FREIE UNIVERSITÄT BERLIN - ADDIS ABABA UNIVERSITY

EPIDEMIOLOGY AND STRAIN CHARACTERIZATION OF OVINE PASTEURELLOSIS INSELECTED SITES OF AMHARA AND SOMALI REGIONS, ETHIOPIA.

A thesis submitted in partial fulfillment for the degree of Master of Science in Tropical Veterinary Epidemiology at the Freie Universität Berlin and Addis Ababa university

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January, 2002

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DEDICATION

This thesis is dedicated to my family: My mother Kuli Gamada, my wife Roman H/selassie, my daughter Meseret Asefa, my son Ibsa Asefa, my brothers Temesgen Woyessa, Merga Deressa and late Father and step-father Deressa Hundera and Woyessa Erena, late sister Malkitu Deressa. For their love and support.

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LIST OF ABBEREVATIONS

AAU	Addis Abeba University
ARTP	Agricultural Research Training Project
Bgvv	Bundesinstitut für gesunheitlichew vebraucherscutz und veterinärmedizin
BOSS	Black head Ogaden Somali Sheep
BgVV	Bundes institut für gesundheitlichen Verbraucherschutz undVeterinärmedizin
CIRAD	Centre International de la Reccherche Agricolepour le Development
CP	Capsular Protein
EARO	Ethiopian Agricultural Research Organization
ELISA	Enzyme Linked Immuno Sorbent Assay
EMVT	Etude de Medicine Veternaire tropicale
FVM	Faculty of Veterinary Medicine
IHA	Indirect Haemagglutination test
ILCA	International Livestock Center for Africa
JIRDU	Jijiga Range Land Development Unit.
LKT	Leukotoxin
NVI	National Veterinary Institute
OIE	Office International des Epizooties
OMP	Outer Membrane Protein
OP	Ovine Pasteurellosis
PCR	Polymerase Chain Reaction
PI-3	Para-Influenza-3-
PPR	Peste Petit Des Ruminantes
RBC	Red Blood Cells
RDC	Respiratory Disease Complex

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ACKNOWLEDGEMENTS

I gratefully acknowledge the Ethiopian Agricultural Research Organization (EARO) through its Agricultural Research Training Project (ARTP) for their kind financial support during the whole period that I undertook my training on the joint Postgraduate Programme between Freie Universität Berlin and Addis Ababa University. Without this support my training would have not been undertaken to its completion.

I wish to sincerely extend my deep thanks to Prof. Dr H.K. Zessin, the Director and Dr. M. Baumann Course Coordinator of the Postgraduate Studies in International Animal Health Freie Üniversitäet Berlin and Drs. Getachew Tilahun, Mekonnon H/mariam from the Dean's office of the Faculty of Veterinary Medicine, Drs. Getachew Abebe and Bayleyegn Molla from the Postgraduate program of the FVM Debre-Zeit for acceptance and granting me the opportunity to work for my Masters degree in this joint program. My special appreciation goes to Dr. M.Baumann who introduced me to this program through the communications we made and Drs. Beyene Kebede, Getachew Tilahun Getachew Abebe and Bayleyegn Molla for their kind discussions they made while I was negotiating for financial support.

My supervisors Dr Lübke Becker, Dr Laekemariam Yigezu and Dr Yilkal Asfaw are acknowledged for their total devotion to the success of my work. Likewise, their encouragement, pledge, supervision, and high insight gave me the strength and confidence.

My special gratitude go to Dr. M.Greiner and M.Kyule for their valuable comments in project design, methodology review and for data analysis.

I wish reach out my acknowledgement to Professor Dr. Schimmel, the director and his team at Bgvv, Germany for the kind provision of referrence strains and their assistance in serotyping of *M.haemolytica*.

I would like to thank Sheno Research Center, National Veterinary Institute, North Shewa and Jijiga zonal veterinary services for administrative and technical assistance during field and laboratory investigations.

Much more thanks are due to w/o Selam Tariku for her help and extra ordinary effort, patience and rich experience during laboratory work. The encouragement and technical assistance of NVI staff is also highly appereciated.

The Animal Health and Animal Production Research Team at Sheno are highly acknowledged for their help at different steps of my study. Especial thanks go to Drs. Teferi Mekonnon and Kassaye Argaw, Ato Mebrat Ejo, Ato Mekbib Worku and w/o Meseret Berhan. I am also thankful for the logistics help of Sheno Center management during field trip.

The services of Mrs. Schmidt and Lebel of Freie universitäet and the staff of Faculty of Veterinary Medicine at DebreZeit are also highly acknowledged.

May I also perceive and continue my acknowledgement to these personalities, Drs, Zinash Sileshi, Sintayehu Abdicho, Adugna Tolera, Ademe Zerihun, Gelagay Ayelet, Yosef Shiferaw, Kassahun Awgichew, w/t Rehrahe Mesfin, Ato Getachew Denu, Ato Mulugeta Mammo, Ato Shiferaw Meleta and Dr. Engineer Hirpa Lemu for their help and encouragement.

The collaboration of the farmers during the interview is highly appearciated.

There are many people who supported me in one way or another, since it is not possible to mention each ones name, may I generally reach out to all who in a way contributed to my success and thank them for their contribution.

ABSTRACT

Epidemilogical survey and strain characterizations of ovine pasteurellosis were carried out in selected sites of Amhara and Somali regions in Ethiopia. The aims were to identify, estimate and characterize *pasteurella* species. In adition, a questionnaire survey was administered in 2 regional states in April and June 2001 to assess the possible predisposing factors of ovine pasteurellosis. A significant ($P<0.05$) difference was observed among the respondents who indicated irregular vaccinations of this disease.

A total of 37 lungs, 27 nasal swabs and 10 tonsils were sampled from clinically healthy and sick sheep at different sites. Bacteriological isolation using the standard protocols established at the NVI at Debrezeit was performed. Further, confirmation of isolates was made at Freie Üniversitäet Berlin, Institut für. Mikrobiologie ünd Tierseuchen using *P.multocida* and *M.haemolytica* specific PCR assays. *M.haemolytica* isolates were further characterized and serotyped at Bgvv Jena, Germany. The proportions of *M.haemolytica* and *P.multocida* specific PCR isolates from lung and nasal swab of clinically healthy and sick sheep from Debreberhan were 25% (95% C.I. 6.71, 9.77) and 4.12% (95% C.I. 21.12, 0.10) respectively. The prevalence in Jijiga was 30.76% (95% C.I. 61.43, 9.09) for *M.haemolytica* and 15.38% (95% C.I. 45.44,1.92) for *P.multocida*.

The *P.multocida* specific PCR capsular type A was isolated from lung tissues of sheep slaughtered at Jijiga abattoir and Debreberhan back yard slab with a prevalence of 8.1% (95% C.I. 21.9, 1.7). Capsular type D was isolated from nasal swabs of sheep from Jijiga abattoir with a proportion of 5.4% (95% C.I. 18.19, 0.6).

Strain characterization showed a prevalence of Serotype A1 of 55.55% (95% C.I. 6.30, 21.20), and A6 of 44.44% (95% C.I 78.79,13.69). Only 2/2 strains of T15 were characterized. These strains were not tested against A2, A8, A9, A13, A14, A16 and A17 sera. The serotyping showed that A1 and A6 were the prominent strains. Among n=15 *M.haemolytica* isolates submitted for serotyping, 26.66% (95%C.I. 55.10, 7.78) were untypable. The prevalence of *P.multocida* capsular A and D isolated from lung tissues and swabs confirmed the less involvment of *P.multocida* in the process of the disease.

These findings suggested that *M.haemolytica* A1 and A6 were the prominent strains and therefore could be recommended as candidates' for the preparation of a poly-valent vaccine against ovine pasteurellosis. This would probably promote the efficacy of the currently produced ovine pasteurellosis vaccine in Ethiopia. In the conclusion, further investigation is recommended to establish whether other strains, for example A2, A8, A9, A13, A14, and A17 exist in Ethiopia. Elucidation of these will nevertheless advance the epidemiology of this disease and its cost-effectiveness in its management.

1.INTRODUCTION AND OBJECTIVES

With the estimated population of 205 million sheep and 147 million goats in Africa of which Ethiopia constitute 15% (Kasahun, 1985), small ruminants are playing a significant role in the socio-economic development of the majority of the African countries. Small ruminants numbering about 40 million in Ethiopia are playing a substantial role in the agricultural economy of the country (FAO, 1993). Unlike large animals, sheep and goats are advantageous in terms of feasible income generation, easy manageability and remarkable adaptability to diversified environmental factors in different production systems. They are important source of meat, milk, skin, and wool fibers and provide manure for maintainence of soil fertility of the soil. About 75% of sheep and 28% of the goats in Ethiopia are raised in the highlands, while the remainders are reared in the low lands (Alemayehu and Fletcher, 1991).

Many breeds of sheep, particularly those that are native to the desert regions of the world, use water very efficiently and can survive and manage for several days without drinking. They can graze far from watering holes and place less stress up on soil and vegetation near water. In Ethiopia, different sheep types (breeds) are identified namely: Menz, Horro, Adal, and Ogaden black head (Kassahun, 1985). Sheep depend mostly on grazing fallow lands and could maintain one to three rams (depending on the size of the flocks) for year round breeding.

Under pastoral system (nomadic, semi-nomadic or transhumance) of Ethiopia, livestock are usually kept with out extra-supplement and with minimum health care. Farmers keep large flocks of sheep and goats, herds of cattle and camel. The surpluses are sold at local markets or trekked to major consumption centers. An average flock size of 0-16 animals per flock, are kept and sheep represent about 57-65% of the total livestock mix in the cool highlands around Debre Berhan (Agyemang et al., 1985). Sheep are raised in different Agro-ecological Zone(AEZ) of the country. The distribution of the animals is, however, determined by two ecological patterns and the density of the population. Three quarter of the national flock is reared in the highland regions, with an average rainfall exceeding 700mm per annum. Three fourth of country's population live in this area. The low lands share a quarter of national sheep stock with annual rainfall pattern ranging 700mm. In general term, sheep play significant role in the national economy. They are raised mainly for meat with exception of the Somali that consume sheep milk. The average

carcass weight of adult sheep is 10kg and the slaughter is 33% (FAO, 1987). About 83 million-kg of mutton is produced annually. Among Ethiopia's export revenue, 43% comes from hides and skins and only 5% from mutton (FAO, 1983). As a whole livestock and livestock products sales provide 87% of the total annual farm cash income, of which 40% consisted of sales from sheep (Gryseels, 1988).

An increase in small ruminant production could contribute to the attainment of food-self sufficiency in the country, especially in protein requirements for the growing human population and to increase export earnings. However, factors such as inadequate nutrition, poor state of health, traditional production system, low genetic potential and the lack of in puts are presumed to be the causes for low productivity. Diseases have a great socio-economic impact on sheep production in Ethiopia, accounting for mortalities ranging 30% in lambs and 20% in adult sheep (Ministry of Agriculture, 1985).

Among major threats to sheep production, multifactorial respiratory disease complex causes substantial losses through morbidity and mortality. Accordingly, infectious and parasitic pneumonia in sheep is a serious problem in the central cool highlands of Ethiopia; accounting for over 38% of the total cases observed (ILCA 1989; Bekele et al., 1992). Economic losses are mainly due to mortality as well as morbidity which leads to decrease in productivity. Scarcity of drugs at local market together with the inability of farmers to afford the cost of treatment made therapeutic disease control impractical. Although vaccination is cost effective means to control contagious diseases, little emphasis has been given as far as development of vaccines for small ruminants is concerned. However, there has been an attempt to control ovine pasteurellosis by vaccinating with *P.multocida* capsular serotype A vaccine produced at National Veterinary Institute (NVI) Debre-Zeit.

Despite the efforts made so far to control the disease by vaccination frequent outbreaks occur.This is presumably due to a wide diversity of *M. haemolytica* serotypes, which are antigenically distinct from that of *P.multocida* capsular serotype A. Eleven serotypes of *M. (P).haemolytica* were isolated from sheep in different regions of Ethiopia (Pegram et al., 1979). Since *M.haemolytica* A2 is the most common isolate from pneumonic lungs of sheep and goats throughout the world, most research activities on the development of Pasteurella vaccine have focused on incorporating either a suitable isolate of *M.haemolytica* A2 or immunogenic antigens

extracted from the serotype (Bahaman *et al.*, 1991a; Moiser, 1993). However, many of the available Pasteurella vaccines, developed using *M.haemolytica* A2 (Gilmour *et al.*, 1979, 1983; Adlam, 1989; Zamri-Saad *et al.*, 1994). were found non protective and cross reactive and visversa. *M. haemolytica* A7 and A9 (Zamiri-Saad *et al.*, 1994) neither nor *M.haemolytica* A1 and A6 (Purdy et al., 1998). Most Pasteurella vaccines incorporate various important serotypes of *M.haemolytica* in order to provide immunity against infections of common serotypes (Moiser, 1993). The outer membrane proteins of *M.haemolytica* A7 were effective in protecting animals against homologous and heterologous infection of live *M.haemolytica* A2, A7 and A9 (Sabri *et al.*, 2000).

Information on the distribution and significance of these serotypes and biotypes of *M.haemolytica* and *P.multocida* is scant and not well documented in different agro-ecologies and production systems of Ethiopia. Preliminary surveys of (Bekele and Gelagay, 1996) and Mekonnen (2000), conducted in the central highlands of Ethiopia revealed a varying sero-prevalence rate of 14 M. haemolytica including strains like A1, A8, A7, and A 6. The existence of these serovariants and bio-variants necessitate carrying out further epidemiological investigation and biotyping. *Pasteurella multocida* and *M. haemolytica* are important etiological factors in the development of pneumonia in cattle and sheep. Safe and effective vaccines against pasteurellosis are still lacking. Until recently there had been no extensive characterization of the organism *P.multocida* at the molecular level (Hunt *et al.*, 2000). Despite effort expended on understanding various etiological, immuniological, and epidemiological aspects of pneumonic pasteurellosis, a reliable preventive tool against the disease is still lacking. Prevention by immunization has been attempted for many years. Field experiences and designed experiments, however, show that *P.multocida* and *M.(P.) haemolytica* bacterins have questionable efficacy in preventing from the disease owing to their poor performance. This could happen when the serotype of Pasteurella, which causes that particular infection, is not contained in the vaccine (Mutters and Bisgaard, 1986).

The opportunistic microorganisms, *(M.)haemolytica* (T and A) and rarely *P.multocida* (A and D) which cause the latent infection of upper respiratory tract, get a chance to come down to the lung and cause severe Pasteurellosis (Gilmour, 1989). A recent reclassification of the genus Pasteurella that based on reference strains of the species *(P).haemolytica* has not categorized the s genus as there was poor DNA-homology, but should instead refer to the genus Actinobacillus (Younan and Wollmann, 1988). Based on the polyphasic investigation performed, a new genus

called *Mannheimia* is proposed for the trehalose-negative *P.haemolytica* complex. At present two previously named species are transferred to this new genus and three new species are described (Angen *et al.* 1999). The number of established serotypes of *M. haemolytica* has subsequently increased to 17 (Pegram *et al.*, 1979; Fraser *et al.*, 1982; Foder *et al.*, 1987; Younan and Wallmann, 1989). Serotypes 3, 4, 10 and 15 are associated with biotype T, and the remaining serotypes with biotype A. Extended phenotypic investigations in the mid-1980s showed that strains from ruminants classified as *M. haemolytica* could be divided in to 12 biogroups based on ornithine decarboxylase and fermentation of L-arabinose, D-sorbitol, glucosides (aesculin, amygdalin, arbutin, cellobiose, gentibiose and salicin) and trehalose (Mutters et al., 1989). DNA hybridizations (Mutters *et al.*, 1986) have shown that some of the biogroups represent separate species and that *M. haemolytica* sensu stricto *M. haemolytica* biogroup 1 should only consist of strains negative for L-arabinose fermentation. *Mannheima (M.) haemolytica*, which causes ovine pasteurellosis, is divided in to two biotypes A and T and further subgrouped in to different serotypes. Biotype A causes pneumonic pasteurellosis, while biotype T causes systemic pasteurellosis in lambs (Radosits, *et al.*, 1994).

