Thesis Ref. No._____

SERO - EPIDEMIOLOGY OF *MANNHEMIA, PASTEURELLA* AND *BIBERSTEINIA* SEROTYPES IN SMALL RUMINANTS IN TANQUA-ABERGELLE DISTRICT, TIGRAY, ETHIOPIA

MSc Thesis

By

Kassaye Berhe

Department of Clinical Studies

MSc Program in Tropical Veterinary Epidemiology

September, 2014

College of Veterinary Medicine and Agriculture, Bishoftu

SERO - EPIDEMIOLOGY OF *MANNHEMIA, PASTEURELLA* AND *BIBERSTEINIA* SEROTYPES IN SMALL RUMINANTS IN TANQUA-ABERGELLE DISTRICT, TIGRAY, 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

Kassaye Berhe

September, 2014

College of Veterinary Medicine and Agriculture, Bishoftu

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 Kassaye Berhe entitled SERO - EPIDEMIOLOGY OF *MANNHEMIA*, *PASTEURELLA* AND *BIBERSTEINIA* SEROTYPES IN SMALL RUMINANTS IN TANQUA-ABERGELLE DISTRICT, TIGRAY, ETHIOPIA and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Science in Tropical Veterinary Epidemiology.

_____	_____	_____
Chair man (title and name)	Signature	Date
_____	_____	_____
External Examiner (title and name)	Signature	Date
_____	_____	_____
Internal Examiner (title and name)	Signature	Date
Dr.Reta Duguma (DVM, MSc, Assistant Professor, PhD Fellow)	_____	_____
Major Advisor	Signature	Date
Girmay W/selassie (DVM, MSc)	_____	_____
Co- Advisor	Signature	Date
Dr. Fufa Abunna (MSc, Associate Professor)	_____	_____
Department Chairman	Signature	Date

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

STATEMENT OF AUTHOR

First, I affirm that this thesis is my *banafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced 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: Kassaye Berhe Signature: _____

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: _____

ACKNOWLEDGEMENTS

First, I would like to praise the Almighty God and his mother, for giving me strength while lacking courage in my journey of life.

Special thanks also for my advisor Dr. Reta Duguma for his valuable comments and critical corrections of this paper in addition to his un reserved courage and sharing knowledge in two years of my study. My heartfelt appreciation and thanks also extend to Management of Arbegona Agricultural Development Office, Sidama Zone of Southern Nations and National Peoples of Regional Government for giving me the promotion of MSc. Program in addition to giving me motivating award in three years of my work time, Mekelle Regional Laboratory (especially, Dr. Girmay Weldeselassie, head of Mekelle Regional Laboratory for his un reserved help in facilitating and financing this research work, Dr. Shiferaw Jenberie, head of National Veterinary Institute Serology Laboratory for his kindness in provision of laboratory facilities and financing this research work and all laboratory technologist found in both laboratories, finally but not least for Tanqua-Abergelle Agricultural Development Office, Central Zone of Tigray Regional state for their highly interested on my work and initiating for the developmental assistances of the district in order to help me in creating awareness for farmers of the district.

ABBREVATIONS

AAU	Addis Ababa University
B.	*Bibersteinia*
CA	Coagglutination test
CCPP	Contagious Caprine Pleuro Pneumonia
CSA	Central Statistics Authority
ELISA	Enzyme Linked Immuno Sorbent Assay
FAO	Food and Agriculture Organization
IHC	Immuno Histo Chemical
IHA	Indirect Haemagglutination Test
LKT	Leuko Toxin
LPS	Lipo Poly Saccaride
M.	*Mannhaemia*
NVI	National Veterinary Institute
P.	*Pasteurella*
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PI	Para Influenza
PPR	Peste des Petits Ruminants
RSV	Respiratory Syncytial Virus
SPP	Species
SRBC	Sheep Red Blood Cell
TBoARDAR	Tigray Bureau of Agriculture and Rural Development Annual Report.
TLU	Tropical Livestock Unit
USA	United States of America

LIST OF TABLES **PAGES**

LIST OF FIGURES **PAGES**

LIST OF ANNEXES **PAGES**

ABSTRACT

A study was conducted from November 2013 up to June 2014 on epidemiology of Mannhaemia, Pasteurella and Bibersteinia serotypes in sheep and goats in Tanqua-Abergelle district, Central Tigray of Ethiopia. Therefore, the aim of this study was to identify the current serotypes circulating, to measure their magnitude and to identify the risk factors in sheep and goats. Cross-sectional study design was employed. Accordingly, serum from 192 sheep and192 goats of jugular vein were aseptically collected and transported to National Veterinary Institute, Bishoftu, Ethiopia. Indirect haemagglutination test was used for serotyping using known antigens for 8 serotypes. Hence, eight serotypes were identified in both species, namely M. haemolytica serotype A1 (79.4%), A2 (73.7%), A7 (65.5%), B. trehalosi serotype T3 (84.4%), T4 (62.5%), T10 (41.4%), T15 (89.6%) and P. multocida type A (66.9%). Mixed serotypes co-infections were quite common in the area in this study. Majority (57%) of the studied shoats had been also infected simultaneously with 6-8 serotypes. Uni-variate logistic regression analysis was employed to screen the risk factor among the collected variables. Only species and peasant association (origin of the animals) were significantly associated to the serotypes infections. Exceptionally, for M. haemolytica serotype A1 age > 2 ½ years was a risk factor, whilst for B. trehalosi serotype T15 body weight (11-20 kg). In multi-variate logistic regression, only peasant association was a significant risk factor for majority of the serotype. However, Spp of the animals in addition to PA was a risk factor for B. trehalosi serotype T3 and T4 infection. In conclusion, shoats of the study area were infected with high overall prevalence of M. haemolytica serotypes (98.7%) and B. trehalosi serotypes (98.7%) than P. multocida type A (66.9%). Peasant association and animal species were potential risk factors for infection. Thus, the currently ongoing monovalent killed P. multocida serotype A-vaccine protection become low in the face of mixed co-infection by different serotypes and virulence factors difference. So multivalent vaccine will help to effectively prevent this disease in the study area and should be developed.

Key words**:** *Epidemiology, Serotypes, Predisposing factors, Small Ruminants, Tanqua-Abergelle, Tigray*

1. INTRODUCTION

Small ruminants play an important role in nutrition and income of people around the world (Habashy *et al*., 2009). They serve primarily as source of meat also provide milk, skin, wool, manure as savings, socio-cultural and ceremonial purposes (Habashy *et al*., 2009; Keba, 2010; Leta and Mesele, 2010). Small ruminants in Africa mainly are very important both for the subsistence and economic development. They are capable of remarkable adaptability to diverse environmental conditions and are amenable ease of management (Yesuf *et al*., 2012).

Africa hosts 205 and 174 million sheep and goats representing approximately 17 and 31% of the world total small ruminant population, respectively (Yesuf *et al*., 2012). The estimated 24 million TLU (Tropical Livestock Unit) for sheep and 23 million TLU for goats in Ethiopia place the country second in Africa and six in the world (CSA, 2004). They supply more than 30 % of all domestic meat consumption and generate cash income from exports of meat, mainly as live animals and skin. Hence an increase in small ruminant production is needed both to maintain self sufficiency and to increase export earnings (Yesuf *et al*., 2012). The small ruminant population of Tigray region of north Ethiopia is estimated to be 1,022,779 sheep and 1,588,779 goats. Small ruminant production in Tigray regional state is playing an important role in the improvement of the incomes for poor farmers, poverty and hunger alleviation and can contribute a major role in the country developmental plan (TBoARDAR, 2012/2013).

In spite of the presence of large number of small ruminant population, Ethiopia fails to optimally utilize this resource as the sector is suffering from lower productivity. The major constraints are inadequate nutrition, poor genetic potentials of the local stock marketing, disease, social factors, structural constraints and shortage of high level of trained human power. Among many factors which limit the economic return from small ruminant production diseases stands in the front line (Leta and Mesele, 2010; Yesuf *et al*., 2012).

One of the diseases that hamper 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). Pasteurellosis is broadly referred to as any of the disease conditions caused by *M. haemolytica*, *P. multocida* and *B. trehalosi* serotypes which is naturally inhabit the upper respiratory system (tonsils and naso-pharynx) of healthy animals (Dziva and Mohan, 2000; Shayegh *et al*., 2009). Healthy sheep and goats carry these serotypes on their tonsils and in their lungs, where they usually do not harm. However, if a trigger factors occur, these serotypes can suddenly multiply rapidly to produce toxins that cause septicemia and often death (Kopcha, 2012).

There are two forms of pasteurellosis in small ruminants, namely pneumonic and septicemic (Amin, 1998; Donachie, 2000). *P. haemolytica (*currently *Mannhaemia haemolytica)*, *P. multocida* and *P. trehalosi* (currently *Bibersteinia trehalosi*) are the three bacteria most commonly cause pasteurellosis in small ruminants (Shayegh *et al*., 2009). The septicemic form is caused by *B. trehalosi* and the pneumonic form by *M. haemolytica* type A and *P. multocida* type A and D (Amin, 1998; Donachie, 2000; Mohamed and Abdelsalam, 2008). But the pneumonic form is regarded as the most important respiratory disease affecting small ruminants (Mohamed and Abdelsalam, 2008; Habashy *et al*., 2009**).**

Pneumonic pasteurellosis (Enzootic pneumonia) is a complex disease that develops when the immune system of the animal is compromised by stress factors such as crowding, transportation, draught, inclement weather, malnutrition, nasopharyngeal colonization, dehydration weaning as well as following concurrent respiratory infestations (Gelagay *et al*., 2004). It causes widespread financial losses because of death, reduced live weight, delayed marketing, treatment costs and unthriftness among survivors (Hawari *et* al., 2008). Generally this disease is a significant cause of decreased productivity and increased treatment costs (Kopcha, 2012).

Despite of annual vaccination programs using *P. multocida* type A vaccine developed in National veterinary institute against pasteurellosis in small ruminants in Tigray, especially in Tanqua-Abergelle, still high outbreaks continue to be observed by farmers and veterinarians. Frequency of the disease outbreak in 2012/2013 in this district was 3-times. The number of cases and deaths of the animals were 362 and 59, respectively (TBoARDAR, 2012/2013). This problem might be due to preconditions and inappropriate vaccine used based on the serotypes found in the district. However, in this area there is limited information on the current field serotypes. This hinders to plan the effective control program of the disease that lead to sustainable development of multi-valent vaccine in our country.

Therefore, the aim of this investigation was:

- To identify *Pasteurella* serotypes circulating and to measure their magnitude in sheep and goats in Tanqua- Abergelle district

- To identify the risk factors of *Pasteurella* infection in sheep and goats in the study area

2. LITRATURE REVIEW

2.1. Epidemiology of Pneumonic Pasteurellosis in Sheep and Goats

2.1.1. Occurrence

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**).**

M. haemolytica, B. trehalosi, and P. multocida are common commensal organisms of the upper respiratory tract (tonsils and naso-pharynx) of apparently healthy sheep and goats. They are distributed worldwide, and diseases caused by them are common in all ages, although the prevalence of serotypes may vary by region and flock (Shayegh *et al*., 2009; Sherrill, 2012).

M. haemolytica is the causative agent of several economically significant veterinary diseases occurring in ruminants and, much more rarely, in other animal species. It accounts for approximately 30% of the total cattle deaths worldwide, and is associated with an annual economic loss of over one billion dollars in North America alone. 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 pasteurellosis is often associated with changes in the environment and occur in spring and summer, but can occur sporadically at any time of the year (Jasni *et al*., 1990; Malone, 1991).

