for the control and prevention of pasteurellosis in small ruminants (Gelagay *et al*., 2004).

2.8.3 Immunity

Colostral immunity in lambs appears to be fairly lasting for approximately 4-5 weeks (Gilmour and Gilmour, 1989). However, the duration of immunity following natural infections has not been investigated adequately. Immunity to *Pasteurella* and *Mannheimia* organisms is thought to be predominantly humoral and may be present up to six months (Gilmour and Gilmour, 1989).

3. MATERIALS AND METHODS

3.1The study area

The study was conducted in Oromia Region; East shewa Zone at Lume district. The area is located at 74km from Addis Ababa and 27 km from Bishoftu, at Longitude between 38º56'E-39 º17'E and Latitude 8º34'N - 8º34'N. The average Elevation of the area is 1780m above sea level. The area have average annual rain fall 969.35mm, and mean annual temperature of 20.4 ºC. Management system of the animals is extensive production system. The main farming system is mixed farming and sheep are the predominant animal species kept in the area. Traditional housing and grazing of natural pasture are the predominant husbandry practices (OBoARDAR, 2012/2013). The district was selected due to having high population of small ruminants, high reports of the pasteurollosis disease challenge and it also the schematic area of the sponsorship.

Table 5. The sheep population from different Kebeles of Mojo district

Districts	**Distance From A.A (Km)**	**Live stock population**			
		Cattle	**Sheep**	**Goats**	**Poultry**
Lume/Modjo	74	99,862	26,693	25,136	64,094

Source: OBOARDAR (2012/2013

3.1 Study animals

For the determination of contributing factors to epidemiology of ovine Pasteurellosis, indigenous sheep breeds belonging all age, sex and health status kept under extensive management system were sampled.The study animals were clinically healthy and sick sheep from five veterinary clinics for nasal swab and serum sampling and apparently healthy and slaughtered sheep from hotels were visited during sample collection. Accordingly 384 nasal swabs, 145 lung lesions and 150 serum samples were collected from the study area.

3.2 Study design

The type of the study was cross-sectional, with simple random sampling technique which was conducted from october 20014- March 20015 to establish the prevalence of *Pasteurella*

trehalosi, *Pasteurella multocida* and *Mannheimia haemolytica* isolated from nasal swab of apparently healthy and sick sheep ,lung lesion from privately owned hotels in Lume district using isolation rates on culture and biochemical tests to identify their distribution among different age groups, sex, and health status and to determine the serotype prevalence of *Mannheimia haemolytica P.trehalosi and P.multocida* from serum samples of sheep collected in the study district.

The study PAs were selected by purposive sampling technique based on sheep population within PAs. The number of sheep to be sampled from each PAs of the districts was determined simply by equally dividing the share of the PAs and finally individual sheep sample was selected purposely from each PAs and then bacterial samples were collected from each sample individual at time of visit. Since the prevalence of ovine pasteurellosis in the selected district were not known, the sample size were determined using the formula given by Thrusfield (2005) based on maximum expected prevalence of 50%. For this study 95% level of significance was considered.

Hence,

$$n = \frac{1.96^2 P_{exp}(1 - exp)}{d^2}$$

n = required sample size

P exp = Expected prevalence (50%)

d = Desired absolute precision (0.5%)

3.3 Sample collection and Laboratory analysis

3.3.1 Nasal Swab collection

Nasal swabs were collected from randomly selected sheep. Before collecting the swabs, the nostrils of the animals were well cleaned with cotton wool soaked in 70 % ethyl alcohol. Then sterile cotton-tipped swabs in screw-capped test tube moistened with tryptose soya broth (Oxoid, Hampshire, England) were inserted into the nostrils of each sheep, and the mucosa surface rubbed by rotating the swabs. The swabs were then placed back into 3 ml of sterile tryptose soya broth (Oxoid, Hampshire, England) in universal tubes and transported packed in ice to the laboratory after collection.

3.3.2 Tissue sample collection

Lung lesion was collected from randomly selected slaughtered sheep at Lume privately owned hotels during the study period. Piece of affected part of lung of the corresponding animals were taken after close inspection and put into separate sterile containers and transported to the laboratory in cool box (Sisay and Zerihun, 2003).

3.3.3 Blood sample

Blood samples for serum extraction were collected directly from jugular vein using sterile needles and plain vacuntainer tubes from randomly selected sheep flock. Up to 5-8 ml of blood was withdrawn and the tubes left to stand in inclined position over night at an ambient temperature to allow clotting, and the sera were collected using sterile Pasture pipette and transferred to sterile testes tubes, labeled and stored at -20 ^{o}C in deep freezer until they were.

3.4 Bacteriological Examination

In the laboratory nasal swabs were incubated immediately at 37 ^{o}C for 24 hours. Whereas lung samples were disinfected with 70 % alcohol and dried, and the samples were cut into pieces with sterile scissors assisted by tongue forceps and put into 3 ml of tryptose Soya broth (Oxoid, Hampshire, England) in universal tubes. The universal tubes were loose capped and incubated at 37 ^{0}C for 24 hours. After 24 hours incubation of the nasal swabs, lung tissues samples in tryptose Soya broth (Oxoid, Hampshire, England), a loop full of culture was transferred onto blood agar (Titan Biotech Limited, Bhiwadi) containing 7% difibrinated sheep blood and MacConkeys agar (Titan Biotech Limited, Bhiwadi), and then streaked by inoculating loop. The plates were incubated at 37 ^{0}C for 24 hours, and after 24 hours incubation, blood agar plates examined for the presence and type or absence of haemolysis and general appearance of the colonies including colour, shape, size and contour. The colonies suggestive of *Pasteurella* and *Mannheimia* were selected and smears were stained with Gram's staining and microscopically examined under oil immersion. Gram-negative, coccobacilli, short rods with or without bipolar staining were subjected for oxidase, urease, catalase, indole and H_2S production tests. Colonies, which were oxidase positive, urease negative, indole negative/positive, catalase positive/negative and yellow (acid) slant, yellow (acid) butt. , H2S negative in TSI agar were subjected for further biochemical tests.

The growth on MacConkeys agar (Titan Biotech Limited, Bhiwadi) was examined for the presence or absence of growth and lactose fermentation as indicated by pink coloured colonies and non-lactose fermentation by absence of pink colour and general appearance of colonies. Accordingly colonies were grouped as lactose fermenters (LF) and non-lactose fermenters (NLF). Plates with *Pasteurella* and *Mannheimia* like colonies were kept for further biochemical testing whereas mixed colonies were further sub-cultured.