Concurrent pathogens like *Chlamydophila psittaci*, viruses like Parainfluenza-3, Reovirus, Adenovirus and Respiratory Syncytial virus (Radostits et al., 1994; O.I.E., 1992; Fenner et al., 1993), Mycoplasma species (*M.ovipneumoniae*, *M.arginini*, *M.agalactiae* and others) and lung worms particularly *Dictyocaulus filaria* take part in Respiratory Disease Complex(RDC) and may suppress the immune mechanisms of the respiratory tract. *(P.) haemolytica* trehalose-negative is reclassified as *Mannheima haemolytica* comb.nov. (P.)*granulomatis*, Bisgaard taxon 20 and *(P.) haemolytica* biovar 3 are reclassified and combined in the species *Mannheima granulomatis comb. nov. Mannheima glucosida sp. nov.* and other *(P.)haemolytica* biogroups (Angen, *et al.*, 1999). Quantitative interpretation of phenotypic data seems to represent a promising method for finding relations among affiliated strains of bacteria and to assist in forming hypotheses for subsequent genotypic investigations (Angen, *et al.*, 1997). *M.haemolytica* produces several virulence factors (leukotoxin, capsule and endotoxin), which are likely to be important in the pathogenesis of pneumonic pasteurellosis. The leukotoxin is cytotoxic to pulmonary macrophages and peripheral neutrophils in vitro, suggesting that *M.haemolytica* leukotoxin may also impair host defenses in vivo and substantially enhance the virulence of the invading organisms (Schewen and Wilkie, 1988; Clinkenbeard *et al.*, 1992).

This study is designed to evaluate biogrouping systems for differentiating bacterial isolates in to definable groups and to evaluate the ecosystem distribution of variant groups and the association of the organisms of each group with disease. Despite the importance of sheep production in the Ethiopian livestock sector and the high prevalence of ovine pasteurellosis the antigen-spectrum of the existing *pasteurella* strains has not been investigated in different sheep populations of different ecological zones.

Based on the previous findings of *Pasteurella* strain identification and bio-var characterization it is hypothesized that field strains of *Pasteurella* isolates are different from the P.multocida A used as vaccinal strain. Hence, this study was proposed with the following general objectives:

- Assess the relationship between *Pasteurella* and Pasteurellosis on sheep husbandry.
- Estimate the prevalence of *Pasteurella* species in sheep.
- Identification, biotyping and strain characterization of *Pasteurella* strains

2.LITERATURE REVIEW

2.1. Definition

Pneumonic Pasteurellosis is acute respiratory disease of sheep, goats and cattle characterized by fever, dyspnoea, nasal discharge, anorexia, toxemia and death with major pathology of lobar and anteroventrally distributed exudative pneumonia and fibrinous pleuritis caused by either (*M.*) *haemolytica* or (*P.*) *multocida* (Gilmour, 1993; Jamaludin, 1993). However, *M. haemolytica* is most frequently isolated from cases of pneumonic pasteurellosis and M. haemolytica A2 has been identified as the most common isolate from pneumonic pasteurellosis of sheep and goats throughout the world (Bahaman *et al.*, 1991b; Gilmour *et al.*, 1991; Mohamad *et al.*, 1993).
Pneumonic pasteurellosis is a stress associated ruminant respiratory disease characterized clinically by acute bronchopneumonia with toxemia and pathologically anteroventrally distributed exudative pneumonia, and in which fibrinous pleuritis is common. Pasteurellosis is a complex disease entity, which develops when the immune system of the animal is compromised by stress factors such as crowding, transportation, and exposure to draught and inclement weather.

2.2. Aetiology

The genus *Pasteurella* was first suggested in 1887 by Trevisan to commemorate the work of Louis Pasteur, following his work in the elucidation of the etiology of fowl cholera. (*P.*) *haemolytica* and (*P.*) *multocida* are frequently associated with respiratory disease syndrome, although some strains like *P. multocida* D, E are known to cause haemoragic septicemia in cattle. But strains vary in their ability to cause disease in different animal hosts (Jaworski et al., 1988). *(P.) haemolytica* was isolated from cattle and sheep in 1932 by Newsom and Cross (De Alwis, 1993).

Table 1. Diseases caused by *P.multocida* and *M.haemolytica*.

Species/Host	Species of Pasteurella	Disease syndromes
Cattle	*P.multocida*,	Haemorrhagic septicaemia
	P.multocida+M.haemolytica	Bovine pneumonic pasteurellosis (shipping fever)
Buffalo	*P.multocida*	Haemorrhagic septicaemia
Sheep and goats	*M.haemolytica*	Pneumonic pasteurellosis, Septicaemic pasteurellosis
Pigs	*P.multocida*	Atrophic Rhinitis, Pneumonia
Poultry	*P.multocida*	Fowl Cholera

Source: DeAlwis, 1993.

Mannheima haemolytica is the primary cause of pneumonic pasteurellosis in sheep and goats while *P.multocida* (A and D biovars) are occasionally associated with systemic infection.

The Gram-negative bacterium recently refered to as *Mannheima haemolytica* is the most important cause of mortality in cattle, sheep and goats in the UK. It is also identified as the major cause of systemic disease in sheep (CVL, 1987-94; VIDA, 1988-95). Within the species there are 12 identified serotypes apparently host specific in disease induction. Serotype A1 is the most commonly isolated serotype cases of bovine pneumonia whereas A2 is the predominant isolate from ovine pneumonia and systemic disease (Frank, 1989; Gilmour and Gilmour, 1989). *M.haemolytica* is carried in the nasopharynx and tonsils of apparently healthy animals and serotype A2 is the most commonly isolated from both sheep and cattle. The organism can be isolatedf from lambs right after birth (Shreeve and Thompson, 1970; AL-Sultan and Atiken, 1985), Pass and Thompson (1971) have shown this carriage to flactuate over time. The adherence of the organism to the nasopharynx of sheep has been shown to coincide with occurrence of disease. However, there is no evidence that indicate whether colonization leads to disease (Biberstein *et al.*, 1970). In the nasal cavity of calves the bacterial flora has been shown to fluctuate in species and number. Although *M.haemolytica* can dominate among the flora, it may get absent for weeks at a time (Magwood *et al.*, 1969). Attempts to culture consistently *M.haemolytica* from swabs taken daily from known colonized animals have failed (Pass and

Thompson, 1971). The mechanisms that *M.haemolytica* possesses to survive in the upper respiratory tract are unknown. The organism must overcome host-response, competition with other resident bacterial flora to remain viable. Nutritional deprivation whether due to a low nutrient environment or host restriction mechanisms, is a situation many colonizing bacteria encounter.

Based on their biochemical characteristics strains of *M.haemolytica* are designated as A or T biotypes. Within biotype A, there are serotypes A1, A2, A5, A6, A7, A8, A9, A11, A12, A13, A14, A16, and A17 (Fodor and Varga, 1987; Younan and Fodor, 1995), and biotype T comprises serortypes T3, T4, T10, and T15, (Fresar, 1982). Strains which are untypable can be isolated with varying frequency, and generally belong to biotype A (Aarself *et al.*, (1970) and (Frank *et al.*, 1980).

2.2.1. General characteristics of family pasteurellaceae

2.2.1.1. Morphology

The family pasteurellaceae are gram negative non motile coccobacilli with bipolar staining or short rods with varying size. Newly isolated strains are capsulated and stain bipolarly, may loose these properties after repeated culturing (Buxton and Fraser, 1977),. 0.3-2μm. Isolates or tissue sample smears are stained with Ramanowfsky stains, like Wright or Giemsa or Leishman's stain (Carter, 1984) *M. haemolytica* is encapsulated and may be short rod, oval-shaped coccobacilli or show pleomorphism. (Merchant and Packer, 1983). Following repeated culture on agar, the organism tends to form longer rods of various sizes and becomes more pleomorphic, forming chains or filaments. Few passage organisms are indistinguishable from *P.multocida*. Bipolar staining is not a constant feature of the organism. Isolation from infected lungs may fail to show bipolarity. (Buxton and Fraser, 1977). Most of the virulent strains of *P. multocida* and (*P). haemolytica* produce capsules of varying size and antigenicity that determine specificity of synthesised antibodies (Adlam, 1989). These capsules can be lost after several sub cultures (Carter, 1984), and old cultures and cells grown under adverse conditions show little cell-associated capsular materials (Jacques, *et al.* 1994 and Veken, *et al.* 1994).

2.2.1.2. Biochemical characteristics

Bacteria of the genera *Pasteurella*, *Mannheim*, *Haemophilus* and *Actinobacillus* and other groups of animal pathogens exhibiting phenotypic and genomic relationship are aerobic or facultatively anaerobic (Younan and Fodor 1993). They grow in tryptose- serum broth or blood agar (OIE, 1992), forming round, smooth, grayish glistering translucent colonies, approximately 1-3 mm in diameter, on agar after 24 hours incubation at 37°C. Only *P. haemolytica* produces a remarkable zone of β-hemolysis on blood agar while the others do not. *P. multocida* does not grow on Mac Conkey's media unlike *M.haemolytica*.

They are Catalase/Oxidasepositive and reduce nitrate to nitrites. Gelatinase-negative, Methyl red and Voges-Proskaure-negative, and lysine and arginine decarboxylase are not produced. Glucose and other fermentable carbohydrates are fermented with the production of acid, but usually no gas (Carter, 1984; Mutters *et al.* 1989. A protease activity on partially purified *M. haemolytica* culture supernatant was observed that cleave Ig G. This phenomenon allows this organism to colonize and proliferate in ovine respiratory tract (Lee and Shewen (1996) in (Brogden *et al.*, 1998). The biochemical characterstics are summarized in Tables 2 and 3.

Table 2. Different biochemical characteristics attributed to Pasteurella organisms.

characteristics	P. multocida	P. haemolytica	P. pneumotropica	P. ureae	P. aerogenes	P. gallinar
Haemolysis	-	+*	-*	-	-	-
MacConkey	-	+	-	-	+	-
Agar growth (Indol)	+	-	+	-	-	-
Urease	-	-	+	+	+	-
Gas from CHO	-	-	-	-	+	-
Catalase	+	+	+	+	+	+
Oxidase	[+]*	+	+	+	+	+
H2S production	[+]	+	+	+	+	+
Nitrate reduction	+	-	+		+	+
Acid production from:						
Glucose	+	+	+	+	+	+
Lactose	-	d	d	-	-	-
Sucrose	[+]	+	+	+	+	-
Fructose	-	-	d	-	+	-
Arabinose	d	d	[-]		+	+
Maltose	d	+	[+]	+		+
Mannitol	+	+	[+]	+		+
Mannose	[+]		[+]			d
Trehalose	[+]	-	[-]	-	+	d
Xylose	[+]	-	[-]	-	+	

Source: Merchant and Parker, 1983; Carter, 1984; Townsend, 1987, Carter and Chengappa, 1991.

(-) Most strains negative; - = all strains negative

*+ = All strains positive; [+] = most strains Positive; d = different among strains; [-] =

Table.3. Different characteristics of biotypes of *M.haemolytica*

Characteristics	*A(P.haemolytica sensu stricto)*	T *(P.trhalosi)**
Fermentation:		
Arabinose	+(7 day)	-
Trehalose	-	+
salicin	-	+ (2days)
Lactose	+/-	-
Catalase	+	-
Esculin	-	+
Lectin agglutination	-	+
Beta-galactosidase	+	-
Colony	Small grey	Larger, brownish centers
Susceptibility to penicillin	high	low
Serotypes	1,2,5,6,7,8,9,11,13,14	3,4,10,15
Viability on media	Early loss of viability	Viablefor longer periods
Principal habitat in healthy carrier	nasopharynx	tonsil
Disease syndrome	Pneumonia in cattle and sheep; septicemia in nursing lambs	Septicemia in feeder lambs

(Merchant Packer, 1983; Carter, 1986; Townsend, 1987;Carter and Chengappa,1991)

2.2.1.3. Biotyping of *P.multocida*

Separation of *P.multocida* in to subgroups or biotypes based up on variations in biochemical characteristics has been reported (Frederikson, 1973; Weaver and Hollis,1980). The subgrouping has been based mostly on reaction patterns observed with acid production from certain pentoses (like xylose and arabinose), disaccharaides (like maltose and trehalose) and polyhydric alcohols (like sorbitol, mannitol and dulcitol). Higher distances of certain fermentation reaction patterns for isolates from a particular animal species have been reported. Acid production from maltose, trehalose and dextron encountered more frequently with isolates from dogs (Heddleston, 1976;

Smith, 1961). No clear-cut correlation established between varying biochemical properties of different strains and pathogenicity.

2.2.1.4. Biotyping of *M.haemolytica*

The formation of a narrow zone of haemolysis on ovine or bovine blood agar is basic to distinguish *M. haemolytica* from other pasteurella species. Thinly poured plates facilitate the observation of this zone which can, in some cases only be detected when colonies are scraped off the surface of the plate (Biberstein *et al.*, 1960). The ability of *M.haemolytica* strains to grow on MacConkey agar (on which lactose positive strains give rise to small pink or reddish colonies), their lack of urease and their ability to produce indole serve as well to distinguish them from other pasteurella organisms. In sugar fermentation two distinct biotypes of *M. haemolytica*, A (majority ferment A rabinose but not Trehalose) and T (all ferment Trehalose), have been differentiated (Smith, 1961) and these are responsible for different clinical forms of pasteurellosis in sheep, goats and cattle. The ability of strains to ferment xylose, salicin and lactose also assists in differentiating between biotypes. Thus A strains are usually salicin negative, xylose positive and lactose positive (apart from serotype A2 strains which are lactose negative) where as T strains are uaually salicin positive and negative for xylose and lactose (Biberstein, 1978). Alternatively, *M. haemolytica* strains may additional be differentiated from pasteurella multocida by their ability to ferment maltose, dextrin and inositol (on old cultures).

2.2.2.Taxonomy

According to DNA hybridization pattern, the genus *pasteurella* comprises several pathogens of animals and man. These include *P.multocida* (with 3 subspecies), *M.haemolytica* (biotype A and T), *P.dagmatis*, *P.gallinarium*, *P.volantium*, *P.canis*, *P.stomatis*, *P.avium*, *P.langaa*, *P.anatis*, *P.caballi*, *P.aerogenes*, *P.antipestfer* and *P.neumotropica* (Quinn *et al.*, 1994; Smith and Phillips, 1990; Carter, 1984). Among these members *P.multocida* and *P.haemolytica* are the most dominant pathogens in domestic animals causing severe diseases and major economic losses in cattle, sheep, pigs and poultry industries (Confer, 1993).

2.2.2.1. Designation of *P.multocida* and *M.haemolytica*

Considerable uncertainity remains regarding taxonomic organization within the family Pasteurellaceae (Carter 1984) and recent data suggest that there is little genetic relationship between the two biotypes of *P. haemolytica* or between *P.haemolytica* and the American reference strains of *Pasteurella multocida* or members of the genus Actinobacillus (Bingham *et al.*, 1990).

Strains of *P.multocida* newly isolated from diseased animals appear as short ovoid rods, measuring 1.0μm by 0.5 –0.8μm, with a tendency to bipolar staining. On repeated passage culture on agar, the organism tends to form longer rods and become more pleomorphic, forming chains, filaments and rods of various sizes. Bipolarity and capsules can also disappear after repeated culturing (Buxton and Frazer, 1977; Smith and Phillips, 1990).

Previous immunological studies conducted on strains of *P.multocida* isolated from a variety of host species employing gel diffusion precipitation tests, capsular swelling tests, indirect haemagglutination tests (Carter, 1984) and protection tests in mice have shown antigenic diversity. However, in most cases antigenic types have been described on the basis of differences in capsular substances, polysaccharides as types A, B, D, E, and F. Capsular types are further subdivided on the basis of somatic antigens. Currently, the common method of designating serotypes is to combine Carter's system of capsular typing and Heddleston's system of somatic typing (Smith and Phillips, 1990); DeAlwis, 1993). Specific serotypes or strains show host specificity and virulence in disease induction as shown on (Table 4).

Table 4. Designation of *P.multocida* serotypes by the Namioka-Carter Method

Capsular type	Somatic type	Serotype	disease
		1:A	Pneumonia (cattle,sheep,pigs)
		3:A	Pneumonia (pigs)
		5:A	Fowl cholera
		7:A	Septicemia (catle)
		8:A,9A	Fowl cholera
B	6,11	6:B	Haemorrhagic septicemia
		11:B	Wound infection(bovine)
D	1,2,3,4,10	1,D,W:D,10:D	Pneumonia (pigs)
		3:D	Pneumonia (cats)
		4:D	pneumonia (Sheep, Pigs)
E	6	6:E	Haemorrhagic septicemia

Source: De Alwis, (1993).

P.multocida doesn't produce exotoxin, but endotoxin is driven from autolysed cells (Merchant and Packer, 1983). However, cell free extracts of certain strains mainly of capsular type D, have been found to contain a toxin that is dermonecrotic for guinea pigs, lethal for mice, and cytotoxic for embryonic bovine lung cells and vero cells (Smith and Phillips, 1990). Sixteen capsular serotypes of *M.haemolytica* and several less clearly defined somatic serotypes are distingushed. Colonial type A contains 12 of the capsular serotypes and the major somatic antigens A and B; and colonial type T contains 4 capsular serotypes 3,4,10 and 15 and the major somatic antigens C and D (Smith and Phillips, 1990). A significant number of isolates of *M.haemolytica* can not be serologically typed and are probably uncapsulated mutants, and they characterstically correspond to biotype A in many aspects (Radostits, 1994). Strains of *M.haemolytica* are now differentiated by a combination of biotype and serotypes and apparent associations of biotypes, serotypes and disease caused as indicated in Table 5.