2.1.1.1. Distribution of *Pasteurella* Species

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 table 1 and 2.

Table 1: Prevalence of *Pasteurella* species in different geographical locations

Country	**Locality**	**Host species**	**MH**	**PM**	**BT**	**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 *et al*. (2006)
		Goats	-	-	26	Shiferaw *et 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 BT = *B. trehalosi*, the numbers indicate prevalence

2.1.1.2. Distribution of *Pasteurella* Serotypes

Although the prevalence is different, *Pasteurella* serotypes in small ruminants is also distributed throughout the world. Records have been reported and among these indicated as shown bellow in table 2, 3 and 4.

Table 2: Epidemiology of serotypes of *M. haemolytica* serotype A

Country	Locality	Host	A1	A2	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A16	Authors
Ethiopia	Quana-Wollo	Sheep	12	4	2	-	6	4	16	-	4	-	10	10	NT	Tesfaye and Abebe (2003)
	Gimba-Wollo	Sheep	16	2	18	6	2	16	2	-	-	-	4	4	NT	Tesfaye and Abebe (2003)
	Debre- Brehan	Sheep	+, ND	36	+, ND	+, ND	+, ND	35	+, ND	+, ND	+, ND	NT	+, ND	+, ND	NT	Gelagay *et al*. (2004)
Sudan		Sheep	14.5	17	8.7	15.5	10	13.5	11	NT	8	14	11	12.7	NT	Hussein and Elsawi Mohamed (1984)
Turkey		Sheep	4.1	20.8	-	12.5	20.8	8.3	-	-	-	-	-	-	-	Kirkan and Kaya (2005)
Hungary		Shoats	++, ND	++, ND	++, ND	++, ND	++, ND	+, ND	+, ND	+, ND	+, ND	+, ND	+, ND	-	+, ND	Fodor *et al*. (1999)

-, + indicates not present and present respectively, while ++ indicates most prevalent, ND = not determined; NT = not tested and the numbers indicate prevalence

Table 3: Epidemiology of serotypes of *P. multocida* types

Country	Locality	Host	A	D	F	Authors
Ethiopia	Debre- Brehan	Sheep	10	NT	NT	Gelagay *et al*. (2004)
	Debre- Brehan	Sheep	+,ND	+, ND	NT	Deresa *et al*. (2010)
	Somali	Sheep	+,ND	+,ND	NT	Deresa *et al*. (2010)
Malaysia		Goat	+,ND	+, ND	NT	Amin (1998)
Turkey		Sheep	6.06	33.3	3.03	Guier *et al*. (2013)
		Goats	-	66.6	-	

-, + indicates not present and present respectively, ND = not determined; NT = not tested and the numbers indicate prevalence.

Table 4: Epidemiology of serotypes of *B. trehalosi*

Country	Locality	Host	T3	T4	T10	T15	Authors
Ethiopia	Wollo (Quana)	Sheep	4	8	-	2	Tesfaye and Abebe (2003)
	Wollo (Gimba)	Sheep	14	8	-	4	Tesfaye and Abebe (2003)
Sudan		Sheep	12.7	11.5	10.5	-	Hussein and Elsawi Mohamed (1984)
Turkey		Sheep	-	8.3	-	-	Kirka and Kaya (2005)

-, indicates not present, T = *B. trehalosi* serotype T and the numbers indicate prevalence.

2.1.2. Hosts

Sheep, goat, pig, poultry, cattle, camel, horse, donkey, dog and cat are hosts of the disease. There are also wild animals like deer, elephant, monkey, rabbit and rodents (Blood, 1994; De Alwis, 1992).

2.1.3. Transmission Methods

The transmission of the disease is by direct contact and aerosol from the diseased to healthy animals. Most of these infectious organisms are spread by direct contact with body fluids (such as saliva, nasal discharge), contaminated feeders, troughs, and equipment status (Brogden *et al*., 1998). The worst epidemics occur during the rainy season, in animals in poor physical condition, stresses are thought to increase susceptibility to infection, and close flocking and wet conditions seem to contribute to the spread of the disease. The majority of *M*. *haemolytica* infections are mostly endogenous, caused by the normally resident bacteria on the upper respiratory tract, although exogenous infections can also occur by direct contact with sick animals or through infected aerosols. Lambs acquire the infection soon after birth, probably transmitted by close contact with their dams (Amin, 1998). Infection of humans is generally associated with some form of animal contact, most commonly a dog and cat bite or scratch (Gerardo *et a*l., 2001).

2.1.4. Associated Risk Factors

2.1.4.1. Predisposing Factors

The reaction of animals to stress is rather variable even within individual animals of the same species. The role of stress in the natural incidence of pasteurellosis was clearly evident by the fact that the disease onset is mainly associated with sudden exposure to stressful situations created by adverse physical, environmental or climatic conditions. The most common examples of these include extremely hot or cold weather with high levels

of humidity, overcrowding in a limited space, poor ventilation, bad management, rough handling and distant transport or shipping (Brogden *et al*., 1998; Mohamed and Abdelsalam, 2008).

Respiratory viruses also predispose to pasteurellosis besides causing respiratory infections and pneumonia. These are *Para-influenza* -3 (PI-3) virus, adenovirus type-6 and respiratory syncytial virus (RSV), and to a lesser extent bovine adenovirus type-2, ovine adenovirus types-1 and 5, and reovirus type-1. More importantly these viruses also 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 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 anti-microbial 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).

2.1.4.2. Pathogenic Virulence Factors

The ability of pathogenic bacteria to cause infection is greatly influenced by certain endogenous factors which can enhance the pathogenicity of the organism and facilitate rapid invasion and destruction of target tissues of the susceptible host. These factors are generally designated as virulence factors. Virulence factors influence the outcome of bacterial-host interactions and they are in fact capable of promoting adhesion, colonization and proliferation of the organism within the animal tissues. In other words, virulence factors are actively involved in conversion of the organism from commensal into pathogen (Hawari *et al*., 2008; Mohamed and Abdelsalam, 2008).

Capsule and lipopolysaccaride (LPS) are the major components of *P. multocida* cell surface. Capsular serogrouping is frequently used for typing of *P. multocida*, and capsular types generally correlate with particular disease and host. Capsule is considered as one of important virulence determinants that allow *P. multocida* to avoid innate host defense systems. Conflicting reports exist on capsular polysaccharides of *P. multocida* as protective antigen. Although pure polysaccharides are nonimmunogenic, antigens responsible for the capsule specific reactions are not completely understood. It was suggested that immunogenicity of capsule is associated with LPS and purified capsule antigens behave as haptens in certain animals (Amin, 1998; Seleim, 2005; Güler *et al*., 2013).

The virulence of *M. haemolytica* and *B. trehalosi* are mediated by the action of several factors, including fimbriae, a polysaccharide capsule, endotoxin (lipopolysaccaride (LPS), and leukotoxin (LKT), that afford the bacteria advantages over host immunity (Seleim, 2005). Fimbriae on *M. haemolytica* may enhance colonization of the upper respiratory tract. Leukotoxin is pore forming cytolysins that affect leukocytes and platelets by altering function at low levels, but causing lysis at high levels and it is particularly important in the pathogenesis because it is specifically toxic to leukocytes resulting in fibrin deposition in lungs and on pleural surfaces.

The endotoxin contributes to adverse reactions in the lungs and also leads to systemic circulatory failure and shock, induce pulmonary reactions in the form of neutrophil and alveolar macrophage reaction changes on the blood capillaries which lead to thrombosis and pulmonary oedema as well as the damages on the pulmonary epithelium (Confer *et al*., 1990; Sherrill, 2012).

The virulent determinants of *M. haemolytica* and *P. multocida* exert their influence not only to produce lesions which include alveolar oedema, 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).

2.2. Etiology of Pneumonic Pasteurellosis and Pasteurella Serotypes

P. haemolytica (currently *M. haemolytica), P. multocida* and *P. trehalosi* (currently *Bibersteinia trehalosi*) are the three bacteria most commonly cause pasteurellosis in small ruminants (Shayegh *et al*., 2009). The disease pneumonic pasteurellosis, observed in sheep and goats is commonly caused by *P. haemolytica* and rarely caused by *P. multocida*. *P. haemolytica* was first identified and recognized in 1932 (De Alwis, 1992), while *P. multocida* was first discovered by Perroncito in 1878 and named after Louis Pasteur who first isolated and described this Gram-negative bacterium as the cause of fowl disease in 1880 and subdivided into four subspecies that include *Multocida, Gallicida, Septica* and recently described *Tigris* (Shayegh *et al*., 2009).

P. haemolytica biotype A was allocated to a new genus and renamed *Mannhaemia* and it was officially assigned to the genus *Mannhaemia* in 1999. The name *Mannhaemia* was given in tribute to the German scientist Walter Mannheim for his significant contributions in the recent taxonomy of the family *Pasteurellaceae*. This new genus now contains several species including *M. haemolytica* and, *M. glucosidal.* In sheep and goats throughout the world *P. haemolytica* type T has been reclassified as *P. trehalosi* (Amin, 1998; Habashy *et al*., 2009**).**

It is an important pathogen of sheep and goats being primarily associated with serious systemic infections in lambs and kids, but also having an association with pneumonia in sheep. The organism, first described as a separate species by Sneath and Stevens (1990), was part of the complex of species once known as the '*P. haemolytica'* complex. *P. haemolytica* type A ferment arabinose, and type T ferment trehalose. The trehalose-

positive can be represent *P. trehalosi* which is recently reclassified as *B. trehalosi* and trehalose-negative organisms represent *M. haemolytica* (Blackall *et al*., 2007).

M. haemolytica is a small encapsulated gram negative cocco-bacillus bacterium. It is non motile, exhibiting slight polymorphism with occasional bipolar staining. It forms a narrow zone of hemolysis on 7% ovine or bovine blood agar which is used to distinguish *P. haemolytica* from other *Pasteurella* species. The ability to grow on MacConkey agar, the lack of urease and the inability to produce indole are additional tests (Amin, 1998); but *P. multocida* is non hemolytic colonies in blood agar, no growth on MacConkey agar, positive for indole, nitrate reduction, oxidase and catalase (Prabhakar *et al*., 2012).

Table 5: Summary of biochemical characteristics *M. haemolytica* and *P. multocida* in sheep and goats

Reaction	***M. haemolytica***	***P. multocida***
Haemolysis	+	-
Motility	-	-
Indole formation	-	+
Litmus milk	Acid	Neutral
Glucose	+	+
Saccharose	+	+
Lactose	+	-
Oxidase	+	+
Catalase	+	+

+, indicates present, -, indicates not present

Source: Hawari *et al*. (2008)

Table 6: Summary of common diseases caused by *Pasteurella* spp in domestic production animals

Hosts	**Name of the disease**
Cattle	Hemorrhagic septicemia; Bovine Pneumonic Pasteurellosis
Buffalo	Hemorrhagic Septicemia
Shoats	Pneumonic Pasteurellosis; Septicemic Pasteurellosis
Pigs	Atrophic rhinitis; Pneumonia; Septicemia (rare)
Poultry	Fowl cholera

Source: De Alwis (1992)

To constitute effective control measures, it is important to know the serotype of the organism (Guler *et al*., 2013). The capsular sero-group of *P. multocida* is generally related to disease predilection. *P. multocida* are classified into 5 sero-groups as A, B, D, E, and F based on capsule structures and further 16 serotypes based on lipopolysaccaride composition (De Alwis *et al*., 1992; Guler *et al*., 2013; Seleim, 2005). *P. multocida*, particularly types A and D has been associated with a pneumonic disease known as pneumonic pasteurellosis or 'shipping fever' (Amin, 1998; Shayegh *et al*., 2009).