3.4.1 Biochemical characteristics

After primary characterization and oxidase, urease, indole, catalase and H_2S test of the isolates, biochemical characterization of the organisms was preformed based on Glucose, lactose, maltose, salicin, sucrose, trehalose and xylose and nitrate reduction tests. *Mannheimia haemolytica* isolates were selected on the basis of xylose and lactose fermentation and lack of fermentation of trehalose and salicin and were catalase positive. Whereas, isolates of *Pasteurella trehalosi* utilised only trehalose and salicin and were catalase negative. *Pasteurella multocida* were selected on the basis of indole production, nitrate reduction, characteristics sweetish odour and absence of growth and haemolysis on MacConkey and blood agar, respectively.

3.4.2 Serotyping

Sera were serotyped for *M. haemolytica* using the indirect haemagglutination (IHA) test introduces by Biberstein (1978) for serotyping *Mannheimia haemolytica.* Serotping was conducted using capsular extract antigen. Briefly, Capsular antigen was extracted from a 24 hr culture of bacteria of known serotypes in tryptose Soya broth, which was inactivated in a water bath at 60 0C for 30 minutes, and centrifuged at 3000 rpm for 30 minutes; the clear supernatant was collected into sterile test tubes to be used as capsular extract antigen. Fresh sheep blood was collected in Alsever‟s solution at proportion of 3:5. The suspension was centrifuged at 2500 rpm for 5 minutes, washed twice with phosphate buffer saline solution (PBSS), and again centrifuged at 2500 rpm for 5 minutes. For sensitisation of the sheep red blood cells (RBC), 50µl of packed RBC were added to 5 ml of capsular extract antigen, and then 50 µl of glutaraldehyde was added and homogenized with gentle shaking, incubated for 1 hr at 37 OC. After incubation the suspension was centrifuged and washed twice with PBSS. Finally, the pellet was adjusted with PBSS to give a1% suspension of RBC. In v bottomed micro-plates 50 µl of PBSS were added to all wells and 50µl of test sera to the first column and serially diluted by pipetting 50µl up to column 12. Fifty microliters of sensitised RBC were added to each well and incubated for one hour at 37 OC. Results were recorded based on complete or more than 50% agglutination seen in each well. The titre showing 1/20 dilution and above were taken as positive.

3.5 Data analysis

For interpretation of the results, after entry of the collected data into the Microsoft Excel sheet, it was summarized by descriptive statistics and then displayed by tables and graphs to illustrate the relationships between the dependent variables (each *Pasteurella* species and their total) and independent variables (PAs, age, sex and health status). Chi-square ($X2$) tests for repeated measures were used to test relationship between dependent variable (*Pasteurella* species distribution) and different independent host and environmental factors. For these analyses SPSS statistics 20 and Epi Info were used. Prevalence of *Pasteurella* and *Mannheimia* isolates was analysed using percentages and serotype distribution compared using percentages and mean percentages.

4. RESULTS

4.1 Characteristics of the isolate

Pasteurella and *Mannheimia* isolates were revealed different morphological features on smear made from fresh isolate cultures. The isolates were Gram-negative, short ovoid rods with an occasional tendency to bipolar staining. Cells arranged in chain were also observed.

The *Mannheimia haemolytica* and *pasteurella trehalosi* revealed moist, smooth greyish, odourless and haemolytic colonies on blood agar, while *P. multocida* revealed round, greyish, non-haemolytic occasionally mucoid colonies. All the isolates of *Mannheimia haemolytica* and *pasteurella trehalosi* were grown on MacConkey‟s agar and showed pink to red small pinpoint colonies. None of P. *multocida* isolates showed growth on MacConkey agar.

A total of 80 isolates from nasal swabs and 35 isolates pneumonic lungs were identified using biochemical tests. All the isolates were positive for oxidase and negative for urease and H_2S production but the *P. multocida* isolates were positive for indole production. All the isolates were able to utilise glucose fermentatively. Bacteriological and biochemical test results are shown in table 6.

Table 6. Summary of biochemical tests for *M. haemolytica*, *P trehalosi* and *P multocida.*

Type of test	Type of species			Total positive
	M.Haemolytica	*P.Trehalosi*	*P.Multocida*	
Haemolysis on blood agar*	60	44	-	104
Growth on MacConkey agar	60	44	-	104
Distinct odour	-	-	11	11
Oxidase	60	44	11	115
Catalase activity	60	-	11	71
Indole production	-	-	11	11
Urease activity	-	-	-	-
H2S production in TSI Slant	-	-	-	-
Nitrate reduction	60	44	11	115
Glucose	60	44	11	115
Sucrose	60	44	11	115
Maltose	60	44	11	115
Lactose	60	-	-	60
Salicin	-	44	-	44
Xylose	60	-	-	60
Trehalose	-	44	-	44

4.2 Bacteriological findings

From 529 samples (384 nasal swabs and 145 lung lesion) collected and cultured, Pasteurella was isolated successfully in 115 sheep. Out of 115, 80 (21.1%) were from nasal cavities and 35 (24.1%) from lungs. The prevalence of *M.haemolytica* 43(11.2%), *P.trehalose* 29(7.6%) and *P.multocida* 8(2.1%) from the nasal swab sample respectively. Whereas in lung lesion, of the samples which were culture positive, 17(11.7%) of the isolate was *M. haemolytica* and 15(10.3%) of the isolate was *P.trehalose* and 3(2.1%) of the isolate was *P.multocida* . On the basis of these results *M. haemolytica* and *P.trehalose* was the most common cause of pasteurellosis in sheep at Lume district regardless of the health status, age, sex and other attributes .

Table 7. Prevalence of M. *haemolytica*, *P. trehalosi* and *P. multocida* isolates of sheep

Type-of sample	No-of sample processed	Total positive	Species identified		
			M.haemolytica	*P.multocida*	*P.trehalosi*
Nasal swab	384	80(21%)	43(11.2%)	8(2.1%)	29(7.6%)
Lung lesion sample	145	35(24.1%)	17(11.7%)	3(2.1%)	15(10.3%)
Total	529	115(21.7%)	60(11.3%)	11(2.1%)	44(8.3%)

From the total isolates *Mannheimia haemolytica, P. trehalosi* and *P. multocida* 43(11.2%), 29(7.6%) and 8 (2.1%) positive in nasal swabs respectively. Out of these isolates, 21(9.3 %) and 60 (38.2%) were isolated from apparently healthy and clinically sick sheep with respiratory syndrome, respectively. There was high significant difference ($P<0.001$) between isolates from nasal swabs of healthy and clinically sick sheep with respiratory syndrome.