Table 5. Designation of types of *M.haemolytica* and disease associations in animals

Biotypes	Serotypes	Designation	Source of Disease
A	1,2,5,6,7,8,9,11-14 and 16	A1	Septicemia (lambs) Pneumonia (cattle)
		A2	Pneumonia (sheep, goats)
		A5-9	pneumonia
		A11-14, A16	Inhabitant of URT of normal sheep,goat and cattle
T	3,4,10,15	T3,T4,T10,T15	

Source: De Alwis, (1993).

In the logarithmic growth phase, *M.haemolytica*, release a heat-labile leukotoxin of high molecular weight. This exotoxin, referred to as cytotoxin-leukotoxin, is antigenic and appears to be produced by all known serotypes of *M.haemolytica* (Smith and Phillip,1990). Extracellular enzymes known as neuraminidase are also produced by *M.haemolytica* strains (Wool cook, 1993).

2.3. Ruminant pasteurellosis

Ruminants develop pasteurellosis following climatic, physiological, nutritional and biological stresses. Depending on circumstances, acute mild or chronic pneumonia may result (Martin, 1996). The *M.haemolytica* and *P. multocida* infection of pneumonic pasteurellosis is important to sheep and goats throughout the world.

In lambs and kids, acute pneumonic pasteurellosis is characterized with fever, listlessness, dysponea, poor appetite and sudden death. Those animals, which survive the acute stage, may recover or become chronically, sick and usually affected by sporadic deaths. Such phenomenon is seen in flocks and herds of small ranches, dairy operations or large feed lots are all affected. Although the role of *Pasteurella* in animal disease has been controversial one, the organisms are

certainly secondary invaders due to inevitable deplitative predisposing factors. Their virulence for specific pathogen free lambs showed their potential pathogens (Gilmour, 1983). The total clinical morbidity and mortality due to disease out breaks usually cause great losses in sheep and goats production in different parts of Ethiopia (Bekele *et al.*, 1992).

2.3.1. Pasteurellosis in sheep

Pasteurellosis is one of the most common infectious bacterial diseases of sheep. It is widespread and occurs in temperate, subtropical and tropical climates. The work of Biberstein in the USA and Smith in Scotland in the 1950s on *M.haemolytica*, especially with regard to serotyping and biotyping, led to a better understanding of the etiology and epidemiology of the disease. Efferts made to develop effective vaccines were challenged by difficulties faced in artificial reproduction of pasteurellosis, which is basic for efficacy tests. However, the commercially -available, empirical, bacterin vaccines which were not very effective initially were improved (Gilmour *et al.*, 1978). Pasteurellosis in sheep occurs principally in two forms: pneumonic form commonly caused by *M. haemolytica* biotype A and rarely by *P.multocida*; and systemic form caused by *M.haemolytica* biotype T (Radostits *et al*, 1994).

2.3.1.1. Pneumonic Pasteurellosis

Pneumonic Pasteurellosis (PP) is an important disease of sheep throughout the world. Its etiologic agents are described as *M.haemolytica* biotype A and *Pasteurella multocida* serotypes A and D. It is an acute bronchopneumonia, often stress-induced. Fever, dysponea, nasal discharge, anorexia, toxemia and death clinically characterize it. Major pathological findings are lobular and anteroventrally distributed exudative pneumonia and fibrinous pleuritis (Radostits *et al*, 1994). In temperate climates, P. *multocida* causes the disease but *M. haemolytica* can cause in rare cases. In New Zealand *M. haemolytica* type A is invariably the cause and serotype A2 is the second common isolate. Research on preventive vaccines against ovine pneumonia is continuing and trials so far conducted using killed *M.haemolytica* A1 and A2 vaccines showed high efficacy against homologous A2 strain challenge (Radostits *et al.*, 1994).

2.3. 1.2. Septicemic Pasteurellosis

M. haemolytica has been isolated from lambs with septicemia, pneumonia, mastitis, arthritis and meningitis (Gilmour 1978; Gilmour and Gilmour 1989). Two biotypes of *M. haemolytica* are isolated and characterized as arabinose (biotype A) and trehalose (biotype T) fermentors. In sheep, the two biotypes of *P.haemolytica* tend to be associated with different clinical conditions. Biotype T mainly causes septiceamia in young weaned sheep (Stamp *et al* 1955; Biberstein and Kennedy 1959; Smith 1961; Hajitos *et al* 1983). So far there have been no precise epidemiological studies of septicaemic pasteurellosis (Gilmour, 1978). The syndrome occurs in all ages groups of sheep at all times of the year but it is prevalent in weaned lambs from 5-12 months of age (Radostits *et al.*, 1994). The pathogenesis is to occur when the bacteria invade the lesions and enter the blood stream via venous and lymphatic routes. They produce embolic pneumonia. The bacteria multiplying in the lungs produce the toxins that elicit inflammation in the lung changes and lead to anoxia and death (Gilmour, 1993; Radostits *et al.*, 1994).

2.3.1.3. Systemic Pasteurellosis

The young lambs are highly susceptible to biotype A infection. The latter causes septicemic pasteurellosis. However, systemic pasteurellosis is a disease associated with infection caused by *P. haemolytica* biotype T strains. All the serotypes of biotype T are known to be responsible for systemic pasteurellosis in sheep (Radostits *et al*, 1994; Gilmour, 1993).

Systemic pasteurellosis appears to be less common than pneumonic one worldwide. It is an important disease of sheep in Britain. Asymptomatic carriage of biotype T strains occurs in the tonsils of many healthy sheep, rather than in the naso-pharynx. Stressful environmental and management factors, such as transport, marketing shearing coupled with movements to better grazing areas have been indicated as predisposing factors for the disease. But, a clinical disease does not invariably follow. (Radostits *et al*, 1994;Gilmour, 1993).

2.3.2. Epidemiology

2.3.2.1.Occurence of *M.haemolytica*

M.haemolytica has been isolated from clinical cases in sheep and goats that occured in various African countries including Kenya (Mwangota *et al.*, 1978), Sudan (Mohammed and Hussein, 1981). In Ethiopia isolation of these organisms has been carried out from diseased and healthy flocks. Accordingly, 11-M. *haemolytica* serotypes were isolated (Pegram *et al.* 1979).

2.3.2.2. Source of infection and mode of transmission

M. haemolytica is a normal inhabitant of the upper respiratory tracts of animals (Radostits *et al.*, 1994). Colonization of the naso-pharynx and tonsil occurs very shortly following birth, probably from the ewe (Shreeve and Thomson, 1970). Apparently healthy carriers are potential sources of infection. Transmission is due to direct contact, which leads to inhalation of aerosols and ingestion of contaminated feed and water (Merchant and Packer, 1983).

2.3.2.3.Host range and susceptibility

Pasteurella organisms have a wide host range and have been isolated for example, from cattle, buffaloes pigs, sheep and goat. Pasteurellosis due to *M. haemolytica* occurs in all age groups of sheep and goats but lambs and kids during the earliest months of life. While in ewes it is prevalent after lambing. (Gilmour and Gilmour, 1989). Septicemic pasteurellosis commonly occurs in conjunction with pneumonic form (Radostits *et al.* 1994).

2.3.2.4. Predisposing (risk) factors

Stress is known as the major predisposing factor for the respiratory tract pasteurellosis in animals and man. (Biondi and Zannino, 1997). However, it is difficult to assess the effect of physiological and environmental stress (Swanson, 1995).

2.3.2.4.1. Animal management factor

Pasteurellosis is often known to be associated with stressful factors. Shipment, handling and transport, crowding of animals, inadequate water and feed supply are some of the predisposing factors of the disease (Carter, 1984; Radostis *et al.*, 1994). In sheep and goats, tolerance of stress varies with age and breed. Under stressful conditions, the pulse rate, body temperature, plasma cortisol, glucose, fatty acids, beta-hydroxybutyrate and urea levels increase while body weight decreases (Biondi and Zannino, 1997).

2.3.2.4.2. Physical environmental factors

Common physical environmental factors, either alone or in combinations, predispose sheep and goats to respiratory infections. These include heat, crowding, limited space, exposure to inclement weather, poor ventilation with high levels of moisture and barnyard gases, excessive dust in feed lots, airway obstruction and inhalation of foreign objects. In calves, experimentally exposed to abrupt changes in temperature have been shown to enhance the *M. haemolytica* colony forming unit (C.F.U.) in the nasopharynx (Jones, 1987). Intensive exercise in animals has been correlated with increased susceptibility to respiratory infection caused by *M haemolytica* (Anderson *et al.*, 1991).

2.3.2.4.3. Viruses as biological factors

Viruses affect the respiratory tract of sheep and goats. Viruses associated with acute infections are parainfluenza-3 (PI-3) virus, respiratory syncytial virus (RSV), adenoviruses, Peste des Petits Ruminant virus (PPRV), Sheep pox and the closely related goat poxvirus, herpesvirus and reovirus (Jacob, 1982). These viruses causeepithelial tissue damage, suppress the local immune mechanism including mucociliarly clearance allowingorganisms reach the lower respiratory tract. Under experimental conditions sheep are first infected with virus and then with *M. haemolytica*. Combined infection of lambs with viruses followed by *M. haemolytica* induces lesions more severe than that seen with either agent alone. These combined infection causes fibropurulent pneumonia with oedema, focal necrosis and pleuritis, resulting in early death of lambs or slow resolution of lesions. Generally, viral infection is thought to create an ideal microenvironment consisting of necrotic cells and proteinaceous fluid in the lung that are conducive to bacterial

growth interference of the mucociliary clearance mechanism of the respiratory tract and, by depressing the capacity of resident lung macrophages to take up and kill bacteria (Jacob, 1982).

2.3.2.4.4. Bacteria as biological factor

Similar to viruses, some respiratory bacterial infections also increase the susceptibility of sheep and goats to secondary *M. haemolytica* A2 infection. Mycoplasmas are commonly infected the respiratory tract of sheep and favour the development of pneumonia.The combination of Mycoplasma ovipneumoniae and *M.haemolytica* A2 induce a proliferative (atypical) pneumonia of lambs (Jakab, 1982). Lesions consist of peri-bronchiolar cuffing and interstitial change characterized by mononuclear cells that cause thickening of alveolar septa (Brogden et al., 1998). *M.ovipneumoniae* may facilitate the pulmonary establishment of *M.haemolytica*, with apparaent exacerbation of the disease in atypical pneumonia. Other bacteria that are isolated with *M. haemolytica* in pneumonic infections include *P.multocida*, *Staphylococcus* species, *Streptococcus* species, *Escherichia coli*, *Chlamydia* and *Haemophilus*.

2.3.2.4.5. Parasitic infections as biological factors

Parasites, particularly lungworms increase the susceptibility of sheep to secondary *M. haemolytica* infection. The lungs of sheep are usually affected by *Dictyocaulus filaria*, *Muellerius capillaris* and *Protostrongylus rufescens* (Radostits *et al.*, 1994). These worms are prevalent in the highlands of Ethiopia (Feseha, and Gebre-Negus, 1977). To date, only *D.filaria* has received more attention, but some reports indicate the prevalence of *M.capilaris* and *P.rufescens* in small ruminants in Ethiopian. *D.filarea* plays a role in development of verminous pneumonia, acute interstitial pneumonia, and secondary bacterial pneumonia (Radostits, *et al.*; 1989).

The distribution and epidemiology of *D. filaria* is worldwide but is highly prevalent in cooler areas with high rainfall. *D. Filaria* is known to be prevail in many parts of Ethiopia, especially the highland areas. It either causes verminous bronchitis or predisposes animals to other lung disease such as *Pasteurella* pneumonia (Progress Report, 1997). Young animals are generally susceptible to the disease compared to the older animals. This has been partly explained by the acquired immunity in older animals. Adult worms of *D. filaria* live in the bronchi and bronchioles, and cause alveolar damage. The resulting blockage of bronchioles by exudate leads

to the collapse of portion of the lung. Clinically the emphasis is on bronchial irritation that results in cough, moderate dysponea and loss of condition. There may be fever and toxemia if secondary bacterial infection occurs (Radostits *et al*, 1989). .

2.3.3. Pathogenecity

Mannheima haemolytica produces leukotoxin, endotoxin and capsular polysaccharide that either directly kill alveolar macrophages (Sutherland, 1985) or suppress function of macrophages (Czuprynski *et al.,* 1987). *M. haemolytica* contains enzymes, which may allow it to proliferate and colonize the respiratory epithelium. These include O-sialoglyco-protein endopeptidase (Abdulah et al., 1992, (Muller and Mannheim, 1995). Furthermore, *M. haemolytica* synthesize inflamatory molecules (capable of inducing tissue damage) including capsular polysaccharide lipopolysaccharide and membrane proteins such as lipopolysacharide associated protein and other surface antigens, proteins used in iron acquisition and periplasmic superoxide dismutase (Brogden *et al.,* 1995). *M. haemolytica* produces an RTX LktA 105 Kda leukotoxin which plays a central role in induction of inflammatory reaction and tissue dammage. Neutrophils and mast cells exposed to Lkt release oxygen-free radicals, proteolytic enzymes and histamine (Adusu *et al.*, 1994). Purified LKt induces lesions in the lungs of ruminants. These lesions consist of consolidated areas with oedema of interlobular septa and haemorrhage (Whiteley, *et al.*, 1992)
M. haemolytica has a primary or secondary role in pneumonia of ruminants. Under normal conditions, lungs are relatively free of pasteurella due to effective lung clearance mechanisms. Hypotheses suggest that a combination of viral infection (like PI-3 virus) of the respiratory tract and devitalizing influences from transportation, temporary starvation, weaning rapid fluctuation in ambient temperature and mixing of flocks from different origins can all collectively promote an increase in density of the organism in the naso-pharynx from which it is then inhaled to the alveoli and not effectively cleared (Radostits *et al.*, 1994; Gilmour, 1993).

M. haemolytica produces several virulence factors like fimbriae, leukotoxins, capsule and endotoxins all of which are likely to be important in the pathogenesis of pneumonic pasteurellosis (Gilmour *et al.*, 1989). Biotype A is particularly associated with the disease, being of a greater incidence due to serotype A2 compared to other serotypes due to its less immunogenecity. Serotype A1 is considered as the most virulent of all serotypes, due to its cytotoxin (Casamtjana, 1994, cited by, Gelagay, 1996). Attempts to understand the pathogenesis of pulmonary lesions

due to M.haemolytica were made by experimental reproduction of the disease following aerosol infection of sheep with PI-3 virus and bovine herpes virus -1 of calves (Blood *et al.*, 1989)

2.3.4. Pathology

During acute pasteurellosis, oral and nasal mucous membranes are congested and muco-purulent sometimes blood smeared discharge comes out of the nostrils. Petechiae and ecchymoses may be found in the subcutis of the neck and thorax. Blood splashing may be seen in the peritoneum, and the liver and kidneys are edematous (Radostits *et al.*, 1994). The most prominent changes, however, are present in the thoracic cavity (Gilmour, 1993; Radostits *et al.*, 1994). Sub-pleural and subepicardial petechiation are common. Varying amounts of a clear, yellowish pleural and pericardial exudate are found. In per-acute cases, pulmonary hepatization is absent, however, the lungs are congested, heavy and the surfaces are red to dark purple with dark patches resembling haemorrhagic infarcts (Gilmour, 1993; Radostits *et al.*, 1994). The cut surface of such lungs exudes pinkish froth and the interlobular and some times lobar septa are thickened and opalescent. In this type and in the less acute pneumonic pasteurellosis, the tracheobronchial linings are red to dark purple and the airways contain pink-stained frothy liquid. In less acute cases, pulmonary consolidation involving large areas of the lung is evident. The pleurae may be thickened and opaque, or clearly oedematous, or may be covered by a thick layer of yellow or greenish clotted exudate. Adhesions between lobes and either the pericardium or to the parietal pleura may be present. The lungs appear bluish to dark red, masking the extent and margins of consolidation (Radostits *et al.*, 1994).

Lesions of spontaneous and experimental pneumonic pasteurellosis in sheep can be distinguished from lesions induced by other Gram-negative bacteria by the deposition of fibrin in the lungs and on the thoracic pleura (Gilmour, 1989). Excess serous fluid is often present in the pleural and peritoneal cavities. Slight hydropericardium can sometimes be seen. Histopathologically, interlobular septa, pleura and peribroncheal interstium are expanded because of edema and fibirin deposition. There are thrombiosis of lymph vessels, hemorrhages, and moderate to marked infiltrations of intact and degenerated neutrophils and macrophages (Gilmour, 1993; Radostits *et al.*, 1994). Changes consist of pneumonitis with multifocal areas of acute fibrino purulent

broncho pneumonia, coagulative necrosis and fibrinous pleuritis. Necrotic areas in alveoli are outlined with congested capillaries and are filled with fibrin, proteinaceous material, bacterial colonies, erythrocytes, neutrophils and macrophages (Gilmour, 1993). Near the lesion margin, exudates containing neutrophils and macrophages form an abrupt transition to normal lung tissue with no fibrinous tissue capsule. Within the zonal lesions, there may be hyperplastic pneumocytes and fibrino-purulent bronchiolitis (Gilmour,1989.