Capsular serotyping provides the primary basis for the classification of strains and epidemiological typing in *M. haemolytica* (Hawari *et al*., 2008) and it has two serotypes A and T *(*Amin, 1998; Seleim, 2005; Akan *et al.,* 2006). A and T serotypes further divide into 17 serotypes based on their surface antigen. Type A comprises A1, A2, A5, A6, A7, A8, A9, A10, A11, A12, A13, A14 and A16, while type T comprises T3, T4, T10, and T15 (De Alwis, *et al*., 1992; Gelagay *et al*., 2004; Akan *et al.,* 2006; Blackall *et al*., 2007). However, serotype A11 was later reclassified as *M. glucosida*. *M. haemolytica* type A usually cause pneumonia (enzootic pneumonia) in all ages of sheep and goats and occasionally cause septicemia in young lambs and kids. In contrast, type T causes a well-defined acute systemic disease in young -adult sheep and goats (Akan *et al.,* 2006; Sherrill, 2012).

Table 7: Summary of common diseases caused by *Pasteurella* serotypes in domestic production animals

Hosts	Name of the Disease	Serotypes
Cattle	Hemorrhagic septicemia (HS)	*P. multocida* serotypes B2 and E2
	Occasionally, HS like septicemia disease	*P. multocida* serotype B3,4
	Bovine pneumonic pasteurellosis	*M. haemolytica* A 1; *P multocida* A
Buffalo	Hemorrhagic Septicemia (HS)	*P. multocida* serotypes B2 and E2
Shoats	Pneumonic pasteurellosis	*M. haemolytica* A
	Septicemia pasteurellosis	*B. trehalosi*
Pigs	Sporadic outbreaks of HS	*P. multocida* serotype B2
	Atrophic rhinitis	Toxigenic strains of *P multocida* type D, occasionally, type A
	Pneumonia	*P. multocida* type A
Poultry	Fowl cholera	*P. multocida* type A (type F in turkeys) and type D are less common.

Source: De Alwis (1992)

2.3. Pathogenesis and Clinical Signs

Sequential development of the pulmonary lesions is highly mediated by complex interactions between the naturally existing causative organism in the upper respiratory tract, the immunological status of the animal and the role of predisposing factors in the initiation of infection. In either situation, the disease is essentially triggered by sudden exposure to a stressful condition or by initial infection with certain respiratory viruses or bacteria like *Mycoplasma* (De Alwis, 1992; Sherrill, 2012).

In healthy animals, the muco-cilliary ladder, the cellular and humeral 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. haemolytica* 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 (Amin, 1998; Sherrill, 2012).

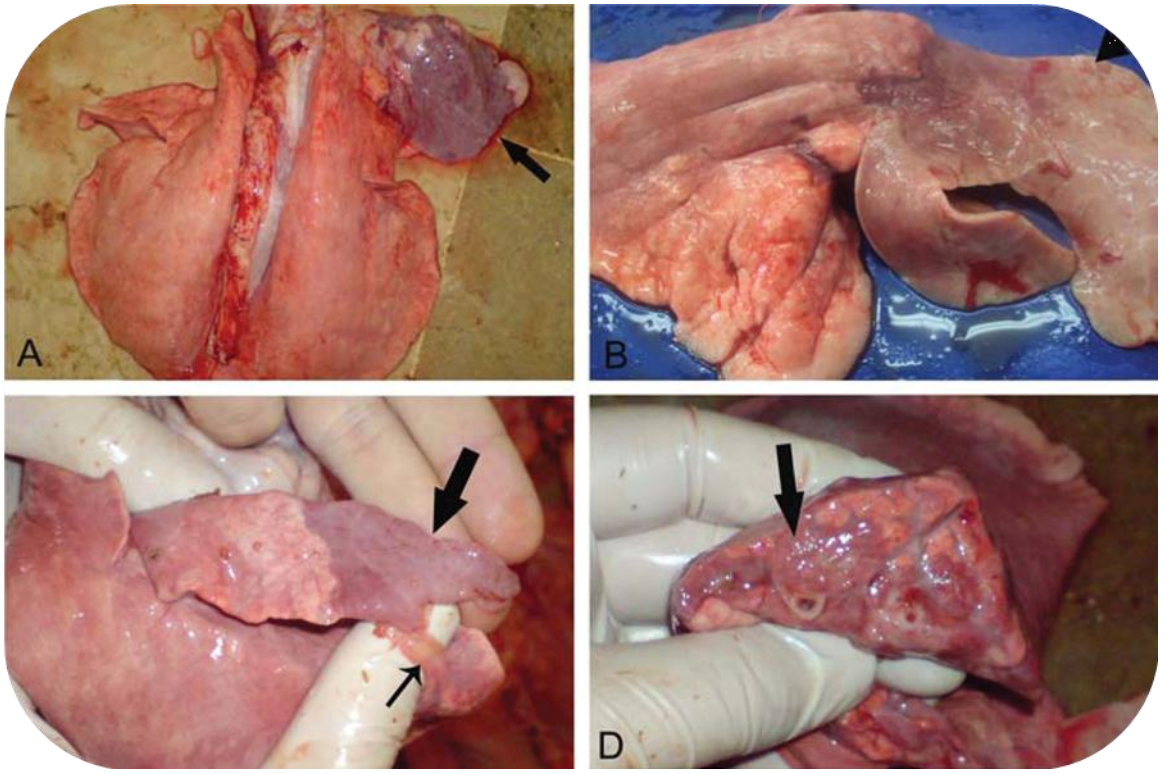
Stress and/or viral infection would eventually impair the local pulmonary defense mechanisms by causing deleterious effects on the cilliating cells and mucous coating of the trachea, bronchi and bronchioles. The causative bacteria from the naso-pharynx will then reach the ventral bronchi, bronchioles and alveoli by gravitational drainage along the tracheal floor and thereby become deeply introduced into the lung tissue (Mohamed and Abdelsalam, 2008).

Endotoxin produced by rapid growth and multiplication of the bacteria in infected lobules will cause extensive intravascular thrombosis of pulmonary veins, capillaries and lymphatics. These vascular disturbances eventually result in focal ischemic necrosis of the pulmonary parenchyma accompanied by severe inflammatory reaction dominated by fibrinous. The virulent determinants of *M. haemolytica* and *P. multocida* exert their influence not only to produce lesions which include alveolar oedema, 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; Mohamed and Abdelsalam, 2008; Sherrill, 2012).

Formation of antigen-antibody complexes may also contribute to the vascular permeability and chemo-taxis of neutrophils. As these neutrophils are lysed, enzymes are released that cause more lung tissue damage. The severity of lesions, however, depends on the rate and extent of bacterial proliferation and the amount of endotoxin released, which in turn depends on the virulence of the bacterial strain and the degree to which the defenses of the host are impaired (Mohamed and Abdelsalam, 2008).

Signs vary in severity and often seen with other types of pneumonia. The affected animals show fever with temperature of 40-41^{o}c, moist, painful cough, and dyspnea (difficulty inbreathing). Examination of the lungs may reveal crackle like sounds, along with nasal and ocular muco-purulent discharge, anorexia (loss of appetite) and depression. Lesions are usually confined to the cranioventral lung lobes on both sides. These areas may appear red to purple and feel firm from consolidation. The pleural cavity may contain variable amounts of straw-colored fluid, and yellow fibrin may cover the pleural surface of affected lung lobes from pleuritis. Chronic cases may have extensive pleural adhesions and multiple abscesses of variable size. These all signs are indicated by Amin (1998), Browning (2007), Mohamed and Abdelsalam (2008) and Sherrill (2012). According to Brogden *et al*. (1998) pneumonic pasteurellosis can be acute in lambs and kids and characterized by fever, listlessness, poor appetite and sudden death. Sheep and goats that survive the acute stage may recover or become chronically affected showing reduced lung capacity and weight gain efficiency and sporadic deaths may occur.

2.3.1. Pathological Investigations of Pneumonic Pasteurellosis in Sheep and Goats



A- Right cranial lobe, lobar pneumonia (arrow), dorsal appearance; B- Right cranial lobe with medial lobe, lobar pneumonia (arrow), dorsal appearance; C- Medial lobe lobular pneumonia (arrow), adhesion on visceral pleura (thin arrow), dorsal appearance and D- The cut section of pneumonic areas

Figure 1. Gross examination of pneumonic lungs

Source: Ozyildiz *et al*. (2013)

Figure 2. Deposits of fibrin in the visceral pleura and excessive straw coloured fluid in the thoracic cavity

Source: Shiferaw *et al*. (2006)

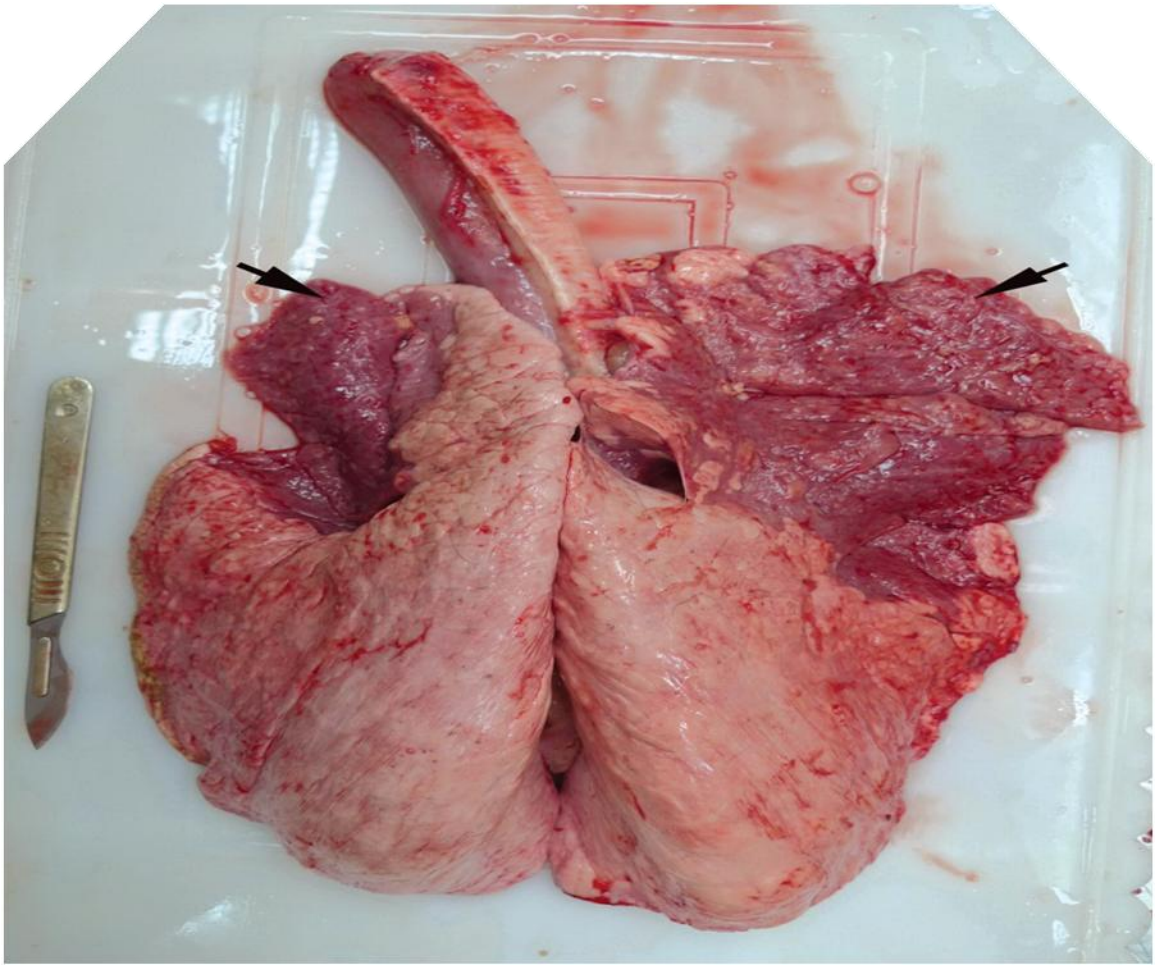


Figure 3. Dark red consolidation of cranio-ventral lobes (arrows)

Source: Azizi *et al*. (2013).