Table 8. The prevalence of *M. haemolytica*, *P. trehalosi* and *P. multocida* from apparently healthy and clinically sick sheep.

Status of sheep	No (%)	Isolated species (%)		
		M.haemolytica	*P.trehalosi*	*P.multocida*
Healthy sheep	227(21)	11(4.8)	8(3.5)	2(0.8)
Diseased sheep	157(59)	32(20.3)	21(13.3)	6(3.8)
Total	384(80)	43(11.2)	29(7.5)	8(2.08)

Regardless of species of the isolates and PAs the prevalence of the isolates in Qoka was 27.2%, Sheran dibandiba 14.2%, Jido 19.4%, Biyo 23.3%and in Mojo 21.1%.

These show there were variations in distribution of the agent among the five study PAs but the variation is not statistically significant because p=0.788.

Table 9. Distribution of total positivity nasal swab among different epidemiological risk factors

Risk factors	Level	No examined	No positive	Prevalence in %	X2	P-value
PAs	Qoka	77	21	27.2	1.17175	0.788
	Sh/dibandiba	77	11	14.2		
	Jido	77	15	19.4		
	Mojo	76	16	21.1		
	Biyo	77	18	23.3		
Age	< 3mth	65	15	23.1	10.24	0.001
	4-6mth	57	16	22.8		
	7-9mth	63	14	20.3		
	10-12mth	54	11	17.3		
	1-3yrs	53	9	17.1		
	4-6yrs	47	8	17.1		
	7-9yrs	44	11	25		
Sex	Female	201	47	23.4	0.0296	0.863
	Male	183	34	18.6		
Health status	Healthy	227	21	10.2	8.858	0.0001
	Sick	157	59	38.2		

Age distribution of the total positivity shows that the isolation rate of the agent varies significantly among different age groups (P=0.001).The isolation rate of the agent increases from age categories 1-3mth (15) to 4-6mth (16), from7-9mth (14) to 10-12mth (11) ,1-3yrs (9)and from4-6year (8) to 7-9yrs (11). Whereas Sex and distribution of agent has no statistical significant variation even if there were variations observed.

When the distribution of the agent by their species was seen among PAs,there were variations observed in distribution of a single species of the isolate among PAs. Even if variation is observed among PAs distribution of isolates was independent of PAs which means they have no

statistically significant association between the isolates and PAs. Likewise age and sex distribution of isolates were examined for similarity in distribution of isolates among different age groups and sex of sheep. A Pearson chi-square test computed for existence of dependency between isolates and age of sheep shows that there was dependency between age of sheep and distribution of the disease agents which implies that at 95% confidence level distribution of isolates is dependent on age of sheep (P-value<0.05). That is statistically there is significant association between distribution of isolates and age of sheep.

Sex isolate cross tabulation shows that there was no significant variation in distribution of isolates both within female and male sheep and also between female and male sheep. That is distribution of isolates was independent of sex of sheep; sex of sheep does not limits distribution of isolates. This implies that sex (host factor) is not a determinant factor of the disease pasteurellosis in sheep.

Table 10. Distribution of Pasteurella spps among different epidemiological risk factors.

Risk factor	Level	No examined	No Positive	*M.haemolytica* No= (%)	*P.multocida* No= (%)	*P.trehalos* No= (%)	X2	P-value
PAs	Qoka	77	21	10(47.6)	3(14.3)	8(38.1)	8.197	0.224
	Sh/dibandib	77	11	5(45.5)	1(9.1)	5(45.5)		
	Jido	77	15	9(60)	1(6.7)	5(33.3)		
	Mojo	76	16	9(56.3)	1(6.3)	6(37.5)		
	Biyo	77	18	11(61.1)	2(11.1)	5(27.8)		
Age	< 3mth	65	15	7(46.7)	2(13.3)	6(40)	2.926	0.232
	4-6mth	57	13	5(38.5)	1(7)	7(53.9)		
	7-9mth	63	14	8(57.1)	1(7)	5(35.8)		
	10-12mth	54	11	7(63.7)	1(9)	3(27.3)		
	1-3yrs	53	9	4(44.4)	1(11.1)	4(44.4)		
	4-6yrs	47	8	5(62.5)	1(12.5)	2(25)		
	7-9yrs	44	11	7(63.7)	1(9)	4(36.4)		
Sex	Female	201	47	27(57.4)	3(6.4)	17(36.1)	2.462	0.292
	Male	183	34	16(47.1)	5(14.8)	8(23.6)		
Health status	Healthy	227	21	11(33.3)	2(9)	8(23.9)	4.398	0.111
	Sick	157	59	32(60)	6(10)	21(40)		

Isolates and health status of sheep shows that there were dependencies between isolates and health status of sheep, indicating that distribution of isolates were dependent of health status of sheep.

For the purpose of *Mannheimia* and *Pasteurella* isolation, lung lesion of sheep were examined from slaughtered sheep at voluntary hotels in which highest number of sheep slaughtered on daily basis were selected for three months, November, December and January during the study period. Sampling of the specimen was performed at a time.

From 145 lung samples collected and cultured, Pasteurella was isolated successfully in 35 (23.4%) sheep. Out of 35, the prevalence of *M.haemolytica* 17(11.7%), *P.trehalose* 15(10.3%)

and *P.multocida* 3(2.1) % from the lung lesion sample respectively. On the basis of these results *M. haemolytica* and *P.trehalose* was the most common cause of pasteurellosis in sheep at Lume district. However, the observed prevalence of isolates from lung in sex and age group has no impact on the isolation of pasteurella species.

Table11. Distribution of total positivity lung lesion among different epidemiological risk factors

Risk factor	**Level**	**No sample**	**No positive**	**Prevalence**	**X2**	**P-value**
Age	young	35	16	45.7	2.7560	0.097
	Adult	110	23	20.9		
Sex	Female	31	8	25.8	1.1348	0.567
	Male	114	31	27.1		

4.3 Sero typing

Sheep Sera collected from Lume districts East shoa Zone of Oromia region, namely Qoka, Sheran dibandiba , Jido, Biyo and Mojo were serotyped using the indirect haemoagglutination (IHA) test as per Biberstein (1978) for serotyping of *M. haemolytica,P.multocida and P.trehalosi*. A total of 150 serum samples, thirty sera from each PAs were examined for specific antibodies. The *M. haemolytica.P.multocida and P.trehalosi* serotype positives have been computed for each PAs.