2.3.5. Immunity

As in other respiratory pathogens, immunity against pasteurella organisms involves mechanical, chemical and cell mediated pulmonary clearance mechanisms (Sharma *et al.*, 1992).

2.3.5.1. Mucociliary factor

Mucociliary clearance is important in removing organisms that reach the respiratory tract and conditions that generally affect ciliated epithelial cells (cold, viruses) and tracheal mucous velocity may result in increased respiratory infections (Brogden *et al.*, 1998). Exposure to cold increases pulmonary adhesion of pathogens, while, simultaneously decreasing mucocilarily clearance of the upper airways, is decreased cold-exposed animals contract respiratory tract infections. Similarly, animals infected with respiratory viruses, which destroy tracheal epithelium, are very susceptible to secondary bacterial infection (Jakab, 1982). Reduced clearance of aspirated upper respiratory secretions containing microorganisms and cellular debris result conditions favoring bacterial growth.

2.3.5.2. Humoral mechanism

Resistance to pneumonic pasteurellosis can be correlated with elevated titers of humoral factors (*imunogllobulins*) like IgA, IgG antibodies (Donachie in Brogden *et al.*, 1998). Therefore the ability of M.haemolytica to cleave immunoglobulins would allow it to colonize and proliferate in the ovine respiratory tract.

2.3.5.3. Pulmonary surfactant

Resident and infalammatory cells, epithelial cells, and serous and mucous secretions in the respiratory tract contain a number of antimicrobial peptides facilitating non-specific protection against bacterial infections. These peptides and some free fatty (neutral) acids are known to enhance the non-specific immune mechanism of the host during a number of bacterial infections (MacDonald *et al.*, 1983). These proteins fix on yeasts and gram negative bacterias and their lipopolysacharides inhibiting their binding on respiratory epithelium. Thus facilitate physical clearance of bacterias by the mucociliuary mechanism and play opsonin like role facilitating phagocytosis. Some individual deficient to these proteins is shown to be more susceptible to pneumonia.

2.3.5.4. Cellular immune mechanism

Alveolar macrophages are instrumental in pulmonary innate immunity serving as phagocytic cells with their capacity to scavenge bacterial particles and other macro-molecular debris. They also serve as accessory cells in nodulation of the immune response, activate and recruit other inflammatory cells for maintenance and repair of the lung parenchyma and normal physiology (Brogden, 1992).

In normal unstressed animals, the cellular defense mechanisms of the lungs and the mucocillary ladder serve to clear pasteurella from aerosols in the nasopharynx. Alveolar macrophages, the principal phagocytic cells of the lungs are the first line of defense against pulmonary bacterial invasions. It is followed by neutrophils as they are breached and inflammation proceeds (Gilmour, 1993).

Stressful factors result in increased cortisole release then by inducing immunosuppresion paving ways for the opportunistic organisms, *M.haemolytica*, to invade the host. Mycoplasms and viruses also impair the respiratory defense mechanisms by enhancing susceptibility to bacterial attachment and colonization, reducing mucocillary clearance, and decreasing surfactant levels and alveolar macrophage dysfunction. When the defense mechanism is compromised, *M.haemolytica* in the upper respiratory tract proliferates, descends in to the terminal bronchioles and alveoli where they multiply again subsequently, the lungs are deluged with large numbers of bacteria they can not cope with (Clinkenbeard *et al.*, 1992).

Once multiplication in the alveoli has begun, *M.haemolytica* virulence factors exert cellular damage. The fimbriae enhances the localization in the upper respiratory tract, the capsule inhibits complement mediated serum killing with phagocytosis and intracellular killing of the organisms; outer membrane proteins act as porins which transport materials in to and out of the cells and facilitate adherence of bacteria to host cells and the leukotoxin.

The maximum production during the phase of growth, is pore forming cytolysin which is cytotoxic to pulmonary macrophages and peripheral neutrophils that enhances the virulence of the invading organisms (Clinkekenbeard *et al.*, 1992; Radostits *et al.*, 1989).

The ability of the leukotoxin to rapidly cross the alveolar wall and interact with multiple cell types as well as humoral mediator systems has an important implication in the understanding of tissue injury that occurs in pneumonic pasteurellosis. In addition to cytolysin mediated damage, leukotoxins can activate macrophages and neutrophils. Subsequently, activated macrophages produce proinflammatory and chemotactic mediators that initiate neutrophil infiltration and fibrin formation in the lungs. Activated neutrophils also release their oxygen radicals, enzymes (lysosomes), and basic proteins which all degrade cellular membranes, increase capillary permeability, which results in fluid accumulation in the interstitium of alveolar wall, intralveolar heamorrhage, alveolar wall necrosis and oedema (Gilmour, 1993; Radostits *et al.*, 1994).

Leukotoxin mediated platelet damage may also contribute to the vascular changes accompanying pneumonic pasteurellosis. Release of fibrinogen and vasoactive compounds from platelets may promote formation of thrombi and accumulation of fibrin in the interstial sites, commonly associated with the disease (Clinkenbeard *et al.*, 1992).

2.3.6. Diagnosis

Diagnosis of pasteurellosis is based on the history, clinical signs, and gross pathological lesions. Species, age affected, season of outbreak and associated factors are also considerable facts giving clue for tentative diagnosis (OIE, 1992) Climatic changes, animal movement and dipping have been reported to be associated with the outbreaks of pasteurellosis in sheep (Gilmour and

Gilmour, 1989). There is general agreement that young animals are more susceptible to both pneumonic and septicemic pasteurellosis. Clinical signs of affected animals and features found at necropsy are important in the diagnosis of small ruminants' pasteurellosis in the field.

2.3.6.1. Clinical

In flock outbreaks, the first manifestations are sudden deaths in the absence of premonitoring clinical signs and very sick sheep that die immediately (Gilmour, 1993). In lambs less than three weeks old the disease is hyperacute, with a generalised infection. Occurrence of sudden death with out prior illiness in lambs may continue through out the outbreak, but signs of acute pneumonia-hyperpnoea and dyspnoea which can be accentuated by driving are present in older sheep (Radostits et al., 1989). As the outbreak progresses, it is replaced by cases with the more obvious signs of pneumonia, including dyspnoea, slight frothing at the mouth, coughing and oculo-nasal discharge. Death may occur as early as 12 hours after the first sign of illiness, but the course in most cases is about 3 days. Infected sheep are usually febrile (40.4-42°C), depressed and anorexic. A typical out break of pneumonic pasteurellosis in adults starts with sporadic deaths. Close inspection of the flock at this stage may reveal some sheep with a slight, serious, oculonasal discharge and with an occasional soft cough. Clinical cases of pneumonia then occur. Affected sheep are dull and anorectic with rectal tempretures ranging from 40.6 to 42.6°c .A proportion of sheep with these signs become recumbent and die within a few hours. Others develops dyspnoea, manifested by exaggerated movement of the abdominal flank muscles. Most sheep which reach these stages die, if untreated, while a small number survive to develop chronic pneumonia and then generally remain unthrifty. Total clinical morbidity and mortality seldom exceeds 10%. The disease in lambs depends on age. It is not common under about three weeks of age, but when encountered is a hyperacute, rapidly fatal septicemia. As the lambs get older, some affected animals may live long enough to appear very dull but quickly become recumbent, collapse and die. By three months of age, higher proportions of cases live long enough to develop the more obvious signs of pneumonia.

2.3.6.2. Laboratory

2.3.6.2.1. Bacteriological

The bacteriological diagnosis of pneumonic pasteurellosis depends on the isolation of the causative organisms, either *M.haemolytica* or *P.multocida*, from sick and/or healthy live animal specimens like exudates, nasal swabs, broncheal lavages and lung tissues of dead animal by cultural, and the identification of the organisms by biochemical and serological methods (OIE, 1992; Quinn *et al.*, 1994).

M.haemolytica is present in very large numbers in lung lesions and exudates. In the very acute and generalized form, it can be recovered from the liver, spleen, kidney, and lymphnodes and heart blood. In subacute cases, the organism is often confined to the lung lesions. Portions of pneumonic lung from the edge of the lesion, and in generalized form pieces of liver, spleen, kidney and lymhnodes could be submitted for isolation.

In the laboratory smooth grayish glistering translucent colonies are characteristic to *M. haemolytica*. The ability of it to grow on MacConkey agar (on which lactose positive stains give rise to small pink or reddish colonies) and formaion of narrow zones of haemolysis on blood agar serve to distinguish it from other Pasteurella (Buxton and Fresar, 1997). They are non-motile and Gram- negative cocco-bacilli with bipolar staining (Merchant and Packer, 1983). Acid with out gas is produced by *M haemolytica* following fermentation of glucose, maltose, saccharose, mannitol, sorbitol, fructose, galactose and xylose. Nitrate is reduced to nitrite while gelatin is not liquidified (Buxton and Freser, 1997; Carter, 1984).

2.3.6.3 Serology

2.3.6.3.1. Indirect haemagglutination test

Indirect haemagglutination (IHA) test has been widely used over a long periods of time for serological diagnosis of pasteurella organisms and for serotyping (Biberstein et al., 1978; Fodor and Varga 1988). Sero-epidemiological investigation of pasteurellosis (Hussein and Mohamed, 1984; Biberstein, 1965) had been established using standard of serum dilution of 1:50 in (Hussein and Mohamed, 1984; Biberstein, 1965) while EMVT, France applied 1/40 dilution (F.Roger, 1996). The highest dilution for positive IHA is 1/256 (Mohammed and Hussen, 1981). The recognized convenience and sensitivity of haemagglutination test encouraged several workers to institute modifications. The source of suitable erythrocytes for haemagglution test is very important considerations since stability and agglutinability of red cells of different animal species

show significant variations. More recently sheep erythrocytes (preferably from male animals) are the most commonly used for IHA tests. They are well agglutinable and require no pre-treatment. One of the earliest agents used to couple antigens to red cells was bis-diazotized benzidine, a molecule with two reactive diazonium groups. But it has not restricted the formation of antigen-antigen, RBC-RBC binding. Among many other chemicals so far used, glutaraldehyde is advantageous in coupling capsular antigens to erythrocytes. Coupling is rapid and complete after few minutes; more over glutaraldehyde and the coated red cells are more stable than the naked RBC for some weeks at 4°C (Sawada et al., 1982).

2.3.7. Differential diagnosis

In acute cases, the necropsy observations may be common to other conditions displaying acute congestion and bacterial examination is essential in differentiation. These conditions include viral infection (PI3, reovirus, adenovirus), mycoplasmal diseases, lungworms, clostridial diseases, phenolic poisoning, aspiration pneumonia, hypostatic pneumonia and post-mortal autolysis. *E.coli* and *Corynebacterium* spp. may cause lung consolidation, pleuresity, and abscess formation, but these infections in sheep are much less common than pasteurellosis and readily differentiated on microbiological grounds.

Subacute and uncomplicated chronic form of the disease can be differentiated histologically from atypical pneumonia. The latter is characterized by a proliferative exudative pneumonia, perivascular and peribronchiolar accumulation of lymphocytes, bronchiolar epithelial cell hyperplasia and hyperplastic bronchiole associated hyaline scar.

Maedi visna virus, pulmonary adenomatosis (retrovirus and herpesvirus) infectiuons and *Dictyocaulus filaria* can cause chronic progressive pneumonia and must be differentiated by their longer course distinctive histological features (Gilmours 1989 and Radostits *et al.*, 1994).

When deaths occur in lambs without prior clinical illness the disease may be mistaken for septicemia caused by Haemophilus agent but in pasteurellosis the rate of spread is the flock is much slower and the flock mortality rate is much less. The Primary differential diagnosis needs to be carried out for systemic pasteurellosis and enterotoxemia caused by *Clostridium*

perferingens type D as both cause rapid death in sheep. Differentiation is done by post-mortem examination (Radostits *et al.*, 1994).

2.3.8. Treatment

Treatment of *Pasteurella haemolytica* infection is largely dependent on the use of antibiotics and sulfonamides. Several antimicrobial agents such as oxytetracycline, penicillin, streptomycin, sulfonamides have been used for longer periods of time in the treatment and prophylaxis of the disease. Tilmicosin (long acting macrolid) at a dose rate of 20 mg/kg was reported to have eliminated *P.haemolytica* from almost all the lungs in experimentally induced pneumonia in calves (Gilmour, 1993). Repeated and inadequate use of these drugs resulted in emergence of resistant Pasteurella species and become a treat to control by chemotherapy. According to invitro sensitivity test study, 70% of *P.haemolytica* isolates were proved to be resistant to oxytetracycline, penicillin and sulfonamides.

2.3.9. Control

2.3.9.1. By vaccination

Since pneumonic pasteurellosis is sporadic, it seems to be unlikely that any long lasting flock resistance develops following disease out break. Many of the predisposing factors are part of the normal environment or management of flocks and some are of infectious nature. There fore prevention of suceptible flock would be achieved by vaccination.

Since the early 1970s, vaccines against pasteurellosis of sheep were commercially available although their efficacy was unclear (Gilmour and Gilmour, 1989). They contained formalin-killed cells and were not poly valent. However, there were no reports of field trials that prove or disprove their efficacy and experiences on experimental bacterin vaccines suggested that protection might be serotype specific.The ability to produce pneumonic pasteurellosis in lambs enabled assessment of novel vaccines A standard protocol was developed, reducing between-experiment variation. Vaccinated and unvaccinated lambs were infected with PI-3- virus intratracheally and intranasally, and one week later exposed to an aerosol of *M.haemolytica* for 15 minutes. The lambs were examined for clinical signs for five or six days and survivors were necropsed on the seventh or eighth day after exposure to the aerosol. In order to have a numeric

indication of the efficiency of vaccination a scoring system was developed, involving specific clinical parameters, a measurment of lung lesions at necropsy and an index of organisms recovered from standard sites. A commercially available bacterin vaccine, has not protected from *M.haemolytica* A2, challenge.

Based on the role of *P.haemolytica* A1 in the disease, process attempts have been made to prevent the disease by vaccination. Several workers have studied the efficacy of a variety of vaccine preparations including live bacteria (Chengappa *et al.*, 1989; Confer *et al.*, 1984; Confer *et al.*, 1986), bacterins (Confer et al., 1987), capsular polysaccharide (CP) (Conlon and Schewen, 1993), outer membrane proteins (Confer *et al.*, 1985; Craven *et al.*, 1991), culture supernatant of *P.haemolytica* (Shewen and Wilkie, 1988; shewen *et al.*, 1988; Conlon *et al.*, 1991), and recombinant leukotoxin (Conlon *et al.*, 1991) with varying results. Some of these preparations have been licensed and are available commercially for use in cattle industry in United States of America.

The immune response to Pasteurella organisms is predominantly humoral. This immunity may persist up to six months. A fluctuating range of serum antibody titer to a range of serotypes is detected by Indirect Haemagglutination test over a period of time in normal flocks. These are probably the response to carriage in the upper respiratory tract and to intermittent mild challenge of the lower respiratory tract (Gilmour and Gilmour, 1989). There have been numerous attempts to develop effective vaccine against pneumonic pasteurellosis since the 1950's, but despite these efforts, a vaccine that provided complete and consistent protection against pneumonic pasteurellosis is not yet available (Brennan *et al*, 1998). An effective vaccine development has been hindered by the presence of multiple serotypes of *P.haemolytica*, lack of cross protection among serotypes and by difficulties with consistent experimental reproduction of the disease (Moiser, 1993).

Field vaccination experience in East Europe showed that there is no specific immunization against ovine pasteurellosis; but is controlled by vaccinating concurrent pathogens like parainfluenza-3 virus vaccine in attempt to immunize lambs against challenge exposure with both the virus and *M.haemolytica* which can result in pneumonic pasteurellosis (Radostits *et al.*, 1994). Experimental studies currently focus on the identification of specific immunogens (CP, OMP, and LKT antigens) of *M.haemolytica* that can be incorporated in vaccines. Hence although,

whole bacterins and live vaccines continue to stimulate interest, future efforts directed towards the development of sub-unit or recombinant vaccines (Brennan *et al.*, 1998; Moiser, 1993).

2.3.9.2. Improved management practices

Management practices such as avoiding over crowding, mixing of different flocks, supplying adequate feed and water, providing ventilated shelter and avoiding from exposure to aerosol infection can reduce incidence of the disease. Vaccination and chemoprophylactic measures at the appropriate time of the year or before exposure to stress factors are crucial in minimizing cases of Pasteurellosis (Radostits *et al.*, 1994; Rimler, 1993).