2.4. 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) and respiratory diseases which are confused with pneumonic pasteurellosis are and Sherrill (2012) such as bacterial pneumonia caused by *Mycoplasma* species (*Mycoplasma ovipneumoniae* and *M. agalactiae*), *Bordetella parapertussis, Chlamydophila abortus*/*Chlamydophila pecorum* and *Streptococcus zooepidemicus,* contagious caprine pleuro pneumonia (CCPP), pulmonary caseous lymphadenitis, lung abscesses caused by *Staphylococcus aureus,* f*usobacterium necrophorum*, *Actinobacillus lignieresi*, tuberculosis and *Corynebacterium pseudotuberculosis* Bell, 2008; FAO, 1999).

The Viral pneumonia also indicated by Sherrill (2012) 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 (PPR). Chronic viral respiratory disease caused by maedi-visna and ovine pulmonary adenocarcinoma., 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 tumours– adenocarcinomas and aspiration pneumonia also indicated by Bell (2008).

2.5. Diagnosis

History of earlier outbreaks, a recent failure to vaccinate, clinical signs and the remarkable lesions like dark red/purple areas and firm to the touch are evident mainly in the anterior and cardiac lobes of the lung may be suggestive diagnosis. Giemsa /Gram stained blood smear, bacteriological identification (Culture and biochemical tests),

molecular (such as PCR) and serological (Such as ELISA and IHA), Histopathological and Immuno-histochemical (IHC) diagnostic methods are confirmatory diagnosis of the disease which have been indicated (Bell, 2008; Kopcha, 2012 and Zafer *et al*. 2013).

2.5.1. Identification Methods of Serotypes

2.5.1.1. Serological Methods

According to Sridhar (2006), serotyping is based on fact that strains of same species can differ in the antigenic determinants expressed on the cell surface. Surface structures such as lipopolysaccaride, membrane proteins, capsular polysaccharides, flagella and fimbriae exhibit antigenic variations. Strains differentiated by antigenic differences are known as 'serotypes'. Serotyping is used in several gram negative and gram positive bacteria. The advantage of serotyping is most strains are typeable, they have good reproducibility and ease of interpretation though some have ease of performance, while the disadvantage is some auto-agglutinable (rough) strains are untypeable, some methods of serotyping are technically demanding, there is dependency on good quality reagent from commercial sources, in-house preparation of reagents is difficult process, serotyping has poor discriminatory power due to large number of serotypes, cross-reaction of antigens and untypeable nature of some strains.

Serotyping is performed using several serologic tests such as coagulation test (CA), indirect haemagglutination test (IHA) and enzyme linked immune-sorbent assay (ELISA). CA and indirect haemagglutination test are accessible tests at any diagnostic laboratory, and they have been proven to be reliable and suitable for serotyping clinical isolates of a variety of gram-positive and gram-negative organisms (Chengappa, 1984; Del Río *et al.,* 2003). IHA have been employed successfully for the identification of serological types of *M. haemolytica* and *P. multocida* and it is most common method (Sawada, *et al.,* 1982). ELISA is easy to perform, cost-effective without particular equipment, high sensitivity and specificity (Peterson *et al.,* 1997).

Several investigators have utilized IHA such as Hussein and Elsawi (1984), Foder *et al*. (1999), Tesfaye and Abebe (2003), Gelagay *et al*. (2004), Kirkan and Kaya (2005).

2.5.1.2. Molecular Methods

Polymerase Chain Reaction (PCR)

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).

A multiplex PCR assay is a rapid alternative to the conventional capsular serotyping system and used for capsular types determination and it is highly specific and its result correlated well with conventional. But real time PCR is highly sensitive than this type (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).

Filed alternation gel electrophoresis (FAGE*)*

This technique is also known as 'pulsed field gel electrophoresis' and it is a method of fingerprinting with high specificity and precision. The major drawbacks of this technique are the requirements of highly purified intact DNA and specialized and expensive electrophoresis equipment, which is generally not available in normal diagnostic laboratories (Ranjan et al., 2011).

2.6. Prevention and Control

Principles for prevention of disease and the utilization of preventive measures faster optimal health and welfare, enhance productivity and economic efficiency, and assure

abundant, safe, and wholesome food. Control and prevention lies with correction of the predisposing factors whenever practical (Kopcha, 2012). Treatment of pasteurellosis using antibiotics can be very effective in control of the disease. Whenever possible, treatment should be based on bacterial culture and sensitivity, especially in flock outbreaks, 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 (Bell, 2008; Kopcha, 2012; Sherrill, 2012).Vaccination is the preferred method of prevention for all forms of pasteurellosis. 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).

Generally proper management and the use of effective vaccine help to reduce the prevalence of the disease and the practical methods are indicated as vaccination for the healthy groups, improve management practices by providing optimal sanitation and air quality in housing, provided adequate colostrum intake for newborns, minimize transportation stress, quarantine new animals before introducing them into the existing herd, administer trace minerals, such as copper, selenium and zinc, to enhance immune function, provide good quality hay and water, and supplement as appropriate, anti-histaminic drugs to reduce lung congestion, keep sick animals in a dry and well ventilated location away from the rest of the herd (Bell, 2008 and Kopcha, 2012).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in five peasant associations from 20 peasant associations of Tanqua-Abergelle district (Figure 4) namely; Lemlem, Negede-Brhan, Gruwure, Mearey and Emba-Rufael. The district was selected purposively due to having high population of shoats and high reports of pasteurellosis disease challenge as indicated in the introduction part, from that the kebeles were randomly selected. Tanqua-Abergelle is one of the districts in Tigray regional state, Ethiopia which is located 863 km north of Addis-Ababa capital city of Ethiopia and 120 km west of Mekelle, capital city of Tigray. Part of the central zone, it is bordered on the south and west by the Amhara region, Tekezé river on the west which separates it from Amhara region and the north western zone, on the north by Kola-Tembien, on the east by Degua-Tembien and on the southeast by the south eastern zone. This district is situated at 13^{o} 14' 06" N latitude and 38^{o} 58' 50" E longitude. The administrative center of this district is Yechila. The elevation of district measures between 938, 2202 meter above sea level, agro climatic zone of the district are mainly lowland (kolla) with temperature from 21^{o}c up to 41 ^{o}c and annual rainfall range is between 400, 650 millimeter and rainfall patterns characterized as low and erratic. There are four seasons in the study area: autumn (meher*)* from September 25 up to November 25, winter (bega*)* from November 25 up to March 25, spring (tsedey*)* from March 25 up to June 25 and summer (kiremt*)* from June 25 up to August 25. Tanqua-Abergelle district has a total population of 92,844, with an annual growth rate of 2.7% and a population density of 64.22 persons per km². Of the total population, 92.43% lives in rural areas, while 7.57% lives in urban areas. The production system in the district is extensive and the important role of the animals is generating cash income, manure, social value, meat, skin, milk and butter (common from goats). The predominant practices are mixed management system, traditional housing and grazing of natural pasture. Total livestock population is estimated as cattle 87,890, sheep 35545, goats 38 744, poultry 78471, mule 6,827, horse 65, donkey 337, camel 50, 56 pigs and 11,220 hives of honey bees. The dominant soil types are vertisols (50% of land area), clay (25%) and silt loam (20%).

The total land area is about 144,564 hectare (1,445.64 km²), of which 29,466 hectare is cultivable land, 15,381.7 hectare is enclosed and the remaining 99,716.3 hectare is uncultivated (includes bare lands, marginal lands, rocky, roads and very steep and unproductive land). The major crops grown in the study area include sorghum, maize, cowpea, groundnut and sesame (oil seed). Crops are grown mainly for their grains and to make use of crop residues for animal feed (Desta *et al*., 2013; TBoARDAR, 2012/2013).

Figure 4. Map of Tigray regional state and study areas

Source: http://www.ocha-eth.org/Maps/downloadables

3.2. Study Animals and Design

Sheep and goats found in the study area of all age and sex groups were included in the study. A cross sectional study was employed for serum collection to characterize the serotypes. The animals were also selected using simple random sampling method.

3.3. Sample Size

50% expected prevalence (p) and a 95% confidence interval (Z= 1·96) with a 5% desired absolute precision (d) was considered to arrive at the required sample size, using the formula (Thrusfield, 2005). Based on Thrusfield (2005) formula, the sample size of study animals was calculated as follows.

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

$$n = \frac{1.96^2 (0.5) (1 - 0.5)}{0.0025}$$

n = 384 (each spp 192)

3.4. Sample collection, Transportation and Laboratory Analysis

The recorded farmers who have sheep and goats in the kebeles had been randomly selected. The species, age, sex and body weight of each study animal were recorded. According to Desta (2009) age of the animals was determined by observing different numbers of erupted permanent incisors whilst the body weight was determined by measuring their heart girth using meter and calculated using the formula indicated in Annex C.

Then, about 10 milliliter blood samples were collected aseptically from jugular veins of the animals using needle and plane test tube (without anticoagulants) from November up to January 2013. Shaking was avoided and the blood was allowed to clot for 2 hours at room temperature, stored horizontally overnight at 4^{o}c and then the serum was separated from the clot standing for 20 minutes at room temperature to allow for clot formation. Serum was separated from the clot by centrifugation at 3000rpm for 10 minutes and transferred to crayovial. Then the separated serum was labeled and kept under refrigeration (-20^{o}c) until transported in the veterinary clinic of the study area. Followed that, the collected 384 sera samples were transported using icebox to National Veterinary

Institute serology laboratory and kept under refrigeration (–20^{o}c) until tested. The type of laboratory test employed was indirect haemagglutination (IHA) test protocol used in NVI as indicated in annex A and an agglutination rate of >50% was taken as positive. Each sample was tested for *M. haemolytica* type A1, A2, A7, *P. multocida* type A and all *B. trehalosi* types based on the procedure indicated in annex A. The source of the reference serotypes were (CIRAD-EMVT, France).