Table 12. Prevalence distribution of *Pasteurella* serotypes in sheep sera in different PAs of Lume districts

Mannhaemia/ Pasteurella serotypes	**Seropositive (%) at dilution ≥1/20**					
	Jido No=30	Qoka No=30	Sh/dibandiba No=30	Mojo No=30	Biyo No=30	Total No (%)
A1	7(23.3)	3(10)	14(46.6)	9(30)	2(6.6)	35(23.3)
A2	17(56.6)	15(50)	11(36.6)	9(30)	13(43.3)	65(43.3)
A7	13(43.3)	7(23.3)	8(26.6)	11(36.6)	9(30)	48(32)
PA	5(16.6)	4(13.3)	4(13.3)	6(20)	3(10)	22(14.6)
T3	18(40)	17(56.6)	15(50)	16(53.3)	11(36.6)	77(51.3)
T4	12(40)	6(20)	9(30)	7(23.3)	8(26.6)	42(28)
T10	8(26.6)	11(36.6)	7(23.3)	9(30)	9(30)	44(29.3)
T15	13(43.3)	8(26.6)	9(30)	7(13.3)	8(26.6)	45(30)

Variation in prevalence among the different serotypes was observed,in mojo district, serotype A1 (30%) followed by A2 (30%) and A7 (36.6%),PA(20%),T3(53.3%),T4(23.3%),T10(30%) and T15(23.3%). in Qoqaa A1 (10%), A2(50) and A7 (23.3 %) PA(13.3%),T3(56.6%),T4(20%),T10(36.6%) and T15(26.6%)., each, in Sheran Dibandiba A1 (46.6%), A2 (36.6 %) , A7 (26.6%), PA(13.3%),T3(50%),T4(30%),T10(23.3%) and T15(30%). In Jidoo A1 (23.3%), A2 (56.6%) and A7 (43.3%), PA(16.6%),T3(40%),T4(40%),T10(26.6%) and T15(43.3%). and in Biyo, A1 (6.6%), A2 (40%) , A7 (30%) , PA(10%),T3(36.6%),T4(26.6%),T10(30%) and T15(26.6%). were the dominant serotypes detected.

In present study results there was higher prevalence of *M. haemolytica* A1 (23.3%), A2 (42.6%), A7 (32%), T3 (51.3) ,T10(29.3) and T15(30%) serotypes were lower prevalence of *P.multocida* biotype A (14.6%) and T4 (16%) were recorded in the study areas.

Table 13. Overall Prevalence of Ovine Pasteurellosis in the Study Districts

Sero-types of ovine pasteurollosis	**Total**	**Prevalence %**
M.hemolytica A1	35	23.3%
M.hemolytica A2	64	42.6%
M.hemolytica A7	48	32%
P.multocida biotype A	22	14.6%
P.trehalosi T3	77	51.3%
P.trehalosi T4	24	16%
P.trehalosi T10	44	29.3%
P.trehalosiT15	45	30%

5. DISCUSSION

Pasteurellosis in sheep caused by *M. haemolytica* and *P. trehalosi* have posed health problem in most part of sheep breeding and rearing regions of Ethiopia due to the significant economic losses they causes through mortality, morbidity, and the high cost of treatment Gelagay, *et al*., (2004). In this study, an attempt was made to differentiate between *M. haemolytica*, *P. trehalosi* and *P. multocida* based on their growth on MacConkey agar, haemolytic pattern, Oxidase and Catalase activity, Indole and H2S production, colony morphology and fermentation of different sugars with acid production Quinn *et al., (*2002) .

The aetiological agent of pasteurellosis in sheep was found to be wide spreading in the study area. Though there were little variation in biochemical characteristics, the results of morphological, staining, colony, cultural and biochemical activities were in total agreement with those documented by Merchant and packer (1983), Carter and Chengappa (1991), Carter (1994) and Quinn *et al.,* (2002).

The haemolytic activity of *M haemolytica* and *P. trehalosi* isolates were lost after subsequent subcultures and were in agreement as reported by Carter and Chengappa (1991), Quinn *et al.* (2002). Two distinct colony types were observed on MacConkey agar, lactose fermenter (*M. haemolytica*) with pink and non-lactose fermenter *(P. trehalosi*) other than pink colour. Whereas the *P. multocida* isolates neither grew on MacConkey agar nor haemolysed sheep red blood cells as observed by Carter and Chengappa (1991) and Quinn *et al* (2002).

The isolates of *M. haemolytica, P. trehalosi* and *P. multocida* were positive for oxidase test and negative for urease, HS2 production in TSI agar slant, and Indole test. Further they utilized glucose fermentatively, but the *P. multocida* isolates were indole positive which coincided with those described by Quinn *et al* (2002*).* The *Mannheimia haemolytica* isolates fermented xylose and lactose, but failed to ferment trehalose and salicin, and were catalase positive, whereas isolates of *P. trehalosi* were fermented. trehalose and salicin, and were catalase negative the results are in agreement with Carter (1984) and Quinn *et al* (2002).

In the present study, *M. haemolytica and P. trehalosi* were isolated at the rate of 11.2% and 7.6% from nasal swabs, 11.7 % and 10.3% from pneumonic lungs, respectively. Isolation of *M.*

haemolytica and *P. trehalosi* from nasal swab and pneumonic lungs may indicate that these species are important species in the induction of pneumonic pasteurellosis in the study area.

The low percent of (2.1%) both from nasal swab and lung lesion P. *multocida* isolates might indicate the occasional involvement of this species in the pneumonic pasteurellosis and similar reports have been made by Merchant and Packer (1983) Carter and Chengappa (1991), Aschalew (1998), Mekonen (2000), Sisay and Zerihun (2003) and Belay (2007). In contrary to our observation Assefa *et al* (2004) and Mesele (2005) reported high incidence rates of 15.4% and 25% of P. *multocida* from sheep slaughtered at Jijiga and ELFORA abattoir respectively. This might be due to the time of sampling and geographical variation, where sampling was conducted in June and sampled sheep were from Somali lowlands where very poor Veterinary infrastructure and vaccination against pasteurollosis (*P. multocida* biotype A) was not conducted for considerable number of years, and animals were transported long distance before being slaughtered at both abattoir, whereas, our study was conducted , where regular vaccination was done in sheep with monovalent *P. multocida* vaccine which is attributed to the low incidence of infection.