3. MATERIALS AND METHODS

3.1. General description of the study area

The study was conducted in selected sites of Amhara and Somali regional states.The selected sites were in three different livestock production systems, the pastoral (nomadic), the agro-pastoral (semi-nomadic) and the cool centeral highland crop-livestock mixed production systems. The two randomly selected zones were North Shewa and Jijiga .The North Shewa (DebreBerhan) area is located about130 km North of Addis Abeba at 9° 36'N and 39° 36'E with altitude range 2200 – 3000m a.s.l.

In North Shewa, small holder mixed crop-livestock farming dominates the agricultural sector. The annual rainfall is of 920mm and the average minimum and maximum temperature is 5.5°C and 20.4°C, respectively with relative humidity of 76.2%. The topography is characterized by flat to undulating and hilly types. Soil degradation and minimal vegetation cover are the main features of the area. The rainfall regime is bimodal. About 70% of the total precipitation occurs from June to September and the rest from February to April.

Jijiga zone is located about 650km east of Addis Ababa at 4° - 10° N and 42° - 48° E with altitude ranging from 1600-1700 m.a.s.l. The average rainfall ranges from 500 to 700mm. The rainfall is unreliable, erratic, and short lasting resulting droughts in the area. Despite the solely dependent crop production on rain fall the zone is characterized by low rainfall of high annual and seasonal variability and low dependability and due to this very fact, the agro-pastoralist squarely rely on livestock breeding. Among livestock sheep and goats are the most prioritized animals in the area and the seasonal mating after rains and when green feed available is the common traditional practice of flock owners similar to the modern synchronized sheep breeding program else where in the world. Therefore, it can be said that livestock herding is a traditional mode of life in Jijiga zone.

In addition to the many virtues and almost indispensable role in the pastoral production system, livestock are conferred a very high social value in the Somali pastoral system. Wealth and social prestige are measured by the size of the livestock herds that an individual or a household owns or

keeps large flock size. The climate is hot, arid and semiarid with scattered shrubs. For the last 3 years rain has been very short and had significant socio-economic impact on animal breeders. The current level of the sector's productivity is low, due to mainly wide range of sheep diseases pneumonic pasteurellosis out break among others is the major health threat to sheep and goats in both production systems (personal communication). The major livestock production in the study sites is sheep production in the highlands and sheep and goat production in the low lands.

3.2. Study animals

Two breeds namely Menz type (Debre Berhan Zone) and Black Head Ogaden Somali sheep (Jijiga Zone) that are reared traditionally in the respective study sites were target animals of this survey. Clinically healthy and sick animals from field and apparently healthy and slaughtere at abattoir and backyard slab were investigated. Based on random sampling 8 veterinary clinics and 8 privately owned hotels from Debre-Berhan zone and one Government owned abattoir from Jijiga zone were visited for tissue sample collection.Accordingly 27 nasal swabs 10 tonsils and 37 lungs were collected from clinically healthy, sick, abattoir and back yard slaughtered sheep of the two zones during the study period.

3.3. Study design

3.3.1. Questionnaire survey

A questionnaire survey totally depended on convinience sampling and willingness of the interviewed farmers in providing the right information. During drafting of the questionnaire format much emphasis was given to the environmental and management predisposing factors in sheep production system (housing, feeding, health care and breeding (Annex 7.1).

The survey was carried out in all villages of the selected sites of North Shewa and Jijiga zones in the months of April and June. Individual flock owners, community leaders and shepherd were the target resource persons to be interviewed. Attempt was made to brief the respondents about the purpose and objective of the survey.

Three individuals from each village and a total of 16 shepherds, 16 flock owners and 16 community leaders were interviewed in the study sites. The areas covered during the questionnaire survey were indicated in Table 6.

3.3.2.Cross-sectional study

This part of the study was undertaken to establish the prevalence of *Pasteurella* species. This survey was planned randomly to detect *Pasteurella* antibodies and fields isolate in apparently healthy and sick sheep population. *Pasteurella* isolation was performed from lung tissue, tonsil and nasal swabs of two different production systems and ecozones regardless of breed, sex and ages. Pasteurella strains were determined from tissue samples collected from sheep slaughtered at the backyard of hotels in Debre-Berhan town and at Jijiga abattoir.Volunteer hotels in which the highest numbers of sheep slaughtered on daily basis were selected for the study. Sampling of different specimen was performed at a time.

3.3.3.Sampling method

Sampling was covered regions, zones, and districts, villages and flock size in that order. A combination of multi-stage and random probability sampling were used.

Sample size

In this case the optimum sample size was determined in terms of the cost of sampling from different clusters relative to the cost of sampling animals in the clusters). A relative formula for a 95% confidence interval was used for clustering number of districts and villages Thrusfield, (1995).

Table 6. Summary of sample size

Zone	No. Districts	No. Villages	No. Flock	Total no of sheep
North Shewa	2	8	16	80
Jijiga zone	2	8	16	80
Sum	4	16	32	160

- 2 Regional states (Amhara and Somali Regions)
- 2 Zones (North Shewa and Jijiga zones)
- 4 districts, two from each zone
- 4 villages from each district and a total of 16 villages
- 2 flocks of sheep from each village and a total of 32 flocks
- 5 sheep from each flock and a total of 160 sheep were sampled.

3.4. Laboratory Investigation

3.4.1. Materials for specimen collection

Swab and tissue specimens from clinically healthy, sick and slaughtered animals were collected and transported from field to National Veterinary Institute, Debre Zeit using the following materials.

- Cotton tipped swabs
- Vacutainers with needles
- Sterile test tubes, pasteur pipettes, empty universal bottles (screw capped)
- Transport media
- Marker, spirit burner, alcohol-soaked cotton
- Scissors, tonge forceps
- Universal bottles containing 3 ml tryptose soya broth.

3.4.2. Materials for Bacteriology

- Tissue samples and nasal swabs
- Tryptose soya broth, tryptose blood agar base, MacConkeys agar
- Bacterial isolates (24 hrs. old)
- Petridishes, sterile pasteur pipettes, incubator
- Binocular compound microscope, microscope slide
- Inoculating loop, marker, spirit burner
- Dyes for Giemsa and Gram staining (Giemsa, Gentian violet, Carbol fuchsin, lug's iodine), and acetone alcohol.
- Distilled water, tap water, xylene, immersion oil, tissue paper, and clean blotting paper.
- A 24 hours pure culture of the isolate
- Capsular antigen extracts of the isolate

3.4.3. Specimen collection procedures

3.4.3.1. Nasal swabs

After the sheep were restrained sterile cotton-tipped swabs (in screw capped test tubes) were moistened with sterile tryptose Soya Broth and were inserted in to the nostrils, and the mucosa was rubbed by rolling the swabs. The swabs were then placed back into 3 ml of sterile Tryptose Soya Broth. Then, the broth was kept in an icebox and transported to NVI laboratory. At arrival samples were immediately incubated at 37°C for 24 hours.

3.4.3.2. Tissue sample

The surface of pneumonic lungs was rubbed with cotton soaked in alcohol to minimize surface contaminants. Using sterile materials this part of the lung was cut with scissors by tonge forceps and put in another sterile screw-capped universal bottles. A pair of tonsils were taken from the same carcass sampled for lungs aseptically and put into sterile screw-capped universal bottles. The two universal bottles, containing tonsil and lung samples from the same animal, were labeled with identical numbers and the type of sample was recorded on the container. Then, the tissues

were transferred to another universal bottles containing 3 ml of Tryptose Soya Broth under sterile environment created by Bunsen burner flame. Before transferring the samples to the broth, they were cut in slices with disinfected scissors. The scissors and tonge forceps were heated over the flame between each sample processing. Then, the samples were transported to NVI with transportation media.

3.4.4. Culture

Regardless of the type of growth in swabs, tonsils and lung tissues were removed and streaked over one half of a petridish plate containing blood agar base supplemented with 7% sheep blood, and MacConkey agar. Two separate streaking were made with an inoculating loop for colony identification. The plates were then incubated at 37°C, aerobically for 24 hours.

The growth on the primary culture plates was characterized on the next day and Pasteurella like colonies were subsequently streaked onto another blood agar and MacConkey agar for further examination. Smears were made and stained with Gram staining procedure and the morphology was characterized by examination in the microscope at 100 X magnification (oil immersion lens).

3.4.5. Pasteurella species Identification by PCR

Twenty five *Pasteurella* species previously isolated as *M.haemolytica* and *P.multocida* at National Veterinary Institute Debre Zeit, Ethiopia were transported to the Institut für Microbiologie und Tierseuchen for PCR assay. Before a direct PCR approach was adopted, a bacterial isolate that had been preserved in a transport media (Paraffin) were subcultured on blood agar for recovery and to counter check the contaminants. Thus, the subcultured isolates were subjected to *P.multocida* and *M.haemolytica* specific PCR assays using standard protocols.

3.4.5.1. Polymerase Chain Reaction (PCR) protocol

Materials

Template DNA
Downstream oligonucleotide primer

Upstream oligonucleotide primer

Taq DNA Polymerase and 10X Reaction Buffer

$MgCl_2$, 25mM

Nuclease-free Water

Nuclease-free light mineral oil

dNTP mix (10mM of each dNTP)

Amplication was done including both positive and negative control reactions.

The first five reaction components (Nuclease-free water, 10XReaction buffer, dNTP mix (10mM of each dNTP), Taq DNA Polymerase, 25mM $MgCl_2$, Downstream primer, Upstream primer and template) were combined in a thin- walled 0.5ml reaction tube. Gently vortex the tube for 10 seconds and briefly centrifuged in a microcentrifuge. The reaction was initiated by adding the template and primers.

Then the reaction was overlayed with 1-2 drops of nuclease-free mineral oil to prevent condensation and evaporation. The tubes were placed in a controlled temperature heat block and procced with thermal cycling profile chosen for the reaction.

The PCR reaction products were analyzed by agarose gel electrophoresis of a 5 /µl aliquot from the total reaction. The products should be readily visible by UV translumination of the ethidium bromide-stained gel. The reaction products were stored at -20°C until needed. The reaction products could be further purified using a number of procedures including the Wizard ™PCR Preps DNA purification system.

3.4.5.2. Specific PCR M.haemolytica and P.multocida assay

The PCR analysis was performed according to standard protocols (Ausbel, 1994). All oligonucleotides used either for the detection of species-specific or virulence associated genes are listed in Table 7.

Pasteurella- multocida-specific assay

The *P. multocida*-specific PCR identified all subspecies of P.multocida through specific amplification of a ~460 bp DNA fragment within the *KMT1/SP6T7* gene.

Capsular typing of *P. multocida* was performed only using the *synth*-PCR. The *toxA*-PCR was used to test if strains can express the dermonecrotic toxin identified

Mannheimia- haemolytica-specific assay

The *Mannheimia- haemolytica*-specific PCR identified Mannheimia-*haemolytica* and *P.trhalosi* through specific amplification of a ~921 bp DNA fragment within the *pomA* gene.

Table 7. Oligonucleotides used for the amplification of species-specific and virulence associated genes of *Pasteurella* and *Mannheimia* species

oligonucleotides	size of product (bp)	gene	reference	sequence reference	species
KMT1/SP6T7	460	*kmt*1-sequence	Townsend *et al.*, 1998	Townsend *et al.*, 1998	*P.multocida*
PomA fwd/rv	921	*pomA*	IMT	Zeng *et al.*, 1999	*M.haemolytica*
ToxA fwd/rv	866	*tox*A	IMT	Petersen, 1990	*P.multocida*
Synth ewd/rv	1264	*pm*HAS	IMT	DeAngelis *et al.*,1998	*P.multocida*

3.4.5.3. Serotyping of *M.haemolytica* field isolates

The PCR isolates of *Mannheimia haemolytica* were submitted to Bundesinstitut für gesundheitlichen Vebraucherschutz und Veterinärmedizin (BgVV) for serotyping. *Mannhemia haemolytica* species serotyping was done by detecting pasteurella capsular antigen using Indirect Haemagglutination Test (IHA). Nine lyophilized reference strains of *M.haemolytica* serotypes, including A1, A5, A6, A7, A11, A12, T3, T4, and T15 from BgVV, (Germany) were used for serotyping. Strains were not tested against A2, A8, A9, A16 and A17.

3.4.5.3.1. Immunization of rabbits

- Rabbits were inoculated with 2.5ml capsular antigen + 12.5ml Adjuvant subcutaneous by (S/C)

- After one week interval, then inoculated capsular antigen of different doses intra-venous by (I/V) and in between each I/V immunization there was 3 days interval.

1. 0.5ml capsular antigen (I/V)
2. 1ml capsular antigen (I/V)
3. 2ml capsular antigen (I/V)
4. 2.5ml capsular antigen +2.5ml free adjuvant(S/C)

Again after a week 2ml of capsular antigen was given I/V. After the last immunization test bleeding was done to establish serum IHA.titres. When serum titres reach below 1:640 four immunizations were followed each of which was 2ml capsular antigen. Finally, the rabbits were completely bled 10-12 days after the last injection, blood was kept overnight at 4°C , for the extraction of serum.

3.4.5.3.2. Capsular antigen extraction

- The pure colony of Pasteurella field isolates was inoculated heavily into Tryptose Soya Broth. incubated overnight at 37°C aerobically.
- The broth was heated in water bath at 60°C for 30 minutes to kill the bacteria
- Centrifuged at 3000rpm for 30 minutes and the clear supernatant was taken as capsular antigen extract.

3.4.5.3.3. Sensitization of Red Blood Cells

- calf blood was freshly collected in Alsevier's solution (Annex 7.3.) at a proportion of 75 ml to 125 from the jugular vein.
- The mixture was then centrifuged at 2000rpm for 10 minutes.
- The packed RBC was washed twice by adding 5ml of PBS and centrifuged as above. There after 50µl of packed RBC's were mixed with 5ml of antigen extract.
- 25ml of gluteraldehyde (50%) was added and homogenized by gentle shaking, incubated for one hour at 37°C with periodical shaking.
- Then, centifuged as above; washed twice with 5ml PBS and centrifuged as above.

- Finally 5ml of PBS was added to the final sediment and adjusted to make a one percent suspension.

3.4.5.3.4. IHAT test proper and interpretation

- 50µl of PBS was distributed to each well except the first column
- 50µl of positive reference sera 1:10 diluted in PBS were added to the first column of the plate.
- The serial two fold dilution (1:10, 1:20...1: 160) was made by transferring 50µl to the penultimate well and the final 50µl diluted sera were kept at 4°C for possible continuation of the assay.
- 50µl of sensitized RBC's was then added to the respective wells, covered with a plastic plate cover and incubated for an hour at 37°C.
- 50% or more complete and course agglutination of RBC indicated positive reaction, small button of deposited cells was a negative reaction.
- Control tests in which sensitized and unsensitized RBC's were added to respective positive serum, were run in parallel with every test.

3.5. Data management and analysis

All data were entered in to Microsoft-Excel database and data on all sorts of information was stored in a computer. Prevalence of Pasteurella species, isolated and serotyped and the distribution were analyzed and compared using chi-sqare frequency rates. It equals the sum over all categorie of the ratio of squared difference between observed frequency and expected frequency. Under the null hypothesis of homogeneity, it is X^2 distributed with degree of freedom df= (m-1) (n-1), where m and n denote the number of categories for the two factors. The computer soft ware gives the calculated X^2 and it's P-value, P which should be greater or equal to 0.05 or greater and equal to 0.01 respectively to accept the null hypothesis at 95% confidence level and 99% confidence level. Relevant predisposing factors mentioned by interviewed farmers, the relationship between occurrence of pneumonic lungs and *Pasteurella* isolates were analyzed using chi-sqare frequency in stat graphics.

Comparable differences and associations between dependent variables of positive and negative swab and organ samples against independent variables (ecosystems, sex, age, health status, and breed of sheep) were also computed using X^2 test in statgraphics.

4. Results

4.1. Questionnaire survey result

The questionnaire survey covered two regional states (Amhara and Somali), two zones (North Shewa and Jijiga), four districts and 16 villages. The response from 48 individuals (3 heads/village) was considered to in the understanding of the major predisposing factors associated with ovine pasteurellosis and to assess their relationship and impacton sheep husbandry. Among the 48 livestock farmers interviewed the relevant responses of Community leaders, flock owners and shepherd were analyzed (Table 10). Accordingly, 27% (n=13) and 33% (n=16) of the community leaders and the shepherd and 25% (n=12) and 31% (n=15) of the flock owners said movement of sheep long distance to market by the means of on hoof transportation was mentioned as stress factor. In contrast none of the community leaders and shepherd indicated transportation by truck as a predisposing factor, but 6% (n=3) of the shepherd and community leaders and 8% (n=4) of the flock owners said on hoof sheep flock movement to the nearby market could be a stress factor.