3.5. Data Analysis and Comparable Variables

The data generated from the field investigations were entered and coded using Microsoft Excel 2007 and analyzed using Epi-info for Windows (Epi-info, CDC). Descriptive statistics were used to summarize the data. Sero-prevalence was calculated by dividing the total number of sero-positive small ruminant by the total number of small ruminants tested. The variables considered as potential risk factors for *Mannhaemia, Pasteurella* and *Bibersteinia* sero-positivity were selected based on existing literature. Association of risk factors (independent variables) with dependent variable (sero-positivity) was assessed initially using cross-tabulation. Strength of association was assessed using uni-variable logistic regression to compute the odds ratio associated with potential risk factors. Non-collinear variables that presented a P value $\leq$ 0.25 in uni-variable analysis were included in the multivariable logistic regression model. In line with this, the sex, age and body weight were not entered in the animal-level multivariable model, due to a P value > 0.25 in the uni-variable analyses. Only animal species and locations (kebeles) were taken to multivariate logistic regression. The 95% confidence level was used and results were considered significant at P value <0·05.

4. RESULTS

4.1. Serotyping

Eight serotypes in both sheep and goats distributing orderly in the study area were identified. The extent to which selected serotypes were only considered was depending up on their current importance in some parts of our country and limited budget.

4.1.1. Serotypes distribution in sheep and *goats*

Of 384 sheep and goats studied, 98.7%, 66.9% and 98.7% of them were infected with *M. haemolytica* serotypes, *P. multocida* serotype A and *B. trehalosi* serotypes, respectively. In total eight serotypes were detected in the study area with varying level of prevalence. The eight serotypes were associated with the species of the animal as assessed by univariate analysis. *M. haemolytica serotype* A1 and A2 as well as *B. trehalosi* serotype T3 and T4 were significantly ($P<0.05$) associated with goats than sheep. However, *M. haemolytica serotype* A7 and *B. trehalosi* serotype T10 were significantly associated with sheep than goats. On the contrary, *P. multocida* type A and *B. trehalosi* serotype T15 did not show any significant ($P>0.05$) difference in sheep and goats (Table 10).

Table 8: *Mannhaemia*, *Pasteurella* and *Bibersteinia* serotypes identified in sheep and goats with reference of sheep in Central Tigray

	Sheep (n=192)	**Goats (n=192)**	**Total (n=384)**	**Goats/Sheep**	
Serotypes	Positive (%)	Positive (%)	Positive (%)	Uni-variate OR (95% CI)	P-value
A1	144 (75.0)	161 (83.9)	305 (79.4)	1.73 (1.04 - 2.86)	0.033
A2	132 (68.8)	151 (78.6)	283 (73.7)	1.67 (1.05 - 2.65)	0.0284
A7	139 (72.8)	112 (58.3)	251 (65.5)	0.52 (0.34 - 0.80)	0.0031
Total A	187 (97.4)	189 (98.4)	379 (98.7)	1.67 (1.05 - 2.65)	0.0284
PA	134 (69.8)	123 (64.1)	257 (66.9)	0.77 (0.50 - 1.18)	0.2333
T3	149 (77.6)	175 (91.8)	324 (84.4)	2.97 (1.63 - 5.43)	0.0004
T4	99 (51.6)	141 (73.4)	240 (62.5)	2.59 (1.69 - 3.98)	0.0000
T10	90 (46.9)	69 (35.9)	159 (41.4)	0.64 (0.42 - 0.96)	0.03
T15	167 (87.0)	177 (92.2)	344 (89.6)	1.77 (0.90 - 3.46)	0.098
Total T	189 (98.4)	190 (99.0)	379 (98.7)	1.51 (0.25 - 9.12)	0.6548

A= *Mannhaemia haemolytica* serotype A, PA = *Pasteurella multocida* serotype A, T = *Bibersteinia trehalosi* serotype T, OR = Odds Ratio, CI = Confidence Interval

4.1.2. Serotypes distribution in different peasant associations

Sheep and goats reared under five different PA (location) had different level of prevalence (Table 9). However, *M. haemolytica serotype* A1 *and B. trehalosi* serotype T15 were not significantly (P>0.05) different among the five PAs. The rest four PA had *M. haemolytica serotype* A2 *and B. trehalosi* serotype T3 which were significantly (P<0.05) different from reference PA (Lemlem) except for Gruwure. *B. trehalosi* serotype T4 was also significantly (P<0.05) different among the four PAs compared to reference PA (Lemlem) except for Emba-Rufael. Additionally *B. trehalosi* serotype T10 was also significantly (P<0.05) different among the four PAs compared to the reference PA (Lemlem) except for Emba-Rufael and Gruwure. However, the four PAs had significantly (P<0.05) different *M. haemolytica serotype* A7 and *P. multocida* type A infections compared to the Lemlem (Table 10).

Table 9: *Mannhaemia*, *Pasteurella* and *Bibersteinia* serotypes identified in five different PAs

	Lemlem (n = 80)	**Negede-Brhan (n = 80)**	**Gruwure (n = 82)**	**Mearey (n = 76)**	**Emba-Rufael (n = 66)**
Serotypes	Positive (%)	Positive (%)	Positive (%)	Positive (%)	Positive (%)
A1	63 (78.8)	62 (77.5)	67 (81.7)	61 (80.3)	52 (78.8)
A2	65 (81.3)	54 (67.5)	75 (91.5)	50 (65.8)	39 (59.1)
A7	80 (10.1)	64 (80.0)	62 (75.6)	63 (82.9)	54 (81.8)
Total A	75 (93.8)	79 (98.8)	81 (98.8)	76 (100.0)	65 (98.5)
PA	25 (31.3)	71 (88.8)	60 (73.2)	57 (75.0)	44 (66.7)
T3	78 (97.5)	60 (75.0)	75 (91.5)	56 (73.7)	55 (83.3)
T4	67 (83.8)	47 (58.8)	57 (69.5)	22 (28.9)	47 (71.2)
T10	11 (13.8)	55 (68.8)	19 (23.2)	56 (73.7)	18 (27.0)
T15	71 (88.8)	76 (95.0)	77 (93.9)	59 (77.6)	61 (92.4)
Total T	80 (100.0)	79 (98.8)	81 (98.8)	73 (96.1)	66 (100.0)

A= *Mannhaemia haemolytica* serotype A, PA = *Pasteurella multocida* serotype A, T = *Bibersteinia trehalosi* serotype T, OR = Odds Ratio, CI = Confidence Interval

Table 10: *Mannhaemia, Pasteurella* and *Bibersteinia* serotypes identified in four different PAs in reference to Lemlem

	Negede-Brhan/ Lemlem		**Gruwure/ Lemlem**		**Mearey/ Lemlem**		**Emba-Rufael/ Lemlem**	
	Uni-variate		Uni-variate		Uni-variate		Uni-variate	
Serotypes	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
A1	0.92 (0.43 - 1.96)	0.8484	1.20 (0.55 - 2.61)	0.63	1.09 (0.50 - 2.39)	0.8151	1.00 (0.45 - 2.22)	0.9956
A2	0.47 (0.23 - 0.99)	0.0486	2.47 (0.95 - 6.43)	0.0635	0.44 (0.21 - 0.92)	0.0302	0.33 (0.15 - 0.70)	0.0039
A7	35.5 (14.24 - 88.48)	0.0000	27.51 (11.32 - 66.84)	0.0000	43.00 (16.74 - 110.50)	0.0000	39.93 (15.26 - 104.49)	0.0000
PA	17.35 (7.49 - 40.16)	0.0000	6.00 (3.04 - 11.84)	0.0000	6.60 (3.27 - 13.32)	0.0000	4.40 (2.19 - 8.83)	0.0000
T3	0.07 (0.01 - 0.34)	0.0007	0.27 (0.05 - 1.36)	0.1141	0.07 (0.01 - 0.31)	0.0005	0.12 (0.02 - 0.60)	0.0092
T4	0.27 (0.13 - 0.58)	0.0007	0.44 (0.20 - 0.94)	0.0348	0.07 (0.036 - 0.17)	0.0000	0.48 (0.21 - 1.06)	0.0714
T10	13.80 (6.24 - 30.48)	0.0000	1.89 (0.83 - 4.28)	0.1263	17.56 (7.76 - 39.70)	0.0000	2.35 (1.02 - 5.42)	0.0448
T15	2.40 (0.71 - 8.16)	0.1584	1.95 (0.62 - 6.10)	0.2500	0.43 (0.18 - 0.06)	0.0670	1.54 (0.49 - 4.86)	0.4557

A= *Mannhaemia haemolytica* serotype A, PA = *Pasteurella multocida* serotype A, T = *Bibersteinia trehalosi* serotype T, OR = Odds Ratio, CI = Confidence Interval

4.1.3. Serotypes distribution in shoats with different age groups

Age of the animals did not have any significant (P>0.05) association with seven of the serotypes studied except with *Mannhaemia haemolytica* serotype A1was significantly higher in age groups of greater than 2½ year compared to the reference age group (less than 1year) (Table11).

Table 11: *Mannhaemia, Pasteurella* and *Bibersteinia* serotypes identified in different age groups with reference of age < 1year

	Age < 1yr (n = 19)	**Age 1-2½ yr (n = 113)**	**Age > 2½ yr (n = 252)**	**Age 1-2½ yr /< 1yr**		**Age > 2½ yr /< 1yr**	
				Uni-variate		Uni-variate	
Serotypes	Positive (%)	Positive (%)	Positive (%)	OR (95% CI)	P-value	OR (95% CI)	P-value
A1	10 (66.7)	70 (77.8)	225 (80.6)	2.69 (0.97 - 7.45)	0.0557	3.17 (1.20 - 8.32)	0.0190
A2	11 (73.3)	67 (74.4)	205 (73.5)	0.76 (0.25 - 2.28)	0.6298	1.14 (0.39 - 3.30)	0.8052
A7	7 (46.7)	56 (62.2)	188 (67.6)	0.90 (0.30 - 2.71)	0.8573	0.58 (0.20 - 1.66)	0.3108
PA	7 (46.0)	62 (68.9)	188 (67.4)	1.62 (0.56 - 4.38)	0.3411	1.45 (0.56 - 3.75)	0.4384
T3	15 (78.9)	100 (88.5)	209 (82.9)	2.05 (0.59 - 7.12)	0.2580	1.29 (0.41- 4.09)	0.6586
T4	11 (73.8)	64 (71.1)	165 (59.1)	0.78 (0.28 - 2.15)	0.6453	1.06 (0.40 - 2.81)	0.8934
T10	5 (33.3)	38 (42.2)	116 (41.6)	1.51 (0.55 - 4.12)	0.4173	1.10 (0.42 - 2.91)	0.8334
T15	11 (73.3)	83 (92.2)	250 (89.6)	1.88 (0.54 - 6.49)	0.3148	2.78 (0.85 - 9.13)	0.0903

yr = year, A= *Mannhaemia haemolytica* serotype A, PA = *Pasteurella multocida* serotype A, T = *Bibersteinia trehalosi* serotype T, OR = Odds Ratio, CI = Confidence Interval

4.1.4. Serotypes distribution in shoats with different body weight

Body weight of the animals did not have any significant (P>0.05) association with seven serotypes studied except *Bibersteinia trehalosi* serotype T15 in animals weighed 11- 20 kg compared to the reference body weight group (1-10 kg) (Table 12).