From total isolates of nasal swabs of clinically sick sheep with respiratory syndrome, *M. haemolytica* and *P. trehalosi* constituted 20.3 % and 13.3% in their proportion, respectively. This result is in agreement with the findings of Tesfaye (1997) and Aschalew (1998) in highlands of central Ethiopia, where they reported 28.8 % and 35 % for *M. haemolytica* and 42 % and 58.3 % for *P. trehalosi*, respectively from nasal swabs of sheep with pneumonic symptoms. The high proportion of *Mannheimia* and *Pasteurella* species in nasal swabs of clinically sick sheep could be due to these organisms are normal inhabitants of the upper respiratory tract of sheep and invasion to the lower tissue (lung) of clinically sick sheep when the immune system of the animals is compromised by different factors (Lopez, 1995) and pneumonia associated with these bacteria species recorded as primary diseases in sheep elsewhere (Shewan, 1986; Gilmour, 1993).

Accordingly, there was a significant difference existing among isolation rates at different time in different areas even if there is no significant difference in isolation rates among the three species in all of the studies at different time and areas. This implies that there must be a continuous survey to be held in different areas to know a recent rate for each of the species implicated for

ovine pasteurellosis and to design a cost effective and efficient prevention and control strategies suited for each area.

Age distribution of the three species associated with ovine pneumonic pasteurellosis disclose that there was an association in distribution of the species among different age groups of sheep and at the same time distribution of the species was also associated with age groups of sheep. According to the present study different age group distribution of the isolates looked like as follows: within age group 1-3mth it was 23.1%, 4-6mth (22.8%), 7-9mth (22.2%), 10-12mth (20.3%), 1-3year (17.1%), 4-6year (17.1%) and 7-9 year it was (25%). This was in agreement with that of the result obtained by Behailu (2012,) kuod (2013). This result ease also in agreement with findings of Gilmour and Gilmour [26], that elucidates pneumonic pasteurellosis occur in all ages of sheep and goats, with the most susceptible in lambs and kids during first life, and dams at lambing but not agreement with Zuber (2009) in Iraq with slight fluctuation in age grouping which was in 1-3mth age category it was 2.1%, 4-8mth (7.7%) and 9-12mth (7.1%).

Sex shows that there was no significant variation in distribution of the species between female and male sex and that the distribution was independent of sex of sheep. In the present study prevalence of the agent in female sheep was 23.4% while it was18.6% in male. This result was in agreement with Behailu (2012) where female 29.2% and male 24.6%, and also with Belay (2007) and Kuod (2013).

The prevalence of *M. haemolytica* , *P.trehalosi* and *P.multocida* was almost similar in nasal swabs(11.2%),(7.6%) and (2.1%) and in lung sample (11.7%),(10.3%) and
(2.1%) respectively. In this study *M. haemolytica* and *P. trehalosi* in pneumonic lungs may indicate that these species are important species in the induction of pneumonic pasteurellosis in the study area. The low percent of (2.1%) P. *multocida* from lung lesion isolates might indicate the occasional involvement of this species in the pneumonic pasteurellosis. No associations were observed between the risk factors and pneumonic pasteurellosis at lung lesion sample ($P > 0.05$).This study result is in agreement with the reports of Marru (2013), *M. haemolytica* (21.96%) and absolute prevalence of *P.multocida* and Kaoud (2010) prevalence of 52% for *Mannheimia* and 42% for *P. trehalosi.*

The serological results showed that the predominance distribution pattern of *M. haemolytica and P.trehalosi* serotypes between the PAs of Lume districts were almost similar. This might be due to similar agro climatic and animal management systems.

The results of IHA test were tabulated to know the overall prevalence and distribution of *M, haemolytica* serotypes in East shoa zone of Lume districts. The prevalence of each serotype was varied. Of 150 sheep sera tested, A1 (60 %), A2 (42.6%), A7 (32%) and PA (14.6%) ,T3(51.3%),T4(16%),T10(29.3) andT15 (30%) were detected. A mong A1 (60 %), A2 (42.6%), A7 (32%) and PA (14.6%) ,T3(51.3%), T10(29.3) and T15 (30%) were the dominant serotypes and *P.multocida* biotype A were the least detected serotypes.

The result of this study is in agreement with the reports of Yeshwas Ferede(2013) P.*haemolytica* A1 (33.1%), A2 (28.5%) and A7 (31.8 %) serotypes where as a lower prevalence of *P. multocida* biotype A (6.6%) were recorded in South Gonder Zone Farta and Lay Gayint districts, the result this study was also in agreement with that of Megersa (1996) and Sisay and Zerihun (2003) who reported that 29 % and 16.9 % prevalence for A1, in East and Northeast Ethiopia, respectively, which was the most prevalent serotype.

Pegram *et al* (1979) was also identified in Ethiopia the most prevalent serotype was A2, while A14 and A9 were the least identified. Hussein and Mohammed (1984) in Sudan identified the predominant A2 and A6 serotypes and A13 and A14 were among the lowest identified serotypes. Frank (1982) in the United States, Frank (1982) in the United States (1988) In Hungary, Prince *et al* (1985) and Gilmour and Gilmour (1989) in New Zealand identified A2 as the most prevalent serotype.

Ayelet (1998) also identifies in central highland of Ethiopia A2 and A6 were the most dominant ones, while A8 was the least screened serotype and Kirkan and Kaya (2005) and Lhan and Keles (2007) in Turkey identified A2 and A6 as most prevalent and A8 was the least identified respectively, which was the most prevalent serotype. This deference in dominance of serotype might be due to geographical location, the time of sampling, serotypes involved in pasteurellosis which varied from year to year, area to area and flock to flock (Gilmour and Gilmour, 1989).

CONCLUSION AND RECOMMENDATIONS

In East shewa zone Oromia region in Lume districts, sheep are the most kept animal than other species and plays an important role in the economy and livelihood of farmers. These small ruminants serve as a principal source of cash income for household expense as well as domestic consumption. However, efficient utilization of this resource is impaired by different factors, such as health problem, poor management and shortage of feed.

Knowing epidemiology of the disease and contributing factors to the disease has a paramount importance in designing a cost effective prevention and control method. For that matter knowing prevalence of the disease to strain level helps one to design a best intervention method using vaccine or antibiotics. Management practices directed at reducing stress are important in preventing *Pasteurella*/*Mannheimia* associated disease in sheep.

Both bacterial and serological results of this study showed that the causative agents of pasteurellosis are prevalent in the area, and serotypes A1,A2, A7, T3,T10 and T15 were dominant over the other serotypes.The current pasteurellosis vaccine, which is used for the control and prevention of pneumonic pasteurellosis, does not include more prevalent agents in the field apart from the strains of *M. haemolytica* which are incriminated in causing pasteurelosis. On the basis of the results obtained the following are recommendations are proposed.

- Extension service should reach public that pasteurellosis is a disease which can be prevented by implementing a management strategy which can reduce/minimize stress.