About 14%(n=9) of the flock owners and shepherd and 20.8% (n=6) of the community leaders reported that out breaks of ovine pasteurellosis occured during cool and chilly weather and this environmental stress factor could be involved in the induction of ovine pasteurellosis. About 18% (n=9) of the flock owners and shepherd and 12% (n=9) of the community leaders answered that hot stress during warm climates can depress the host and aggravate the coughing and nasal discharge of the sheep which leads to the infection of commensal microorganisms. About 27% (n=13), 33% (n=16), 25% (n=12) of the community leaders, flock owners and shepherd said that inadequate supply of feed and water could be predisposing factors for coughing and nasal discharge of sheep. None of the flock owners responded that adequate feed and water supply can be a stress factor, where as 6% (n=3) of the community leaders and 8% (n=4) of the shepherd said adequate feed and water supply with no balance of diet could be a stress factor.

About 22.9% (n=11) of the community leaders and 27.5% (n=13) of the flock owners and 20% (n=10) of the shepherd answered that parasitic infections like lungworm (locally known as'Engib') were associated with the coughing and nasal discharge of sheep as secondary infection. About 12% (n=6) of the shepherd, 10% (n=5) of the community leaders and 6% (n=3) of the

flock owners reported that other bacterial and viral diseases could also be considered in aggravating the disease. 29% (n=14) of the flock owners, 22.9% (n=11) of the community leaders, and 4%(n=2) of the shepherd reported that hyperventilation through not well constructed sheep barns can induce coughings and nasal discharge of sheep. 14% (n=7) of the sheepherd 10% (n=5) of the community leaders and 4% (n=2) of the flock owners responded as inadequate ventilation in very closed sheep barns can cause safocation and contribute to the induction of nasal discharge and coughing of sheep. About 29% (n=14) of the flock owners, 22% (n=11) of the community leaders and 14% (n=7) of the shepherd said traditional housing structure for keeping sheep mixed livestock species can cause the disease. 10% (n=5) of the community leader and 4% (n=2) of the flock owners and the shepherd said housing the sheep in wooden fencing and karrel in the open air can expose the host to hyper ventilations and trigger to the disease. About 22.9% (n=5) of the community leader 8% (n=4) of the flock owners and 4% (n=2) of the Shepherd answered that shearing of animals during the hursh cold weather can activate the opportunistic microorganisms of the host. 29% (n=14) of the shepherd, 25% (n=12) of the flock owner and 23% (n=11) of the community leaders reported that docking of sheep in addition to other managemental factors could be a predisposing factor to nasal discharge and coughing of sheep.

About 22.9% (n=11) of the community leaders, 8% (n=4) of the flock owners and 4% (n=2) of the Shepherd said there was a regular vaccination regime to control the diseae in the village. In contrast 29% (n=14) of the Shepherd, 25% (n=12) of the flock owners and 8% (n=5) of the community leaders talked about irregular vaccination status can contribute to the susceptibility of the sheep to the disease. In general, there was no significant difference among the interviewed groups in the response of variables except on vaccination status. The fact that most of the flock owners and shepherd have said irregular vaccination could be a problem favouring respiratory disease in sheep unlike the community leaders (Table 10).

The flock size owned varies in the two production systems, about 5-100 in the highlands as compared with 50-300 in the agro-pasoralist system. In the surveyed districts of North Shewa highland livestock species like cattle, sheep, goat, equine and chicken are widely raised with exception of camels. In the low lands of Jijiga Sheep, goat, cattle, camel and equine are largely raised except chicken. The livestock and population size is shown on Tables 7, 8 and 9.

Table.7. Estimated livestock population in DebreBerhan and Jijiga zones.

Eco-zone	sheep	goat	cattle	camel	equine	chicken
D/Berhan	1290757	490696	1159255	3775	229333	1138126
Jijiga	1316000	548000	439000	129000	42000	-

Table. 8. Estimated livestock population in two districts of North shewa zone:

Woreda/district	sheep	goat	cattle	camel	equine	chicken
B/warana	17526	86907	78584	-	104433	56985
A/Asagirt	99035	61769	109743	-	160804	614114

Table. 9. Estimated livestock population in two districts of Jijiga Zone:

district	sheep	goat	cattle	camel	equine
Harshin	660000	276000	217500	66500	19000
jijigaZuria	656000	272000	221500	62500	23000

Source: Zonal agriculturalBeauro of Jijiga and North Shewa.

Table 10. Chi-square frequency of the questionnaire survey on the environmental, biological and management predisposing factors for the induction of ovine pasteurellosis.

variable	Comm.leader				Flock owner				shepherd				X^2
Distance	long		Near by		long		Near by		long		Near by		
to market	n	%	n	%	n	%	n	%	n	%	n	%	
	13	27	3	6.3	12	25	4	8	13	27	3	6	0.2
Transport	On hoof		By truck		On hoof		By truck		On hoof		By truck		
means	n	%	n	%	n	%	n	%	n	%	n	%	
	16	33	0	0	15	31	1	2	16	33	0	0	0.2
Hursh	Cool+chilly		Hot stress		Cool+chilly		Hot tress		Cool+chilly		Hot stress		
weather	n	%	n	%	n	%	n	%	n	%	n	%	
	10	20.8	6	12	7	14	9	18	7	14	9	18	1.5
Feed+	adequate		inadequate		adequate		inadequa		adequate		inadequate		
H2O	n	%	n	%	n	%	n	%	n	%	n	%	
supply	3	6	13	27	0	0	16	33	4	8.3	12	25	4.3
Sec.inf.	parasitic		other		parasitic		other		parasitic		other		
	n	%	n	%	n	%	n	%	n	%	n	%	
	11	22.9	5	10	13	27.5	3	6	10	20	6	12	1.4
Housg	Adequ.vent.		Inadeq.ven		Adequ.vent.		inadeq.vt		Adequ.vent.		Inadeq.ven		
	n	%	n	%	n	%	n	%	n	%	n	%	
	11	22.9	5	10	14	29	2	4	2	4	7	14	3.8
Housg	traditonal		fence/karrl		traditonal		fence/kar		traditonal		fence/karrl		
Structur.	n	%	n	%	n	%	n	%	n	%	n	%	
	11	22	5	10	14	29	2	4	2	4	7	14	3.8
Anim.mgt.	shearing		docking		shearing		docking		shearing		docking		
	n	%	n	%	n	%	n	%	n	%	n	%	
	11	22.9	5	8	4	8	12	25	2	4	14	29	0.8
Vacc.stat	regular		irregular		regular		irregular		regular		irregular		
	n	%	n	%	n	%	n	%	n	%	n	%	
	11	22.9	5	8	4	8	12	25	2	4	14	29	12

**significant (P =<0.05) difference.

4.2. Major diseases of sheep and causes of death in the study areas

Table.11. Vernacular names of the endemic diseases in the study area

vernacular name	Veterinary equivalent	species affected
Engib/Sambab	Pasteurellosis	cattle, sheep, goats
Abasenga	anthrax	cattle, sheep, goats, equines
Abagorba	black quarter	cattle
Fentata	sheep pox	sheep, goats
Chok	foot rot	sheep goats
Odoma	facioliasis	cattle, sheep, goats
Hukissa (kissat)	internal parasites	all farm animals
Ekek	itching, skin disease	all farm animals

Table. 12. Summary of questionnaire survey on the major causes of morbidity and mortality of sheep in the study area.

Variable	Comm.leader		Flock owner		Shepherd		Total response	
	Count (n)	%	Count (n)	%	Count (n)	%	Count (n)	%
Cough,N.d.,diarr., *major syndromes	15	31.3	14	29.2	15	31.3	44	91.8
Itching,bloat, abort,suddeathas minor. syndromes	1	2.08	2	4.17	1	2.08	4	8.33
Cough, N.d.,diarr.major causeuse of death	14		13		16		43	89.6
Itching,bloat,sudd.death minor cause of death	1	4.17	2	6.25	1	0	4	10.4
Vet clinic visit by flock owners	14	29.2	14	29.2	15	31.3	43	89.5
Vet clinics with no visit	2		2		1		5	10.4

*N.disch. =Nasal discharge, diarr.= diarrhea, Sudd.=sudden death

The response on the cause of morbidity and mortality showed that out of 48 individuals (3 heads/village) interviwed 91.8% (n=44) said coughing and nasal discharge of respiratory disease complex (RDC) and diarrhea are the major clinical signs during disease out break (Table12). Majority of the respondents 89.6% (n=43) have claimed on respiratory disease as economically important disease and main cause for death. Only 8.33% and 10.4% (n=4) among the interviewed farmers said that itching, bloat abortion and sudden death were the major manifestations respectively. 89.5% (n=43) of the totally interviewed indicated that flock owners visited veterinary clinics and 10.4% (n=5) did not visit The disease out break was noted from October to January in highlands around DebreBerhan and April to June in the low lands around Jijiga / Harshin.

Fascioliasis, lungworm, coenurosis, sheep pox and foot rot were the second important infections usually encountered by farmers in the Debreberhan while skin disease was more important in Jijiga area as compared to respiratory disease. Diseases like pasteurellosis and PPR (Peste des Petits Ruminants) are prevailing due to abscence of irregular vaccination number of Anthrax and FMD (Foot and Mouth Disease) cases increase due to irregular vaccinations implemented in the area.

4.3. Livestock management practice

According to the questionnaire result, the livestock production system is traditional and extensive way of management. In the highlands the main livestock grazing land include swampy and water logged areas, forest margin, hilly tops and mountain sides, stony and infertile lands and road sides In semi-nomadic and nomadic pastoralist areas thorny bushes are topographic features.

4.2. Bacteriological findings

4.2.1. Isolation and Strain characterization

Growth on blood agar was detected after 24 hours of culturing at 37°C aerobically. Weak and strong haemolytic mostly greyish, rounds with raised surface and glistening appearance small to medium sized colonies were observed. *P.multocida* forms smooth, greyish glistening translucent colonies, approximately 1 mm in diameter, on blood agar after 24 hours incubation at 37°C. the haemolytic capacity was usually reduced and totally lost after two or three subcultures.

Growth on MacConkey was observed after 24 hours culture at 37°C aerobically, and was characterized by the presence of two colony types. Lactose fermenters manifested by pink coloration around colonies and non-lactose fermenters were small, round with raised surface or mucoid, flat with smooth appearance. Similar colonies were observed for lactose fermenters.

The *Pasteurella multocida* isolates were not haemolytic, no growth on MacConkey agar with mucoid, smooth, greyish and glistening on blood agar.

4.2.2. Isolation of Pasteurella from lung, tonsil and nasal swab

A total of (n=74) specimen were collected for bacteriological examination from sheep of different origins and epidemiological units in North Shewa and Jijiga zones out of which (n=20) isolates were identified and documented for *M.haemolytica* and *P.multocida* (Table14). Prevalence of *Mannheima haemolytica* and *Pasteurella multocida* isolates varied. Thus, the over all prevalence was 20.27% (95%C.I 31.22, 11.18) for *M. haemolytica* specific PCR and 6.75% (95%C.I15.06, 2.23) for P.multocida specific PCR respectively (Figure. 6).

Out of 37 lungs, 10 tonsils and 27 nasal swabs examined 27% (95% C.I. 44.1, 13.8) and 8.11% (95%C.I. 21.9, 1.7) have shown the prevalence of *M. haemolytica* specific PCR and *P.multocida* specific PCR from lung respectively. The Positivity rate of *M.haemolytica* and *P.multocida* for specific PCR was 1.11% (95%C.I 29.16, 2.3) and 7.41% (95%C.I 24.28, 0.9) respectively. There was a 20% (95%C.I 55.6, 2.5) positive for *M.haemolytica* specific PCR for isolates from tonsil.and there was no positive for *P.multocida* specific PCR of the same sample (Figure1).

The bacterial isolation result of sheep lung from Debreberhan zone has shown that 25% (95%C.I 46.71, 9.77) and 4.12% (95%C.I 21.12, 0.10) for *M.haemolytica* and *P.multocida* respectively. However, nasal swab of on-farm sheep flock collected from this zone were negative (Figure 2). The isolation rate for *M.haemolytica* from the same specimen and the same zone was 16.66% (95%C.I 48.41, 2.08). In Jijiga Zone the bacteriological findings showed 30.76% (95%C.I 61.43, 9.09) and 15.38%(95%C.I 45.44, 1.92) for both *M.haemolytica* and *P.multocida* isolated from lung of sheep respectively. The nasal swab isolates of *M.haemolytica* and *P.multocida* in this zone indicated 6.66% (95%C.I 31.94, 0.1) and 13.33% (95%C.I 40.46, 1.66) respectively (Figure 3). *M.haemolytica* and *P.multocida* specific PCR conducted on isolates of tonsil of sheep from Jijiga abattoir revealed the abscence of *P.multocida* but, gave a 20% (95%C.I 55.6, 2.5) positivity for *M.haemolytica* (Figure 4). Lung and nasal swab isolates of *P.multocida* from Jijiga abattoir and D/Berhan back yard slaughter indicated the prevalence of *P.multocida* capsular A type 8.1% (95%C.I 21.9, 1.7) and toxi positive of which one isolate was from D/berhan back yard. The capsular D type of P.multocida was isolated from Jijiga abattoir waiting yard and the frequency of the isolate was 5.4% (95%C.I 18.19, 0.6) and it was toxi positive (Figure 5). Similarly in Debreberhan zone the prevalence of *M.haemolytica* and *P.multocida* isolates showed 25% and

4.12%from lung and 16.66% isolate of *M.haemolytica* from swab of on-farm sheep flock. No isolate of *P.multocida* was found from nasal swab in this zone. The specific PCR for *M.haemolytica* and*P.multocida* carried on isolates from pneumonic lung revealed a 24.3% (n=9) prevalence as compared with 8.1% (n=3) for healthy sheep. For all bacteriologically tested lungs 13.5% (n=5) and 2.7% (n=1), that gave growth of the two strains are associated with hepatization the lungs. The *M.haemolytica* and *P.multocida* specific PCR result on isolates from lung and swab of different origin, age, sex and breed has no significant ($p>0.05$) difference. However, there was higher positivity recorded by PCR specific *M.haemolytica* and *P.multocida* in young sheep 13.5% (n=5) and 5.41% (n=2) as compared to 10.8% (n=4) and 2.71% (n=1) in adult sheep

Strains were not isolated from non-pneumonic lung except one isolate of (*M. haemolytica)* and three isolates of *P.multocida* from clinically sick sheep (Figures1). In general, there was no significant difference observed in the frequency distribution of pasteurella isolates by altitude, breed, age and sex of the animal.