Table 12: *Mannhaemia, Pasteurella* and *Bibersteinia* serotypes identified in shoats in different body weight with reference of 1-10kg

	Body-wt, 1 - 10kg (n = 15)	**Body-wt, 11 - 20kg (n = 90)**	**Body-wt, > 20kg (n = 279)**	**Body-wt, 11 - 20kg /1-10kg**		**Body-wt, > 20kg/1-10kg**	
				Uni-variate		Uni-variate	
Serotypes	Positive (%)	Positive (%)	Positive (%)	OR (95% CI)	P-value	OR (95% CI)	P-value
A1	10 (66.7)	70 (77.8)	225 (80.6)	1.75 (0.53 - 5.71)	0.3537	2.08 (0.68 - 6.34)	0.3537
A2	11 (73.3)	67 (74.4)	205 (73.5)	1.05 (0.30 - 3.65)	0.9274	1.01 (0.31 - 3.26)	0.9902
A7	7 (46.7)	56 (62.2)	188 (67.6)	1.88 (0.62 - 6.71)	0.2598	2.36 (0.83 - 6.71)	0.1070
PA	7 (46.0)	62 (68.9)	188 (67.4)	2.53 (0.83 - 6.71)	0.1006	2.36 (0.83 - 6.71)	0.1070
T3	15 (78.9)	100 (88.5)	209 (82.9)	2.25 (0.53 - 9.50)	0.2698	1.20 (0.32 - 4.42)	0.7809
T4	11 (73.8)	64 (71.1)	165 (59.1)	0.89 (0.26 - 3.06)	0.8600	0.52 (0.16 - 1.69)	0.2818
T10	5 (33.3)	38 (42.2)	116 (41.6)	1.46 (0.47 - 4.27)	0.5186	1.42 (0.47 - 4.27)	0.5292
T15	11 (73.3)	83 (92.2)	250 (89.6)	4.31 (1.08 - 17.13)	0.0379	3.13 (0.93 - 10.48)	0.0636

wt = weight A= *Mannhaemia haemolytica* serotype A, PA = *Pasteurella multocida* serotype A, T = *Bibersteinia trehalosi* serotype T, OR = Odds Ratio, CI = Confidence Interval

4.1.5. Serotypes distribution in shoats of different sex

Sex of the animals did not have any significant (P>0.05) association with any of the 8 serotypes studied (Table 13).

Table 13: *Mannhaemia, Pasteurella* and *Bibersteinia* serotypes identified in shoats in different sex with reference of male

	Male (n = 77)	**Female (n = 307)**	**Female/Male**	
			Uni-variate	
Serotypes	Positive (%)	Positive (%)	OR (95% CI)	P-value
A1	62 (80.5)	243 (79.2)	0.91 (0.49 - 1.72)	0.7909
A2	50 (64.9)	233 (75.9)	1.70 (0.99 - 2.90)	0.0523
A7	53 (68.8)	198 (64.7)	0.83 (0.48 - 1.41)	0.4963
Total A	76 (98.7)	300 (97.7)	0.56 (0.06 - 4.63)	0.594
PA	55 (71.4)	202 (65.8)	0.76 (0.44 - 1.33)	0.3486
T3	66 (85.7)	258 (84.0)	0.87 (0.43 - 1.78)	0.7175
T4	45 (58.4)	195 (63.5)	1.23 (0.74 - 2.06)	0.4112
T10	30 (39.0)	129 (42.0)	1.13 (0.68 - 1.89)	0.6262
T15	69 (89.6)	275 (89.6)	0.99 (0.43 - 2.25)	0.9931
Total T	75 (97.4)	304 (99)	2.70 (0.44 – 16.45)	0.2808

A= *Mannhaemia haemolytica* serotype A, PA = *Pasteurella multocida* serotype A, T = *Bibersteinia trehalosi* serotype T, OR = Odds Ratio, CI = Confidence Interval

4.1.6. Distribution of Mixed Serotype co-infections *distribution in shoats*

Forty three animals (11.19%) of the 384 studied had been infected with seven serotypes (A1A2A7PAT3T4T15) simultaneously whilst 19 (4.95%) animals again infected with another set of seven serotypes (A1A2A7PAT3T10T15). Thirty four (8.85%) animals had been infected with all of the 8 serotypes studied simultaneously. Details of the lists of co-infection had been illustrated (Table 14).

Animal species and origin (kebele) of the animals did not have any significant (P>0.05) association with any of the mixed serotypes co-infections (Table 15).

Table 14: Mixed *Mannhaemia*, *Pasteurella* and *Bibersteinia* serotypes co-infections identified in shoats in Central Tigray

Mixed serotype infection	Total (%)	Mixed serotype infection	Total (%)	Mixed serotype infection	Total (%)
A1A2A7PA (n = 4)	1 (0.26)	A1A7T3T4T10T15 (n = 6)	1(0.26)	A1A7PAT10 (n = 4)	1(0.26)
A1A2A7PAT10T15 (n = 6)	12 (3.13)	A1A7T3T4T15 (n = 5)	5 (1.30)	A1A7PAT10T15 (n = 5)	4 (1.04)
A1A2A7PAT3 (n = 5)	1(0.26)	A1A7T4T10T15 (n = 5)	2 (0.52)	A1A7PAT15 (n = 4)	2 (0.52)
A1A2A7PAT3T10 (n = 6)	4 (1.04)	A1A7T4T15 (n = 4)	1(0.26)	A1A7PAT3T10T15 (n = 6)	3 (0.78)
A1A2A7PAT3T10T15 (n =7)	19 (4.95)	A1PAT3 (n = 3)	1(0.26)	A1A7PAT3T15 (n = 5)	1(0.26)
A1A2A7PAT3T15 (n = 6)	6 (1.56)	A1PAT3T10T15 (n = 5)	3 (0.78)	A1A7PAT3T4T10T15 (n = 7)	6 (1.56)
A1A2A7PAT3T4 (n = 6)	1(0.26)	A1PAT3T15 (n = 4)	5 (1.30)	A1A7PAT3T4T15 (n = 6)	8 (2.08)
A1A2A7PAT3T4T10T15 (n = 8)	34 (8.85)	A1PAT3T4T10T15 (n = 6)	1(0.26)	A1A7PAT4T10 (n = 5)	1(0.26)
A1A2A7PAT3T4T15 (n =7)	43 (11.19)	A1PAT3T4T15 (n = 5)	9 (2.34)	A1A7PAT4T10T15 (n = 6)	3 (0.78)
A1A2A7PAT4T10 (n = 6)	1(0.26)	A1T3T15 (n = 3)	4 (1.04)	A1A7PAT4T15 (n = 5)	1(0.26)
A1A2A7PAT4T10T15 (n = 7)	4 (1.04)	A1T3T4T15 (n = 4)	3 (0.78)	A1A7T3T10 (n = 4)	1(0.26)
A1A2A7T10T15 (n = 5)	1(0.26)	A2A7PAT10 (n = 4)	1(0.26)	A2T3T4T15 (n = 4)	3(0.78)
A1A2A7T3T10T15 (n = 6)	3 (0.78)	A2A7PAT10T15 (n = 5)	2 (0.52)	A2T4T15 (n = 3)	1(0.26)
A1A2A7T3T15 (n = 5)	3 (0.78)	A2A7PAT3T10 (n = 5)	1(0.26)	A7 (n = 1)	2(0.52)
A1A2A7T3T4 (n = 5)	2 (0.52)	A2A7PAT3T10T15 (n = 6)	5 (1.30)	A7PAT10T15 (n = 4)	4 (1.04)
A1A2A7T3T4T10T15 (n = 7)	1(0.26)	A2A7PAT3T15 (n = 5)	1(0.26)	A7PAT15 (n = 3)	3 (0.78)
A1A2A7T3T4T15 (n = 6)	22 (5.73)	A2A7PAT3T4 (n = 5)	1(0.26)	A7PAT3 (n = 3)	1(0.26)
A1A2PA (n = 3)	1(0.26)	A2A7PAT3T4T10T15 (n = 7)	4 (1.04)	A7PAT3T10T15 (n = 5)	4 (1.04)
A1A2PAT3 (n = 4)	1(0.26)	A2A7PAT3T4T15 (n = 6)	5 (1.30)	A7PAT3T15 (n = 4)	1(0.26)
A1A2PAT3T10 (n = 5)	2 (0.52)	A2A7PAT4T10T15 (n = 6)	1(0.26)	A7PAT3T4T10T15 (n = 6)	2(0.52)
A1A2PAT3T10T15 (n = 6)	4 (1.04)	A2A7T3T15 (n = 4)	2 (0.52)	A7PAT4T15 (n = 4)	4 (1.04)
A1A2PAT3T15 (n = 5)	5 (1.30)	A2A7T3T4T10T15 (n = 6)	1(0.26)	A7T10 (n = 2)	1(0.26)
A1A2PAT3T4T10T15 (n = 7)	10 (2.60)	A2A7T3T4T15 (n = 5)	4 (1.04)	A7T15 (n = 2)	1(0.26)
A1A2PAT3T4T15 (n = 6)	13 (3.39)	A2A7T4T10T15 (n = 5)	1(0.26)	A7T3T15 (n = 3)	1(0.26)
A1A2T3 (n = 3)	1(0.26)	A2PAT10T15 (n = 4)	1(0.26)	PAT3T15 (n = 3)	1(0.26)
A1A2T3T10 (n = 4)	1(0.26)	A2PAT3T10T15 (n = 5)	3 (0.78)	PAT3T415 (n = 3)	1(0.26)
A1A2T3T10T15 (n = 5)	2 (0.52)	A2PAT3T15 (n = 4)	1(0.26)	T3 (n = 1)	1(0.26)
A1A2T3T15 (n = 4)	5 (1.30)	A2PAT3T4 (n = 4)	1(0.26)	T3T15 (n = 2)	1(0.26)
A1A2T3T4 (n = 4)	3 (0.78)	A2PAT3T4T15 (n = 5)	1(0.26)	T3T4 (n = 2)	1(0.26)
A1A2T3T4T10T15 (n = 6)	1(0.26)	A2T15 (n = 2)	1(0.26)	T3T4T15 (n = 3)	2(0.52)
A1A2T3T4T15 (n = 5)	30 (7.81)	A2T3 (n = 2)	1(0.26)	T3T4T10T15 (n = 4)	1(0.26)
A1A7T3T15 (n = 4)	1(0.26)	A2T3T4 (n = 3)	2 (0.52)	T4	1(0.26)
A1A7T3T4T10 (n = 5)	1(0.26)	A1A7PA (n = 3)	1(0.26)	**Grand total**	**384**

Table 15: Mixed *Mannhaemia*, *Pasteurella* and *Bibersteinia* serotypes co-infections identified in shoats with five different kebeles of Central Tigray

	Mixed serotypes infection Positive (%)			**Uni-variate OR (95% CI)**	**P-value**
	1-2	**3-5**	**6-8**		
Spp					
Sheep (n=192)	6 (3.1)	79 (41.1)	107 (55.7)	1	
Goats (n=192)	4 (2.1)	76 (39.6)	112 (58.3)	1.51 (0.42 – 5.45)	0.52
Total (n = 384)	10 (2.6)	155 (40.4)	219 (57.0)		
PA					
Lemlem (n = 80)	4 (5.0)	54 (67.5)	22 (10.0)	1	
Negede-Brhan (n = 80)	2 (2.5)	23 (28.8)	55 (68.8)	2.05 (0.36 - 11.53)	0.4143
Gruwure (n = 82)	1 (1.2)	20 (24.4)	61 (74.4)	4.26 (0.46 -38.99)	0.1992
Mearey (n = 76)	3 (3.9)	30 (39.5)	43 (56.6)	1.28 (0.46 - 38.99)	0.7515
Emba-Rufael (n = 66)	0 (0.0)	28 (42.4)	38 (57.6)	53.54 (0.0000 - >1.0E12)	0.9647

A= *Mannhaemia haemolytica* serotype A, PA = *Pasteurella multocida* serotype A, T = *Bibersteinia trehalosi* serotype T, OR = Odds Ratio, CI = Confidence Interval

4.1.7. Further Analysis by Multi-variate Logistic regression

Uni-variate analysis result indicated that of the risk factors studied; only Spp and PA (location) of the animals were significantly associated to the infection of the major serotypes (*M. haemolytica serotype* A1, A2 and A7, *B. trehalosi* serotype T3, T4, T10 and *M. haemolytica serotype* A2, A7, *B. trehalosi* serotype T3, T4, T10) respectively. Exceptionally, for *M. haemolytica serotype* A1 age group greater than 2 ½ years was a risk factor, whilst for *B. trehalosi* serotype T15 infection body weight (11-20 kg) was a risk factor in uni-variate analysis.