- Extensive /comprehensive investigation should be taken for the determination of the magnitude and the distribution of the various serotypes of *Mannheimia* and *Pasteurella* in different parts of the country.

- Developing a polyvalent vaccine consisting of the most predominant serotypes of *Mannheimia haemolytica* which are circulating in sheep in the study area is of paramount importance

6. REFERENCES

Ackermann, M.R., Brogden, K.A., (2000): Response of the ruminant respiratory tract to *Mannheimia (Pasteurella) haemolytica. Microbes and Infection*, **2**, 1079-1088.

Ames, T. R., R. J. F. Markham & A. J. Opuda.,(1985). Pulmonary response to intratracheal challenge with Pasteurella haemolytica and Pasteurella multocida. Canadian Journal of Angen O., R. Mutters, D. A. Caugant, J. E.Olso

Angen, O., Mutters, R.,Cuagant, D..A., Olsen, J.E. and Bisgaard, M. (1999): Taxonomic relation of the *(Pasteurella) heamolytica* complex as evaluated by DNA-DNA hybridizations and 16s rRNA sequencing with proposal of *Mannheimia haemolytica* gen. nov.comb.nov., *Mannheimia granulomatis* comb,nov., *Mannheimia glucosida* sp.nov., *Mannheimia ruminalis* sp.nov. and *Mannheimia verigena* sp. Nov. int. *J. Syst. Bacteriol*., **49**:67-86.

Anon (2004): State of Ethiopians Animal Genetic Resource Country Report. Addis Ababa, Ethiopia: A contribution to the First Report on the State of the World's Animal Genetic Resource. Institute of Biodiversity Conservation (IBC):**74**.

Aydın N(1997): Pasteurellaceae family ası. In, Arda M, Minbay A, Leloğlu N, Kahraman M, Akay Ö, Ilgaz A, İzgür M, Diker KS (Eds): Özel Mikrobiyoloji, Epidemiyoloji, Bakteriyel ve Mikotik İnfeksiyonlar. 4. Baskı, s. 64-74. Medisan,Ankara,.

Ayelet,G.(1996):Epidemiology and serological investigationon mutifactorial ovine respiratory disease and vaccine trials on the highlands of north shoa (Ethiopia) DVM thesis.AddisAbaba University,Faculty of Vet .Medicine ,Debrezeit.

Belay, M. (2007)Study of Ovine Pasteurollosis in Wollo Zone of Amhara region,Ethiopia. DVM thesis, Addis Ababa,FVM,Debrezeit.

Bell, S. (2008): Respiratory disease in sheep; Differential diagnosis and epidemiology.In Practice. *Apr*., **30**: 200-207.

Biberstein, E. L. & D. C. Hirsh, (1999). Pasteurella. In: Veterinary Microbiology, eds D. C. Hirsh & Y. C. Zee, Blackwell ScienceInc., pp. 135.

Biberstein, E. L. and Thomson, D. A. (1996): Epidemiological studies on *Pasteurella haemolytica* in sheep. *J. Comp. Pathol*., **71:**83–94.

Biberstein, E. and Gills, M. (2002): The Relationship of the Antigenic Types of the A and T Types of *Pasteurella haemolytica. J Comp Path;* **72**, 316-320.

Biruk, T. and Tesfaye, S. and Genene, T. (2013): Diversity of bacterial species in the nasal cavity of sheep in the highlands of Ethiopia and first report of *Histophilus somni* in the country. *Trop. Anim. Health Prod*., **45**:1243–1249.

Bisgaard, 1999a. Taxonomic relationships of the [Pasteurella] *haemolytica* complex as evaluated by DNA-DNA hybridizations and 16S rRNA sequencings with proposal of Mannheimia haemolytica gen. nov., comb. nov., *Mannheimia granulomatis* comb. nov., *Mannheimia glucosidal* sp. nov., *Mannheimia ruminalis* sp.nov. and *Mannheimia varigena* sp. nov. International Journal of Systematic Bacteriology,**49**, 67−86.

Bisgaard, M., Philips, J. E. and Mannheim, W. (1986): Characterization and identification of bovine and ovine *Pasteurellaceae* isolated from the oral cavity and rumen apparently normal cattle and sheep. *Acta. Pathol. Microbiol. Immunol. Scand*., **94:**9-17

Blood, D.C. and Radostits, O.M. (1994): Disease caused by Pasteurella species. A Textbook of Cattle, Sheep, Pigs , Goats and Horses. 8th ed. Brailliere Tindal, London, pp: 748–785.

Boyce, J. D., Chung, J. Y. and Adler, B. (2000): *Pasteurella multocida* capsule: composition, function and genetics. *J. Biotech.*, **83**:153–160.

Brogden, K. A., Lehmkuh, H. D. and Cutlip, R. C. (1998): *Pasteurella haemolytica* complicated respiratory infections in sheep and goats. *Vet*. *Res*. *May-Aug*., **29**(3-4): 233 54.

Carter, G. R. (1984): *Pasteurella, Yesinia* and *Francisella*. In: Diagnostic Procedures in Veterinary Bacteriology and Mycology .4th ed. Charles, C. Thomas publishing, U.S.A, Pp 111-118.

Kisiela, D. and Czuprynski, C. *(2009):* Identification of *Mannheimia haemolytica* Adhesins Involved in Binding to Bovine Bronchial Epithelial Cells. *Infect Immun*, **77**, 446-455.

De Alwis**,** M.C.L. (1992): Pasteurellosis in Production Animals. An international workshop sponsored by ACIAR held at Bali, Indonesia, pp: 1-39**.**

Donachie, W. (2000): Pasteurellosis; Diseases of sheep. Blackwell Scientific Oxford, pp: 191-197.

Elsheikh, M. H. and Hassan, O. S. (2012): Pneumonia in Goats in Sudan. *Int. J. of Ani.* and *Vet. Advances*, **4**(2): 144-145.

Eshetu,M.(1991):Pneumonic pastuerollosis in sheep slauthered at An abatoir.DVM.thesis,AddisAbaba university Faculity of Vet.Medicine.Debrezeit.

FAO (Food and Agriculture Organization of the United Nations (1999): Recognizing Peste Des Petits Ruminants. A Field Manual. www.fao.org/docrep/003/x1703e/x1703e00.htm

Gautam, R., Kumar, A. A. Singh,V.P. Singh,V.P., Dutta, T.K. and Shivachandra, S.B. (2004): Specific identification of *Pasteurella multocida* serogroup-A isolates by PCR assay. *Res.* in *Vet. Sci.* **76**: 179–185.