Table. 13. Summary of specific PCR *M.haemolytica* and *P.multocida* positives and negatives

		Pasteurella multocida/ Mannheima haemolytica				
Code	^Species (original)	material	Origin	P.multocida specific PCR	M.haemolytica specific PCR	Remarks
P1	P.multocida	lung	abattoir Ji-ga*	+	-	Capsular Type A
P2	M.haemolytica	lung	abattoir ji-ga		+	
P3	P.multocida	lung	D/B B/Y**	+	-	capsular type A,tox +
P4	P.sp.	lung	abattoirJi-ga			no growth
P5	M.haemolytica	lung	D/B/ B/Y	-	+	
P6	M.haemolytica	lung	D/B B/Y	-	+	
P7	M.haemolytica	lung	B/yard D/B	-	+	
P8	M.haemolytica	lung	abattoir J-ga	-	+	
P9	P.multocida	lung	abattoirJi-ga	+	-	Capsular type A
P10	M.haemolytica	lung	backyard D/B*	-	+	
P11	M.haemolytica		D/B/ B/Y	-	+	
P12	M.haemolytica	lung	abattoir Ji-ga	-	+	
P13	M.haemolytica	lung	abattoir JI-ga	-	+	
P14	P.sp.	lung	D/B/ B/Y	-	+	
P15	P.sp.	nasal swab	D/B/ on-farm	-	-	no pasteurella sp.
P16	M.haemolytica	nasal swab	D/B/on-farm***	-	+	
P17	P.multocida	nasal swab	abattoir Ji-ga	+	-	Capsular type D, to '+
P18	P.sp.	nasal swab	D/B/on-farm	-	-	no pasteurella sp.
P19	P.sp.	nasal swab	Ji-ga abattoir	-	-	no pasteurella sp.
P20	M.haemolytica	nasal swab	D/B/on-farm	-	+	
P21	P.sp.	nasal swab	abattoir Ji-ga	-	-	no pasteurella sp.
P22	P.multocida	nasal swab	abattoir Ji-ga	+	-	Capsular type D, to '+
P23	M.haemolytica	nasal swab	abattoir Ji-ga	-	+	
P24	M.haemolytica	tonsil	abattoir Ji-ga	-	+	
P25	M.haemolytica	tonsil	abattoir Ji-ga	-	+	
Total isolates				5	15	

* Jijiga abattoir

**Debreberhan backyard slaughter

***Debreberhan on-farm

Table 14. Summary of bacteriological findings on *Pasteurella multocida/M. haemolytica*

Origin of sample	Material	Sampled	PCR M.h	PCR P.m.	% total isolate	
					Mh	Pm
Jijiga abattoir	lung	13	4	2	30.76	15.38
D/B/b/yard	lung	7	2	0	28.57	0
D/B/B/yard	lung	17	4	1	23.53	5.88
Sub total		37	10	3	27	8.11
D/Bon-farm	N/Swa	12	2	0	16.6	0
Jijiga abattoir	N/swab	15	1	2	6.6	13.33
Sub total		27	3	2	11.11	7.41
Jijiga abattoir	tonsil	10	2	0	20	0
Overall total		74	15	5	20.27	6.75

Table.15. Chi-square frequency of *P.multocida* and *M.haemolytica* specific PCR on isolates from lung of sheep of different origin, age, sex and breed

ategories	Specific PCR *M.haemolytica*				Specific PCR *P.multocida*			
	+ves	%	X2	P-value	+ves	%	X^2	P-value
Young	n=5	13.5	0.0	1.00	n=2	5.41	0	1.00**
Adult	n=4	10.8	-	-	n=1	2.71		
Female	n=2	5.41	0.02	0.88	n=1	2.7	0	1.0
Male	n=7	18.9			n=2	5.4		
Menz type	n=3	8.11	0.24	0.62	n=1	2.7	0	1.0
BOSS*	n=6	16.2	-	-	n=2	5.4		
D/Berhan o back yard	n=3	8.11	1.13	0.57	n=1	2.7	1.6	0.44
D/Berhan back yard	n=2	5.4			n=0	0		
Jijiga abattoir	n=3	10.8			n=2	5.41		

*_____Black head Ogaden Somali Sheep

**_____There was no significant difference (P>0.05) in between categories.

Table.16. Chi-square frequency of *P.multocida* and *M.haemolytica* Specific PCR isolates from nasal swab of sheep of different origin, age, sex, breed and health status categories

Categories	Specific PCR *M.haemolytica*				Specific PCR *P.multocida*			
	+ves	%	X2	P-value	+ves	%	X^2	P-**value
Young	n=1	3.7	0.0	0.94	n=0	0	0.6	0.43
Adult	n=2	7.4	-	-	n=2	7.4		
Female	n=1	3.7	0.12	0.72	n=0	0	1.05	0.3
Male	n=2	7.4			n=2	7.4		
Menz type	n=2	7.4	0.04	8.4	n=0	0	0.3	0.56
BOSS*	n=1	3.7		-	n=2	7.4		
A/healthy	n=1	3.7	0.04	0.8	n=2	7.41	0.33	0.56
Sick	2	7.4			n=0	0		
D/Berhan on-farm	n=2	7.4	0.04.	0.83	n=0	0	0.33	0.56
Jijiga abattoir	n=1	3.7			n=2	7.41		

*___Black head Ogaden Somali Sheep

**___There was no significant (P>0.05) difference of isolates in between categories.

Table.17. Chi-square frequency of specific *P.multocida* and *M.haemolytica* on isolates from pneumonic lung of sheep

category	Specific PCR *M.haemolytica*				Specific PCR *P.multocida*			
	+ves	%	X2	P-value	+ves	%	X^2	P-value
A/healthy	n=9	24.3			n=3	8.1		
Lung worm	n=3	8.11	0.45	0.5	n=1	2.7	0	1.0*
No lung worm	n6	16.2			n=2	5.4		
Hepatized	n5	13.5			n=1	2.7		
Hydatid cyst	n=2	5.41	0.14	0.93	n=0	0	1.24	0.54
Emphysematous	n=2	5.41						

*There was no significant (P>0.05) difference

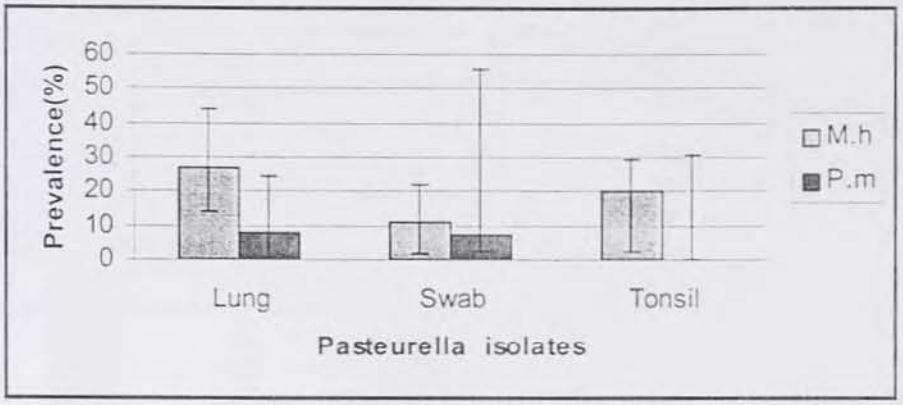


Figure 1. Prevalence of Pasteurella isolates from lung, nasal swab and tonsil in DebreBerhan and Jijiga zones, Ethiopia.

There were some variations in prevalence of specific PCR *M.haemolytica* and *P.multocida* isolates from different specimens. Thus, the sub total prevalence of *M.haemolytica* from lung of sheep was 27% (95% CI 44.1, 13.8) and 8.1% (95% CI 21.9, 1.7) for *P.multocida*. The sub total prevalence of *M.hahemolytica* from nasal swab was 11.11% (95% CI 29.16) and 7.4% (95% CI 24.28, 0.9) for *P.multocid*. There was no isolate of *P.multocida* from tonsil. There was 20% (95%CI 55.6, 2.5) prevalence of *M.hahemolytica* isolates from tonsil of sheep.

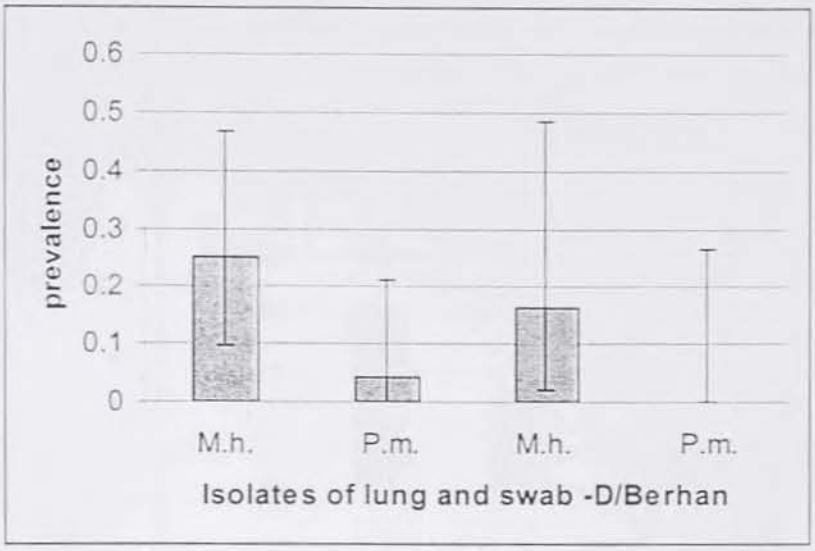


Figure 2. *M.hemolytica* and *P.multocida* specific PCR isolates of lung and nasal swab from DebreBerhan back yard slaughter and on-farm sheep flock

The prevalence of *M.haemolytica* and *P.multocida* specific PCR isolates of lung and nasal swab from D/Berhan showed that 25% (95%C.I 46.71, 9.77) and 4.12% (95%C.I 21.12, 0.10) respectively. However, the prevalence of *M.haemolytica* and *P.multocida* specific PCR isolates of nasal swab indicated that there were no isolates of *P.multocida* from swabs of on-farm sheep flock, but there were 16.66% (95%C.I 48.41, 2.08) for *M.haemolytica*.

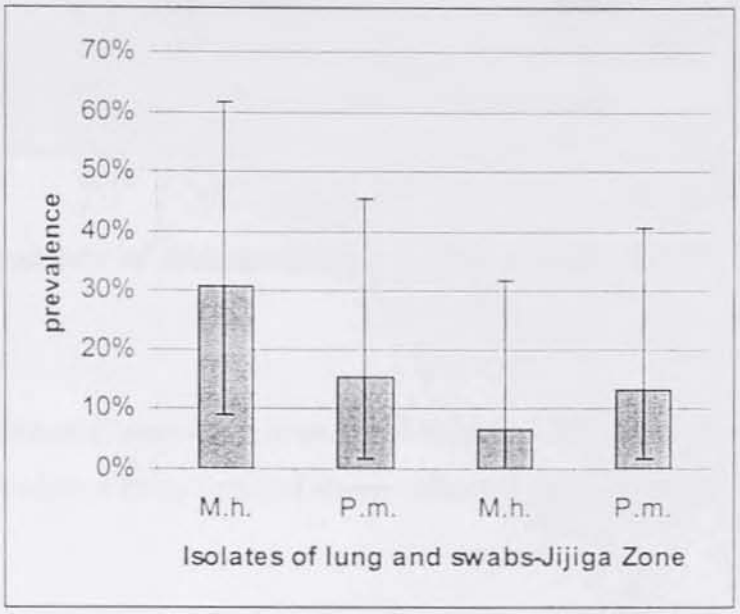


Figure 3. *M.haemolytica* and *P.multocida* specific PCR result on isolates of lung and nasal swab from Jijiga abattoir

The prevalence of *M.haemolytica* and *P.multocida* from lung and nasal swab specimens collected from Jijiga zone indicated that 30.76% (95%C.I 61.43, 9.09) and 15.38% (95%C.I 5.44, 1.92) respectively. The prevalence of swab isolate for *M.haemolytica* and *P.multocida* was 6.66% (95%C.I 31.94, 0.1) and 13.33% (95%C.I 40.46, 1.66) respectively.

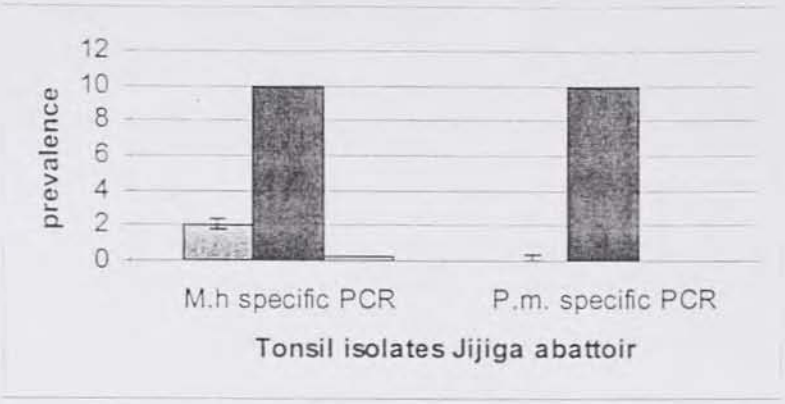


Figure 4. The frequency of *M.haemolytica* and *P.multocida* specific PCR isolates from Jijiga abattoir.

There was no isolate of *P.multocida* from tonsil of sheep, but there were 20% (95%C.I 55.6, 2.5) isolates of *M.haemolytica* from tonsil of sheep collected from Jijiga abattoir.

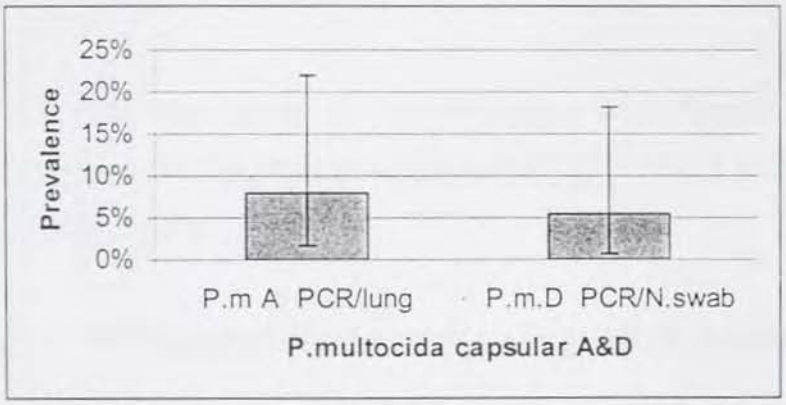


Figure 5. Comparative result for *P.multocida* capsular type A and D from Jijiga abattoir and D/Berhan back yard

The prevalence of *P.multocida* capsular type A isolate from lung of sheep slaughtered at Jijiga abattoir was 8.1% (95%C.I 21.9, 1.7) of which one isolate was from D/Berhan back yard. 5.4% (95%C.I 18.19, 0.6) was isolate of capsular D type from nasal swab of sheep sampled from Jijiga abattoir.

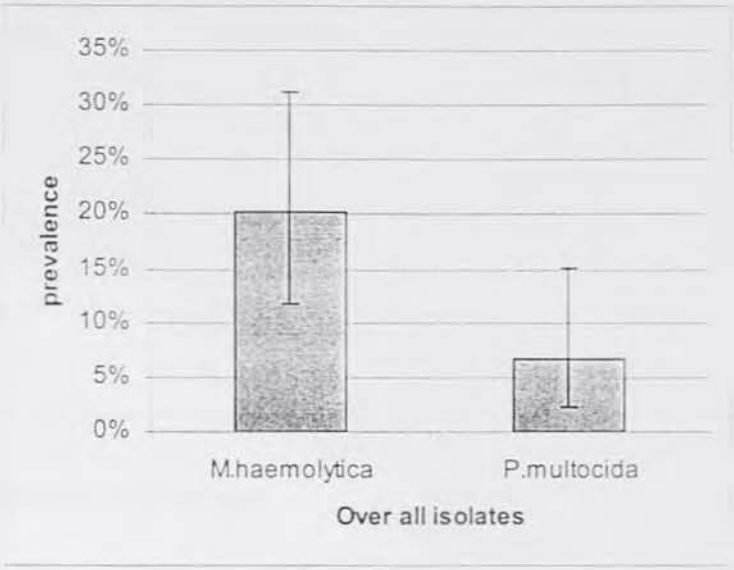


Figure 6. The over all specific PCR result for *M.haemolytica* and *P.multocida* on isolates from DebreBerhan and Jijiga Zones

The overall prevalence of *M.haemolytica* and *P.multocida* isolates from sheep in both Debreberhan and Jijiga zones showed 20.27% (95%C.I 31.22, 11.18) and 6.75%(95%C.I 15.06, 2.23) respectively.

4.2.3. Biotyping and serotyping of *M.haemolytica*

As indicated in Table19, the most prevalent *M haemolytica* serotypes were A1 (55.6%) and A6 (44.44%). A range of specimen that yield different serotypes were characterized from lung (A1, A6, and T15) and Nasal swabs (A1, T15). The characterized serotype from tonsil was only A1. Serotype with low frequency of characterization was T15. The Serotypes A5, A7, A11, A12, T3 and T4 were not characterized from all specimens using the available antisera. Furthermore, the strains A2, A8, A9, A13, A14, A16 and A17 have not been tested.

Table 19. Frequency of *M. haemolytica* isolates serotyped from lung, tonsil and nasal swab of sheep in DebreBerhan and Jijiga zones

M.haemolytica A & T Biotype	Isolated from:			No. strains	% strains
Biotype A	Lung	Nasal swab	Tonsil		
A1	3	1	1	5	55.55
A6	4	-	-	4	44.44
sub total	7	1	1	9	-
Biotype T					-
T15	1	1	-	2	-
sub total	1	1	-	2	-

Table 20 Serotypes of *M.haemolytica* isolates

Code	*M.haemolytica* specific PCR	Biotype A	Biotype T
P2	+	A1	
P5	+	A6	
P6	+	A6	
P7	+	A6	
P8	+	A6	
P10	+	A1	
P11	+		T15
P12	+	untyped	
P13	+	A1	
P14	+	untyped	
P16	+		T15
P20	+	untyped	
P23	+	A1	
P24	+	untyped*	
P25	+	A1	

As shown in (Table 20) out of 15 *M.haemolytica* isolates subjected to serotyping only 11(n=9 from biotype A and n=2 from biotype T) were characterized using standard protocol of IHA at Bgvv. The other four were the untypable isolates.

Table 21. Frequency of *M.haemolytica* serotypes from clinically healthy and sick sheep

Health status of sheep	biotype A		biotype T
	serotypes		serotype
	A1	A6	T15
A/healthy	2	1	1
Sick	3	3	1

Table 21 showed out of N=15 *M.haemolytica* isolates characterized by serotyping 13.33% (95%C.I 40.46, 1.65) of strain A1 was from apparently healthy and 20% (95%C.I 48.08, 4.33) was from clinically sick sheep respectively. Strain A6 was characterized 6.66% (95%C.I. 31.94, 0.1) from Apparently healthy and 20% (95%C.I 48.08, 4.33) from clinically sick sheep respectively. Only 6.66% (95%C:I 31.94, 0.1) of strain T15 was characterized both from apparently healthy and sick sheep respectively.