Assessment of the risk factors using multi-variate logistic regression showed that only PA (origin of the animals) was a significant risk factor for majority of the serotypes (*M. haemolytica serotype* A2 and A7, *B. trehalosi* serotype T3, T4 and T10), whilst for *M. haemolytica serotype* A2 Negede-Brhan, for *B. trehalosi* serotype T3 and T4 Gruwure and for *B. trehalosi* serotype T10 Gruwure and Negede-Brhan were not a risk factor. However, species of the animals in addition to PA was a risk factor for *B. trehalosi* serotype T3 and T4 infection. This implied that goats were at higher odds of having the infection by these two serotypes compared to sheep (Table 16).

Table 16: *Mannhaemia, Pasteurella* and *Bibersteinia* serotypes identified in species with reference of sheep and in different kebeles with reference of Lemlem in Central Tigray

	Serotypes, Multi-variate															
	A1		**A2**		**A7**		**PA**		**T3**		**T4**		**T10**		**T15**	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95%CI)	P-value
Spp																
Sheep (n,192)	1		1		1		1		1		1		1		1	
Goats (n,192)	1.85 (1.1 - 3.1)	3.135	1.50 (0.91-2.46)	0.107	0.87 (0.51 -1.50)	0.639	1.04 (0.63-1.69)	0.87	2.46 (1.30-4.64)	0.00	1.98 (1.24-3.14)	0.00	0.87 (0.54-143)	0.606	1.48 (0.72-3.05)	0.284
PA																
Lemlem (n ,80)	1		1		1		1		1		1		1		1	
Negede-Brhan (n,80)	1.02 (0.47 - 2.18)	0.954	0.50 (0.24 -1.05)	0.07	35.40 (14.18-88.38)	0.00	17.46 (7.51-40.56)	0.00	0.08 (0.01-037)	0.001	0.29 (0.14-0.63)	0.00	13.56 (6.12-30.03)	0.00	2.56 (0.75-8.77)	0.132
Gruwure (n,82)	1.36 (0.61-2.99)	0.441	2.68 (1.02 -7.04)	0.044	27.29 (11.19-66.54)	0.00	6.04 (3.04-12.01)	0.00	0.32 (0.06-1.62)	0.17	0.49 (0.23-1.07)	0.07	1.84 (0.81-4.20)	0.143	2.11 (0.66-6.67)	0.203
Mearey (n,76)	1.42 (0.63 - 3.21)	0.394	0.52 (0.24 -1.12)	0.097	41.39 (15.75-108.76)	0.00	6.71 (3.22-13.97)	0.00	0.10 (0.02-0.45)	0.00	0.10 (0.04-0.22)	0.00	16.66 (7.21-38.53)	0.00	0.51 (0.20-1.30)	0.164
Emba-Rufael (n, 66)	1.18 (0.52 - 2.67)	0.688	0.368 (0.17- 0.78)	0.009	39.26 (14.90-103.46)	0.00	4.44 (2.18-9.03)	0.00	0.15 (0.03-0.75)	0.02	0.56 (0.25-1,28)	0.17	2.27 (0.97-5.29)	0.056	1.71 (0.53-5.50)	0.361

A= *Mannhaemia haemolytica* serotype A, PA = *Pasteurella multocida* serotype A, T = *Bibersteinia trehalosi* serotype T, OR = Odds Ratio, CI = Confidence Interval

5. DISCUSSION

IHA have been employed successfully for the identification of serological types of *M. haemolytica* and *P. multocida* and it is most common method (Sawada, *et al.*, 1982). Several investigators have also utilized this test (Hussein and Elsawi, 1984; Foder *et al*., 1999; Kirkan and Kaya 2005). So far, this test is the only serological method utilized for *Pasteurella* serotyping in our country (Tesfaye and Abebe 2003; Gelagay *et al*., 2004; Yeshwas *et al.*, 2013).

The predominant serotypes identified in this study were *B. trehalosi* serotype T15 (92.2%, 87.0%), T3 (91.1%, 77.6%), T4 (73.4%, 51.6) and T10 (35.9%, 46.9%) in goats and sheep, respectively. In goats, *M. haemolytica* serotype A1 was predominant (83.9%) followed by A2 (78.6%) and A7 (58.3%), whilst in sheep A1 (75.0%), A7 (72.8%) and A2 (68.8%). This finding is higher than the findings of Hussein and Elsawi Mohamed (1984) who reported *B. trehalosi* serotype T3 (12.7%), T4 (11.5%) and T10 (10.5%), while *B. trehalosi* serotype T15 was not detected in sheep in Sudan; Tesfaye and Abebe (2003) who reported *B. trehalosi* serotype T3 (14%), T4 (8%) and T15 (4%) in sheep of Quana district of Wollo in Northern part of Ethiopia. It was also higher than the same authors finding in Gimba district of Wollo in Northern part of Ethiopia T4 (8%), T3 (4%) and T15 (2%). The authors did not detect *B. trehalosi* serotype T10 in both districts of Wollo. However, Kirka and Kaya (2005) reported *B. trehalosi* serotype T4 (8.3%) was the only serotype present in sheep in Turkey. The most probable explanation for the discrepancy of the current findings from elsewhere could be due to weather condition, nature of the pathogen host immunity, overcrowding in a limited space, bad management, rough handling and distant transport or shipping (Brogden *et al*., 1998).

The prevalence of *M. haemolytica* and *B. trehalosi* serotypes studied in this work were higher than the findings in Wollo areas of Ethiopia (Tesfaye and Abebe, 2003) and in Sudan (Hussein and Elsawi Mohamed 1984). In this line, the report was A2 (17%), A1 (14.5%) and A7 (10%) in sheep in Sudan (Hussein and Elsawi Mohamed 1984) and A1 (12%), A7 (6%) and A2 (4%) in sheep of Quana district of Wollo in Ethiopia (Tesfaye

and Abebe, 2003). It was also higher than the finding of the same authors in Gimba district of Wollo in sheep of Northern part of Ethiopia A1 (16%), A2 and A7 (2%). Lower prevalence was reported for A2 (36.1%), A7 (5.9%) and A1 (5.3%) in sheep of Northern Nigeria (Odugbo *et al*., 2003). The prevalence of A2 and A7 (20.8%) and A1 (4.1%) was lower than the current findings in sheep of Turkey (Kirkan and Kaya, 2005) and A1 (33.1%), A7 (31.8%) and A2 (28.5%) in sheep of Farta and Lay-Gayint districts of South Gonder, Northwest Ethiopia (Yeshwas *et al.* (2013).

In the current study, *P. multocida* type A (66.9%) was lower in prevalence than the *M. haemolytica* serotypes (98.7%) and *B. trehalosi* serotypes (98.7%) counterparts. However, this finding was higher than the 10% prevalence in sheep of Debre- Brehan in Central part of Ethiopia (Gelagay *et al*., 2004); 6.1% in sheep of Turkey (Guier *et al.*, 2013) and 6.6% in sheep of Farta and Lay-Gayint districts of South Gonder, Northwest Ethiopia (Yeshwas *et al.,* 2013). Therefore, this prevalence difference was most probably due to difference in environmental condition, immunity of the animals, circulation of particular serotypes in the area and the reaction of animals to different levels of stress. The reaction of animals to stress is rather variable even within individual animals of the same species (Brogden *et al.* 1998; Mohamed and Abdelsalam 2008).

Mixed serotypes co-infections were quite common in the area in this study. Forty three animals (11.19%) of the 384 studied had been infected simultaneously with seven serotypes (A1A2A7PAT3T4T15) whilst 19 (4.95%) animals again infected with another set of seven different serotypes (A1A2A7PAT3T10T15). Thirty four (8.85%) animals had been infected simultaneously with the 8 serotypes studied. The effect of such diverse serotypes co-infection on the pathophysiology of the animals is hard to predict as we don't know whether the serotypes cooperate or compete for existence in the face of immune response of the host. Under the current prevalent malnutrition, drought and mismanagement condition in Ethiopia, the serotypes can readily shift from commensal life to pathogenic form. In this line, virulence factors are actively involved in conversion of the organism from commensal into pathogen (Hawari *et al*., 2008). These factors include leukotoxin, lipopolysaccaride, adhesins, capsule, outer membrane proteins, and

various proteases (Singh *et al*., 2011). The virulence factors of the serotypes and immune response of the animals to different serotypes is different. For example, capsule and lipopolysaccaride are the major components of *P. multocida* cell surface, whilst lipopolysaccaride and leukotoxin are major components of *M. haemolytica* (Güler *et al*., 2013) and virulence factors exert their influence 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).

Spp, age, sex, body weight and PA (location) had been evaluated as a risk factor for pasteurellosis in this study. Of these, only PA and Spp were a risk factors using multivariable logistic regression analysis. PA (location) was a significant risk factor for majority of the serotypes (*M. haemolytica serotype* A2 and A7, *B. trehalosi* serotype T3, T4 and T10). However, for *M. haemolytica serotype* A2 Negede-Brhan, for *B. trehalosi* serotype T3 and T4 Gruwure and for *B. trehalosi* serotype T10 Gruwure and Negede-Brhan were not a risk factor. However, species of the animals in addition to was a risk factor for *B. trehalosi* serotype T3 and T4 infection. This implied that goats were having higher infection by these two serotypes compared to sheep. Elsewhere investigation on serotypes and their prevalence were computed across study location, breed, age and sex in sheep of Farta and Lay-Gayint districts of South Gonder, Northwest Ethiopia (Yeshwas *et al.,* 2013). In their findings, there was no significant association ($P>0.05$) among computed parameters. In another study in Ireland, *M. haemolytica* serotypes (A1 up to A14) and *B. trehalosi* serotypes (T4, T10 and T15) were isolated from different age groups in sheep and cattle (Ball *et al.,* 1993). In their findings, majority of the pasteurellosis prevalence was higher in sheep than cattle and also higher in young age groups (<1 year) than adult ages. These differences might be due to adaptability of the serotypes and immune response of the animals.

6. CONCLUSION AND RECOMMENDATION

384 Sera sample were tested to identify ovine and caprine *Manheamia*, *Pasteurella and Bibersteina* serotypes circulating in the study area. In addition to that age, sex, species, body weight and kebele (location) of the animals were compared to identify the risk factors associated to the level of infection. Eight serotypes were identified in both species of the animals, namely *M. haemolytica* serotype A1 (305(79.4%), A2 (283(73.7%), A7 (251(65.5%), *B. trehalosi* serotype T3 (324(84.4%), T4 (240(62.5%), T10 (159(41.4%), T15 (344(89.6) and *P. multocida* type A (66.9%). Shoats of the study area were infected with high overall prevalence of *M. haemolytica* serotypes (379(98.7%) and *B. trehalosi* serotypes (379(98.7%) than *P. multocida* type A (257(66.9%). Mixed serotypes co-infections were quite common in the area in this study. Two hundred and nineteen animals (57%) of the 384 studied had been infected with six up to eight serotypes simultaneously and 155 (40.4%) animals infected with three up to five serotypes whilst 10 (2.6%) animals infected with one up to two serotypes. Therefore, the monovalent killed *P. multocida* biotype A-vaccine might not be protective to the diverse serotypes affecting single animal. Only PA (location) and Spp were a risk factors using multivariable logistic regression analysis. PA (location) was a significant risk factor for majority of the serotypes (*M. haemolytica serotype* A2 and A7, *B. trehalosi* serotype T3, T4 and T10). However, Spp of the animals in addition to PA was a risk factor for *B. trehalosi* serotype T3 and T4 infection.