Gentry, M. J., Confer, A. W. and Craven, R., C. (1987): Effect of repeated in vitro transfer of *Pasteurella haemolytica* A1 on encapsulation, leukotoxin production, and vaccine. *J. Clin. Microbiol*., **25:**142-145.

Gilmour NJL., Gilmour JS., (1989): Pasteurellosis of sheep. In, Adlam CF, Rutter JM (Eds): Pasteurella and Pasteurellosis. pp. 223-262. Academic Press Inc, New York

Habashy, H.F., Fadel. N.G. and El Shorbagy. M.M. (2009): Bacteriological and Pathological Studies on the Causes of Mortalities among Sheep in Sharkia-Governorate Farms. *Egypt. J. Comp. Path.* and *Cli. Path*, **22**(1): 130 – 146.

Hassan, S. A. O., (1999). Aerobic Bacteria Associated with Goat Pneumonia in Sudan. M.V.Sc. Thesis, Faculty of Veterinary Science, University of Khartoum, Sudan. Hayashidani, H., E. Honda, T. Nakamura, Y. Mori, T. Sawada & M. Ogawa, 1988. Outbreak of pneumonic pasteurellosis caused by Pasteurella haemolytica infection in Shiba goats in Japan. Japanese Journal of Veterinary Science, **50**,960-962.

Hawari, A.D, Hassawi, D.S. and Sweiss, M. (2008): Isolation and Identification of *Mannheimia haemolytica* and *Pasteurella multocida* in Sheep and Goats using Biochemical Tests and Random Amplified Polymorphic DNA (RAPD) Analysis. *J. of Biol. Sci.*, **8** (7): 1251-1254

Higlander,S.K.(2001):Molecular genetic analysis of Mnahemia or Pasteurella.In Frountiers in Bioscience **6**,Pp228-115.

Hussein, A.M., and Elsawi Mohamed, O. A. (1984): Serological survey of sheep sera for antibodies to *Pasteurella haemolytica* serotypes in Sudan. *Rev Elev Med Vet Pays Trop.*, **37**:418–421.

Jasni, S., Zemari-Saad, M., Kamal- Hizat, A., Mutalib, A. R., Salim, n. and Shekih- Omar, A.R. (1990): Seasonal occurrence of caprine pneumonic pasteurellosis in central Peninsular Malaysia. *J. Vet. Malaysia*, **2**(2): 147-148.

Kaoud, H., El-Dahshan, A.R., Zaki, M.M. and Nasr, S. A. (2010): Occurrence of *Mannheimia haemolytica* and *Pasteurella trehalosi* Among Ruminants in Egypt. *NewYork Sci. J*.**,** **3**(5):135-141

Kilian, M. and Fredericksen, W. (1981): Identification Tables for the *Haemophilus*-Pasteurella-Actinobacillus group. In: Kilian, M., W. Frederiksen, and E. L. Biberstein (eds) *Haemophilus*, *Pasteurella* and *Actinobacillus*, Academic Press, London, Pp 281-290.

Kopcha, M. (1997): *Pasteurella* and *Mannheimia* Pneumonias in Sheep and Goats. Merck Veterinary Manual, White house Station, N.J., U.S.A.

Kusiluka, L. and Kambarage, D. (1996): Diseases of Small Ruminants. Hand Book, P: 25.

Laurence etal.,O.W., Maheswern, S.K., Weis,D.s.and Ames, T.R.(1990):Immunohitological localization of *P.haemolitica* A1-derived endotoxin and Capsular polyacchrides in experimental bovine pneumonic pasteurollosis.Vet Path.**27**.Pp.150-161.

Lhan, Z and Keles, H. (2007): Biotyping and serotyping of *Mannheimia* (*Pasteurella*) *haemolytica* isolated from lung samples of slaughtered sheep in the Van Region. *Turk. J. Vet. Anim. Sci*., **31:**1-5.

Malone, F.E. (1991): A study of respiratory disease in housed fattening lambs. FRCVS Thesis, Royal College of Veterinary Surgeons, London, pp: 1-7.

Michael, W. (2008): Pasteurellosis Transmission Risks between Domestic and Wild Sheep. CAST Commentary. Pp 325.

Mohamed, R. A. and Abdelsalam, E. B. (2008): A review on Pneumonic Pasteurellosis (respiratory *mannheimiosis*) with emphasis on pathogenesis, virulence mechanisms and predisposing factors. *Bulg. J. Vet. Med*., **11** (3): 139−160.

Mokonnen,T.(2000):An Epidemiological study on Ovine Pasteurollosis Arsi,South east Ethiopia.DVM thesis ,AAU,FVM.Debrezeit.

Moredun: Controlling Pasteurella Pneumonia in Sheep,(2009). Edition of the Sheep farmer ww.moredun.org.uk/webfm_send/340.

NCBI (2004): The NCBI taxonomy browser, ttp://www.ncbi.nlm.nih.gov/taxonomy

OBoARDAR (2013): Oromia Bureo of Agriculture and Rural Development Annual

OIE, (1992): Diagnosis of pasteurollosis In: Manual of stand for diagnostic test and vaccine in inter.animals.**2**, Pp, 1-27.

Prabhakar, P. Thangavelu, A., Kirubaharan, J. J. and Chandran, N. D.J. (2012): Isolation and Characterisation of *P.Multocida* Isolates from Small Ruminants and Avian Origin. *Tamilnadu J. Vet.* and *Ani. Sci.*, **8** (3): 131-137.

Quinn, P. J., Carter, M. E., Markey, B. and Carter, G. R. (1994): *Pasteurella* species .in: Clinical Veterinary Microbiology. Wolfe publishing. Mosby, London, Pp 254-258.

Quinn, P. J., Morkey, B. K., Carter, M. E., Donnelly, W. J. C., Leonard, F. C. and Maguire, D. (2002): *Pasteurella* species and *Mannheimia haemolytica.* In: Veterinary Microbiology and Microbial Disease. Blackwell publishing company: Blackwell science. London, Pp. 137-142.

Quire, M.W., Donachie, W. and Gilmour, N. J. L. (1986): Serotypes of *Pasteurella haemolytica* from cattle. *Vet. Rec*., 19:93–94.

Radostits, O. M., Blood, D. C., Gay, C. C. (1994): Diseases caused by *pasteurella* species. In: Veterinary Medicine, Textbook of the Disease of cattle, sheep, pigs, goats and horses. 9th ed. Bailliere Tindall, London, Pp 770-774.

Ragy, S. (2005): Major Pathogenic Components of *Pasteurella multocida* and *Mannheimia (Pasteurella) haemolytica* Isolated From Animal Origin. *Vet Micro, **17**, 312-356.*

Ranjan, R., Panda, S.K. Acharya, A.P., Singh, A.P. and Gupta, M.K. (2011): Molecular Diagnosis of Haemorrhagic Septicaemia - A Review. *Vet. Worl*., **4**(4):189-192.

Rimler,R.B.(1993):Laboratory technique for Pasteurollosis typing and diagnosis. In:past in production animals, Areview in :pattern,B.E.,Spencer,T.,and Jonson,R.B (ed).ACIAR,Australian,Conbera.**43**,Pp,136-200.

Richard, W. (2009): Outer Membrane Proteomics of *Pasteurella multocida* Isolates to Identify Putative Host-Specificity Determinants. *Bioscience Horizons,* Volume2, Issue1, pp1-12.

Sarah, J. (2011): Adherence of *Mannheimia haemolytica* to Ovine Bronchial Epithelial Cells. *Bioscience Horizons,* **4** (1), 50-60.

Sherrill, A. (2012): Overview of Pasteurellosis of Sheep and Goats. Merck Veterinary Manual, White house Station, N.J., U.S.A.

Shiferaw, G.,Tariku, S., Gelagay, A , Abebe, Z. (2006): Contagious Caprine Pleuropneumonia and *Mannheimia haemolytica*-associated acute respiratory disease of goats and sheep in Afar Region, Ethiopia. *Rev. sci. tech. Off. int. Epiz*. **25** (3):1153-1163.

Sisay.T.and Zerihun ,A.(2003):Diversity of *M.haemolytica* and *P.treholsi* serotypes from apparent healthy sheep and abattoir specimens in the high land of Wollo ,north east Ethiopia .Vet.Reserch. **27**,Pp.3-14.

Sloxome, R. F., J. Malark, R. Ingersol, F. Derksen & N. Robinson, (1985). Importance of neutrophils in the pathogenesis of acute pneumonic pasteurellosis in calves. AmericanJournal of Veterinary Research, **46**,2253-2258.

Smith. R. and Phillips, J.E.(1990): *Pasteurella* and *Actinobacillus*. In: Parcker M. T Duerden B. I.(eds): Topley and Wilson‟s. Principles of Bacteriology, Virology and Immunology. 8th ed. B.C Decker Inc.U.S.A. Pp 383-399.

Tesfaye M (1997): Serological and bacterial investigation of *P.haemolytica* serotypes in sheep in high land of Wollo (North east Ethiopa).DVM thesis, Addis Ababa,FVM,Debrezeit.

Zeleke, A. (1998): A study of ovine pneumonic pasteurellosis in North Shoa. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

Zuber, I. (2009): Epidemiological Study of Pasteurellosis in Lambs in Erbil Governorate, Northern Iraq. *UDC***, 619**, 579.843.

7. APPENDIES

Annexe 1. Procedure of IHA

According to Sawada (1982) the procedures are indicated as follows:

Antigen preparation

- Extract the antigens by heat extraction method followed by centrifugation.
- Seed reference strains on tryptose-serum agar and incubate at 37°C for 18 -20 hours (four serotypes deal with at a time)
- Alternatively, culture the serotypes in tryptose-serum broth and incubated for 18-20 hours at 37°C
- Harvest the growth in PBS in proportion of 20 to 30 colonies in 10 ml PBS
- Centrifuge cultures at 2000 rpm for 20 minutes and re suspend the sediment in equal volume of PBS
- Heat this suspension in water bath at 60°C for an hour to kill viable organisms, centrifuge at 5,500 rpm for 15 minutes at 4°C by refrigerated centrifuge
- Discover clear supernatant fluid and used as capsular antigen extract

Sensitisation of SRBC

- Draw blood from the jugular vein of sheep freely flowing into a syringe containing Alsever`s solution, take 75 ml sheep blood in 125ml Alsever`s solution. Add small amount of crystalline penicillin to avoid bacterial contaminants. Store at +4^{o}C at least one day overnight, the blood can be used for about 2 weeks
- Wash three times in PBS by centrifugation at 2000 rpm for 10 minutes
- Add 100 µl of packed (PCV) RBCs to 10 ml of each antigen
- Add 50 µl of 50% gluteraldehyde and homogenise by gentle shaking and incubated for one hour at 37°C with periodical shaking
- Centrifuge at 2,000 rpm for 10 minutes and wash two times in PBS by centrifugation
- Finally add 10 ml of PBS to the final sediment and made up to 1% suspension

Test procedure

- For screening positive sera, add 95 µl of 1XPBS into micro plate (control) rows A1-A12, C1-C12, E1-E12, G1-G12, and add 5 µl of test sera in the same wells from the pre plates. The final dilution is $1/10^{th.}$ Transfer 50 µl diluted test sera in the (test samples) rows B1-B12, D1-D12, F1-F12, H1-H12, and add 50 µl of sensitized SRBCs to respective wells. Add 50 µl of unsensitized 1% SRBC to the control micro plate rows in parallel and incubate in moist chamber for one hour at 37°C
- Add 100 µl 1/10 dilution in PBS to the first rows of the plate in duplicates
- Transferring 50µl to the other wells (1:10, 1:20, 1:160, etc…) make a serial double fold dilution and discard the final 50µl dilution sera
- Add Control tests, in which sensitised and unsensitised SRBC's to respective positive and negative sera parallel in every test
- Cover the plates with micro plate sealer to prevent evaporation and incubated at 37^0C in moist chamber for 60 minutes with constant agitation
- Complete and coarse agglutination of red cells indicates a positive reaction; small button of deposited cells are a negative reaction.
- ≥ 50% agglutination rate is taken as positive

Annex 3. Serum Sampling Format

Date_____

Animal ID: _____

Owner's name: _____

Address: District/Kebelle: _____

Breed_____

Spp: _____

Sex: _____

Age: _____

Heart girth measure (cm): _____

Annex 4. Determination of body weight and body condition score

According to Hamito (2009) and Sezenler *et al.* (2011) the formula is describes as follows:

LW (Kg) = -30.77+0.82HG (cm) for male

LW (Kg) = -31.0+0.80HG (cm) for female

BCS = LW/-30.77+0.82HG for male

BCS = LW/-31.0+0.80HG for female

Annex 5. Determination of age with different numbers of erupted permanent incisors

Source: Hamito (2009).

No. of permanent incisors	Estimated age range	
	Sheep	Goats
0 pair	Less than 1 year	Under 1 year
1 pair	1-1½ years	1-2 years
2 pair	1½-2years	2-3 years
3 pair	2½-3years	3-4 years
4 pair	More than three years.	More than four years
Broken mouth	Aged	Aged