Based on the above concluding remarks, the following recommendations are forwarded:

- ✓ All *Pasteurella* serotypes, virulence factors and their pathogenicity should be investigated in future study

- ✓ Multivalent vaccine that effectively prevent the major identified circulating serotypes in the area should be developed

- ✓ Detailed epidemiological and risk factor study should be conducted for each serotypes in sheep, goats and other livestock species.

7. REFERENCES

Akan M., Oncel T. Sareyyupoglu B. Hazıroglu R. Tel, O.Y. and Cantekin Z. (2006): Vaccination studies of lambs against experimental *Mannheimia* (*Pasteurella*) *haemolytica* infection. *Smal. Rumint. Res.*, **65**: 44–50.

Amin M.M. (1998): In Vitro and in Vivo Establishment of *Pasteurella Haemolytica* A2 in the Lungs of Goats. Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Science. Faculty of Veterinary Medicine and Animal Science Universiti Putra Malaysia, pp: 1-12.

Azizi S. Korani F. and Oryan, A. (2013): Pneumonia in Slaughtered Sheep in South-Western Iran. Pathological Characteristics and Aerobic Bacterial Aetiology. *Vet. Itali.*, **49** (1):109-118.

Ball H.J, Connolly M. and Cassidy, J. (1993): *Pasteurella Haemolytica* Serotypes isolated in Northern Ireland during 1989-1991. *Br.vet. J*, 149: 561.

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

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.

Blackall P. J., Bojesen A.M., Christensen H. and Bisgaard M. (2007): Reclassification of (*Pasteurella*) *trehalosi* as *Bibersteinia trehalosi* gen. nov., comb. nov. *Int. J. of System.* and *Evol. Microbiol.*, **57**: 666 – 674.

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.

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): 233-254.

Browning M. **(**2007): Bacterial Pneumonia in Goats. Alabama Cooperative Extension System. New March, UNP-91. Alabamaa and Mandauburn Universities, pp: 1-4.

Chengappa M. M., Carter G. R. and Bailie W. E. (1984): Comparison of Indirect Hemagglutination and Rapid Plate Agglutination Tests with Counterimmuno-electrophoresis for typing *Pasteurella haemolytica. J. of Cli. Microbiol.*, **20** (4): 660-663.

Confer A.W., Panciera R.J., Clinkenbeard K.D. and Mosier D.A. (1990): Molecular aspects of virulence of Pasteurella haemolytica. *J. Vet. Res. Ap*r., **54** (l): 48-52.

CSA (Central Statistics Authority) (2004): The 2001/02 Ethiopian Agricultural Sample Enumeration (EASE), Executive Summary. Addis Ababa, Ethiopia.

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

Del Río M. L. Gutie´rrez C. B. and Rodríguez Ferri E. F. (2003): Value of Indirect Hemagglutination and Coagglutination tests for Serotyping *Haemophilus parasuis. J. of Cli. Microbiol*. *Feb*., **41** (2): 880–882.

Deressa A., Asfaw Y., Lubke B. Kyule M. W. Tefera G. and Zessin K. H. (2010): Molecular Detection of *Pasteurella multocida* and *Mannheimia haemolytica* in Sheep Respiratory Infections in Ethiopia. *Inte. J. Appl. Res. Vet. Med*., **8** (2): 101-108.

Desta D., Hagos H., Belay, S., Gizaw S. and Legese G. (2013): Analysis of goat value chains in Tanqua-Abergelle district, Tigray, Ethiopia: Research Program on Livestock and Fish Document, pp: 4-5.

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

Dziva F. and Mohan, K. (2000): Pasteurellosis and *Pasteurellae* in Zimbabwe. An Update, *Zimbabwe Vet, J.*, 31(1): 1-10.

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

FAO (Food and Agriculture Organization of the United Nations (1999): Recognizing Peste Des Petits Ruminants. A Field Manual, pp: 1-8.

Fodor L., Varga J., Hajitos L. and Molnar T. (1999): Serotypes of *Pasteurella haemolytica* and Pasteurella trehalosi isolated from farm animals in Hungary. *Zentralbl. Vet .med. B. May*, **46** (4):241-7.

Gelagay A., Laekemariam Y. Esayas G., Selam T. and Kassahun A. (2004): Epidemiologic and Serologic Investigation of Multifactorial Respiratory Disease of Sheep in the Central Highland of Ethiopia. *Int. J. Res. Vet. Med*., **2** (4): 274-278.

Gerardo S.H., Citron D.M., Claros M.C., Fernandez H.T., Goldstein E.J.C. (2001). *Pasteurella multocida* subsp. *multocida* and *P. multocida* subsp. *septica* differentiation by PCR fingerprinting and a-glucosidase activity. *J. of Cli.Microbiol.* **39**: 2558–2564.

Güler L. Gündüz K., Sarişahin A. S. (2013): Capsular Typing and Antimicrobial Susceptibility of *Pasteurella multocida* Isolated from Different Hosts. *Kafkas Univ. Vet. Fak. Derg.*, **19** (5): 843-849.

Gautam R., Kumar A. A. 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.

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.

Desta H. (2009): Estimation of weight and age of sheep and goats, Technical Bulletin No. 23, pp: 1-9.

Hawari A.D, Hassawi D.S. and Sweiss M. (2008): Isolation and Identification of *Mannhaemia 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.

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.

Keba T.M. (2010): Identifying breeding objectives of smallholders/pastoralists and optimizing community-based breeding programs for adapted sheep breeds in Ethiopia. Doctoral Thesis, University of natural resource and Life Science Viena, pp: 1-17.

Kirkan P.Kr and Kaya O. (2005): Serotyping of *Mannheimia haemolytica* Strains Isolated from Pneumonic Lungs of Sheep in the Aydyn Region of Turkey. *Turk J. Vet Anim Sci.*, **29**: 491-494.

Kopcha M. (2012): *Pasteurella* and *Mannheimia* Pneumonias in Sheep and Goats. Merck Veterinary Manual, White house Station, N.J., U.S.A. pp: 1-7.

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

Leta S. and Mesele F. (2010): Prevalence of Small Ruminant Trypanosomosis and Tsetse Fly Challenge in Upper Didessa Valley, Ethiopia. *Global Veterinaria*, **5** (4): 215-219.

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

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

Odugbo M.O., Odama L.E., Umohc J.U. and Makinde A.A. (2003): Serotypes of *Pasteurella haemolytica* from pneumonic lungs of sheep in northern Nigeria. *Smal. Rumint. Res.*, **48**: 239 - 243.

Ozyildiz Z., Tel, O. Y., Yilmaz R., Ozsoy S.Y. and Keskin K. (2013): Pathological and Microbiological *Investigations* of Pneumonic Pasteurellosis in Sheep. *Kafkas Univ Vet Fak Derg*., **19** (1): 103-108.

Peterson R. R. Deeb B. J. and Digiacomo R. F. (1997): Detection of Antibodies to *Pasteurella Multocida* by Capture Enzyme Immunoassay Using a Monoclonal Antibody against P37 Antigen *J. of Cli. Microbiol. Jan*., **35** (1): 208–212.

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.

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.

Sawada T., Rimler R. B. and Rhoades K. R. (1982): Indirect Hemagglutination Test that uses Glutaraldehyde- Fixed Sheep Erythrocytes Sensitized with Extract Antigens for Detection of *Pasteurella* Antibody. *J. of Cli. Microbiol.*, **15** (5):752-75.

Seleim R.S. (2005): Major Pathogenic Components of *Pasteurella Multocida* and *Mannheimia (Pasteurella) Haemolytica* Isolated From Animal Origin. Dokki, 12311. Cairo, Egypt.

Shayegh J., Sharaf J. D., Mikaili P. and Namvar H. (2009): Pheno- and genotyping of *Pasteurella multocida* isolated from goat in Iran. *Afr. J. Biotechnol*., **8** (16): 3707-3710.

Sherrill A. (2012): Overview of Pasteurellosis of Sheep and Goats. Merck Veterinary Manual, White house Station, N.J., U.S.A. pp: 1. 3.

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.

Singh K J. W. Ritchey J.W. and A. W. Confer A.W. (2011): *Mannheimia haemolytica*: Bacterial-Host Interactions in Bovine Pneumonia. *Vet. Path*., **48** (2): 338-348.

Sneath P.H.A. and Stevens M. (1990): Actinobacillus rossii sp. nov., *Actinobacillus seminis* sp. nov., nom. Rev.,*Pasteurella bettii* sp. nov., *Pasteurella lymphangitidis* sp. nov., *Pasteurella mairi* sp. nov. and *Pasteurella trehalosi* sp. nov. *Inte. J.of System. Bacteriol.*, **40**: 148-153.

Sridhar Rao P.N. (2006): Typing Methods Manual. *JJMMC, Davangere.* pp: 1-4.

Terry M. Lo, Thomas W. and Inzana J. (1998): Detection and Identification of *Actinobacillus pleuropneumoniae* Serotype 5 by Multiplex PCR. *J. Clin. Microbiol*., *Jun*., **36** (6): 1704–1710.

TBoARDAR (2013): Tigray Bureo of Agriculture and Rural Development Annual Report.

Tesfaye S. and Abebe Z. (2003): Diversity of *Mannheimia haemolytica* and *pasteurella trehalosi* serotypes from apparently healthy sheep and abattoir specimens in the highlands of Wollo, North East Ethiopia**.** *Vet, Res. Commu., Jan.,* **27** (1): 3-14.

Thresfield M. (2005): Veterinary Epidemiolgy, 5th edition. U.K. Black well science.

Yeshwas F., Shigdaf M., Hailu M. and Agraw A. (2013): Sero-Typing and Evaluation of the level of Protective antibody titer in Northwest Ethiopian Sheep before and after Ovine pasteurellosis Vaccination *Int. J. Pharm. Med. & Bio. Sc.* **2** (4): 57-58.

Yesuf M., Mazengia H. and Chanie M. (2012): Histopathological and Bacteriological Examination of Pneumonic Lungs of Small Ruminants Slaughtered at Gondar, Ethiopia. *American-Eurasian J. Scie. Res.*, **7** (6): 226-231.

Zafer O., Osman-Yasar T., Rahsan Y., Sule Yurdagul O. and Oktay K. (2013): Pathological and Microbiological Investigations of Pneumonic Pasteurellosis in Sheep. *Kafkas Univ. Vet. Fak. Derg., Jan/Feb.,* 19.

8. APPENDICES

Annex **A:** 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 procedures

- 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 dilutions 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 **B.** Determination of age with different numbers of erupted permanent incisors

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

Source: Desta (2009)

Annex **C**. Determination of body weight and body condition score

According to Desta (2009) and the formula is describes as follows:

LW (Kg) = -30.77+0.82HG (cm) for male, -31.0+0.80HG (cm) for female