5. DISCUSSION AND CONCLUSION

The present epidemiological study and strain characterization of pasteurella species in sheep has shown the prevalence of the microorganism, their distributions and some predisposing factors associated with active Pasteurellosis in the selected study sites.

Observation of predisposing factor

Previous works of Biondi and Zaninno (1997), Anderson *et al.* (1991)) indicated that common physical environmental stresses, either alone or in any combination, predispose many sheep to respiratory infections. These could be heat, crowding (limited space), exposure to inclement weather, poor ventilation with high levels of moisture and barnyard gases or smokes, handling and transport, castration and docking. Besides, weaning and change in feed exhaustion and hunger during transportation, excessive dust in the barn and feed lots, high loads of parasites and mixing of animals from different sources were reported as predisposing factor. In line with this report attempt was made to observe the environmental, biological and management factors under the farmer's situation on the ground

Distance to market point as a factor

This observation was made on the response whether trading sheep flock closer or longer to market could have an effect on respiratory disease complex. All interviewed farmers positively responded that there were few market places, which could meet their price demand and hence forced to travel long distance in search of good market. Thus, trading sheep long distance to market has an effect of coughing and nasal discharge after arrival. As far as transport stress is concerned farmers gave equal importance to movement on hoof or by truck. The reseason could be that the farmers usually use both means of transportation during holidays to transport to large markets as far as the interviewed farmers were from Peri-urban and market oriented areas. The means of transportation and migrating animals' long distance could also predispose to the disease.

Hursh weather condition as a factor

Considering ecological differences farmers were interviewed to get information on respond the peak time of the disease outbreak. Accordingly, 14% of the flock owners and shepherd and 20.8% of the community leaders responded that the disease out break occured during cool & chilly whether in the highlands where as the rest 18% and 12% said during warm climate and heat stress in the lowlands. Nevertheless the response of the farmers among each other has no significant ($P=>0.05$; $X^2=1.5$) difference. This survey work is consistent with reports of Gelagay and Bekele (1996).

According to Gelagay (1996) and Bekele (1996). In the highlands of North Shewa chilly and cool weather are the main features with extremes in October and January that are associated with out break of ovine pasteurellosis. The environmental features in mid and low lands of jijiga were warm to hot climate, cold temperature, and windy with hyper ventilation's in months of April and June which was conducive for opportunistic infections. In the present survey work the observation time did not coincided with the observations made in the highlands but agrees with low lands report.

Animal management as a factor

During the survey all factors such as feed supply (whether adequate and balanced, or in adequate ,housing with adequate or in adequate ventilation and whether sheep were kept overnight mixed with large ruminants in the same pen or not was also assessed. Moreover, the structure of the house whether it was traditional shade or an open air housing with some fences and kraal, the shearing of animals in cool or warm seasons, docking of female and castrating the male sheep, tethering in a limited space during rainy season were entirely discussed and observed as a factor. Correspondingly, 27%, 33% and 25% of the farmers suggested that these factors could predispose to the chronic coughings and nasal discharges of their sheep flock during out breaks. The response of the regular and irregular vaccination in which all community leaders responded irregular vaccination was not a predisposing factor in contrast to the flock owners and Shepherd could be due to the leadership role attempted to say that veterinary regulations are respected in the community. Further more, the flock owners and Shepherd reported that due to the fact that many sheep flock in the off roads and remote areas were not vaccinated regularly. Therefore the

type of irregular and all of a sudden approach of mass vaccination can depress and triggers commensals of the host.

Biological factors

The effect of secondary infection has also pronounced equally by respondents in such a way that internal parasitism particularly the load of lungworms in the highlands and the gastrointestinal parasites in all selected study sites contributed to the expression of pathogenicity of *Pasteurella* microorganism. This is in line with works of (Brogden et al., 1998). He reported like viruses, some respiratory bacterial infections increase the susceptibility of sheep to secondary *M.haemolytica* infection. *Mycoplasma species and D. filaria* are common in the respiratory tract of sheep and may contribute to the occurence of pneumonia.

According to the response of the farmer sheep and cattle in the highlands (Debreberhan) and sheep and goats in the lowlands (Jijiga) are the principal livestock assets. Among the health problems of livestock production in selected study sites respiratory disease complex and parasitism were the major constraints All respondents (n=48) reported that respiratory diseases manifested by nasal discharge and coughing were the major ones. This piece of observation is in agreement with the previous reports of Mekonnon (2000). He reported that the predisposing factors have the potential to compromise the respiratory defence mechanism and enhance the pathogenicity of opportunistic microorganisms.

Of interviewed farmers 80% asserted the vital role and socio-economic impact of livestock ingeneral and sheep and goat sales as seasonal cash generating income in particular. This interview report was consistent with that of Alemayehu et al. (1993) who have shown that sheep had high economic value particularly for cool highland farmers where they provide 63% of the cash income and 23% of the food subsistencevalue from livestock production.

Prevalence of pasteurella isolates

The over all prevalence of *M.haemolytica* and *P.multocida* shown by specific PCR on isolates from the total sampled specimen revealed that *P.multocida* constitute only 6.75%. This may indicate that *M.haemolytica* is the major causative agent involved in ovine pneumonic pasteurellosis in the selected study sites. There was some variation in frequency of isolation of pasteurella species.from lung, swab and tonsil (Figure 1). The differences and dominance of *M.haemolytica* over *P.multocida* could be attributed to different pattern of ovine pasteurellosis. Further more, it could be due to the competition existing between strains, the host and environment in such circumstances, some of them find a selective advantage (Biberstein *et al.*, 1970).

The fact that the variation showed a lesser extent of *P.multocida* involvment in the induction of pneumonia compared to *M.haemolytica* was in a good agreement with literature (Gilmour, 1978;Radostits *et al.*, 1994; DeAlwis 1993). Although the number of isolates were relatively small 20/74 (27%) as compared to the retrospective studies in Ethiopia it is in consistent with reports of Tesfaye (1997) in which the prevalence of pasteurella in slaughtered sheep was reported as 30%.

The absence of *P.multocida* isolate from tonsil and the presence of *M.haemolytica* may be due to a potent leukotoxin of *M.haemolytica* which kills ruminant leukocytes and enhances pathogenecity (Gonzalez and Maheswaran, 1993).

The geographical distribution of pasteurella isolates was different in North shewa and Jijiga zones. There were 30.76% *M.haemolytica* and 15.38% *P.multocida* isolate of lung from Jijiga abattoir and 6.66% *M.haemolytica* and 13.33% *P.multocida* isolate from nasal swab of sheep in Jijiga zone. On the other hand, 25% isolate of *M.haemolytica* and 4.12% isolate of *P.multocida* from lung and 16.66% isolate of *M.haemolytica* from swab in Debreberhan zone.

The numerical variation in distribution pattern of the isolates in both regions can be attributed to the season of sampling with high stressful conditions. In this regard, the lowlands of Jijiga around Harshin area was the typical out break season in June. During this period flock owners migrate their flock to another grazing area or to the urban market point for long distance of about 150

kms on hoof. This type of flock migration could be either in search of green feed or for the supply of sheep to urban market to safeguard from the disease situation.

The situation in highland of North Shewa could be associated with the infection of lungworms, hepatized lung, and hydatid cyst than the situation in mid and low altitude. The fact that the pathogenesis of pasteurellosis is complex and multifactorial some management practices, environmental stress factors influence the pulmonary defense mechanisms and all contribute to disease development. Although these array of factors equally predispose the host to both pasteurella species severe clinical disease was caused by *M.haemolytica* and rarely *P.multocida* is well addressed by (Moiser, *et al.*, 1989). In comparison to the number of isolates from previous works there is a strain variation from locality to the host and season of sampling.

The present study was much lower in prevalence of *M.haemolytica* isolate as compared to the previous survey reports of South Wollo Gimba area in Ethiopia.from lung of slaughtered sheep. This variation of isolation frequency from year to year and season to season was explained by (Gilmour, 1993). Furthermore, the animals in each specific locality might carry these organisms and reservoirs may exist for their potential involvement in the process of the disease. The specific *P.multocida* and *M.haemolytica* isolates from pneumonic lung of sheep were 2.7% (n=1) and 8.11% (n=3) for the lung affected by lungworm (*D.filaria*) respectively. The specific *P.multocida* and *M.haemolytica* isolates from pneumonic lung of sheep were 16.2% (n=6) and 5.4% (n=2) for not affected lung with no lungworm. This could be attributed to the isolation of the microorganism from apparently healthy sheep has no remarkable variation with the isolation of pasteurella species from lung with no lungworm.

The Chi-square frequency of *P.multocida* and *M.haemolytica* specific PCR isolates from lung and nasal swab (Table 15,16) have shown that there were no *P.multocida* isolates from Debreberhan back yard slaughter slabs, but there were 5.4% (n=2) and 7.4% *M.haemolytica* isolates. The absence of *P.multocida* isolate from Debreberhan and the dominance of *M.haemolytica* in the present study could be explained that *M.haemolytica* is more important agent in these localities and this could give a clue as back feed information for the approach of cocktail vaccine production in the future. Further more, the presence of both *P.multocida* and *M.haemolytica* isolates from both lung and nasal swab in Jijiga abattoir could be associated with the risk factors and multifactorial effect of the opportunistic pasteurella species. On the other

hand, keeping animals in the open air over night without housing and migrating them long distance for long period of time could complicate and trigger both microorganisms.

The Chi square frequency of the independent variable age has no effect at 95% confidence interval (X^2=0; P-value=1.00) on both *P.multocida* and *M.haemolytica* isolates of lung. However, there was variation numerically among age categories. The numerical variation of young sheep 13.5% (n=5) for *M.haemolytica* and 5.4% (n=2) for *P.multocida* might be due to the sampling rate, in which more lung samples were taken carcass of young ones. The other reason could be due to the susceptibility of the young animals to infection. The absence of *P.multocida* isolate from nasal swab of young sheep might be associated again with the sampling rate in which few young sheep were sampled for this study and in addition the total sample taken was equally very few because of the logistics and convenience sampling.

The observed prevalence of isolates from lung in sex groups has no impact on the isolation of pasteurella species. The differences in percentage might be due to more sampling of male sheep from the hotels of the study zones. Similar observation was made for both isolates from nasal swab in both sex and age groups. However, there were higher isolates of *M.haemolytica* and *P.multocida* from adult sheep. This could be explained that because of the visible nasal discharge during sampling time more swabs were taken from male sheep.

The Chi-square frequency has also revealed that breed of sheep has no impact on the isolation rate of both pasteurella species. The fact that the Black Head Somali Sheep showed higher numerical difference than that of Menz type for both strains is related with sampling time. The sampling time for black head ogaden Somali sheep (BOSS) in Somali region was during the disease out break peak time in June, where as in Menz sheep area, though, the right sampling time was from October up to January during which animals suffer due to high parasite burden. The slight numerical differences in both breeds showed that more sick animals were sampled from black head ogaden sheep. Confounding factors like region, clustering effect or production systems contribute in one way or another to differences among all categories on the isolation of *Pasteurella* species from nasal swab could be the explanation.

Strain characterization of *M.haemolytica*

In the present strain characterization with IHA a system of reclassification of *P.haemolytica* to *M.haemolytica* based on DNA-DNA hybridization was used. According to the new nomenclature the former two biotypes A and T on the basis of DNA relationships *P.haemolytica* was reclassified and a new species *P.trehalosi* (Annex 7.4) was formed from the biotype T strains of *P.haemolytica* (Sneeth and Steeve, 1990) leaving the former biotype A strains in the *P.haemolytica* species. Reinvestigation of the trehalose negative *(P.) haemolytica* complex recently resulted in reclassification of these organisms and a new genus, *Mannheimia*, containing at least 5 species was established (Angen et al., 1999).

These changes were based on an extensive investigation using quantitative evaluation of phenotypic data, ribotyping, multilocus enzyme electophoresis, 16S rDNA sequencing and DNA-DNA hybridization (Angen *et al.*, 1997a, b, c). Accordingly, *Mannheimia haemolytica* (earlier *(P.) haemolytica* biogroup 1) represents only strains negative in fermentation of L-arabinose and contains reference strains of serotypes 1,2,5-9,12-14, 16. In the new reclassification biotype A represents serotypes 2,5-9,11-14,16,17, biogroup 1.3.5-12 and biogroupe 1 is *M.haemolytica*. On the other hand, biotype T contains serotypes 3,4,10,15, biogroupe 2 and 4 are *(P) trehalosi* (Annex 7.4). Generally, *P.haemolytica* has 13 serotypes and *P.trehalosi* 4, however with the IHA test used for serotyping *P.haemolytica* and *P.trehalosi* nontypable strains also occur.

Results of serotyping of the 15 *M.haemolytica* isolates were summarized in table 19. The test indicated that among 15 *M.haemolytica* isolates subjected to serotyping in Bgvv both biotypes A & T of *Mannheimia hemolytica* were identified. The predominant serotype identified was A1 with subsequent prevalence of 55.55% (n=5) .The second most frequent serotype was A6. There were only two serotypes of T15 from biotype T. Analysis of serotypes isolated from various geographical areas revealed that 7.5% of the cases in Hungry as well as in other countries appeared as Pneumonia or Pleuropneumonia due to biotypes. This is in agreement with the current study. Our result differ some what from those of Biberstein and Thompson (1966), Thompson *et al.* (1977) and Fraser *et al.* (1982b) isolated T biotypes much more frequently than the current result. In the findings of the previous work A biotypes A2 was the most common, while with us A1 was the most frequent serotype from sheep. Among the recently described serotypes A13 and A14 (pegram *et al.*, 1979) none of them were found in the present study, but

serotype T15 (Fraser *et al.*, 1982b) occured more frequently and seems to be an important pathogen not only in Scotland but also in Hungary. This finding is not in agreement partially with the current finding. The fact that serotype T15 was identified from lung and swab of sheep from Debreberhan was in agreement while the less frequency of serotypes disagrees with the previous work of Gelagay (1996).

The prevalence of serotypes in apparently healthy and clinically sick sheep showed (Table 21) that out of n=15 *M.haemolytica* isolates characterized by serotyping 13.33% (95%C.I 40.46, 1.65) of strain A1 was from apparently healthy and 20% (95%C.I 48.08, 4.33) was from clinically sick sheep respectively. Strain A6 was characterized 6.66% (95%C.I. 31.94, 0.1) from A/healthy and 20% (95%C.I 48.08, 4.33) from clinically sick sheep respectively. Only 6.66% (95%C:I 31.94, 0.1) of strain T15 was characterized both from apparently healthy and sick sheep respectively.

These results substantiated as the high prevalence of strains A1 and A6 (20%vs13.33% and 6.66%) in sick sheep than healthy could be attributed to immuno-incompetence of the host due to the involvement of secondary infections. The concurrent mycoplasmal or viral immunosuppresive agents like PI-3, PPR, Adenovirus and high burden of D.filaria can trigger the opportunistic pasteurella microorganism. This finding is in agreement with the previous works of (Biberstein and Thompson 1966). The previous observation suggested that healthy sheep in normal flock tend to have relatively low incidence of *P.haemolytica* carriers as measured by single sampling like our finding. A rise in carrier rates of *pasteurella* and a simultanneous narrowing of the type spectrum in diseased flocks recieved considerable support which associated with the lesions in-contact sheep. One also would suspect from these observations that the introduction or sudden proliferation of a particular virulent and communicable strain was not a factor in the pathogenesis of the disease out break (Shreeve and Thompson (1970).

The most important finding in this survey was the most frequent distribution of biotypes, than T biotypes, which were more common in lung of sheep around Jijiga area and in rare cases from nasal swab and tonsil of the same area. T biotypes found only from one lung and one swab sampled from DebreBerhan study area.

On the basis of this result one can argue that A biotype strains must be more selective pathogenic than T biotype strains of lung from healthy sheep and nasal swab of clinically sick ones. The predilection site of the two types might give a clue to the different syndromes of Pneumonia and septicemia produced by these types. In an approximately comparable survey, Biberstein and Thompson (1966) found that 26% of their total isolates were of the T biotype compared with the current study only two isolates of T biotype were identified Furthermore, in the present study unlike the previous workers biotype serotypes A1 and A6 were the most prominent findings.

These findings suggest that the proportion of A biotype strain involved in fatal cases of ovine pasteurellosis. These results may be of significance in the formulation of vaccines against ovine pasteurellosis as they give an indication of the serotypes most commonly found in disease conditions. The relative importance of *M.haemolytica* serotypes in lungs and nasal swabs have shown that serotypes A1 and A6 were the most predominant ones and the least was T15. This is partially in agreement with the work of (Pegram *et al.*,1979) in Ethiopia; Fraser(1982) in the United Kingdom and Hussein and Mohammed (1984) in Sudan.

Out of 15 *M.haemolytica* submitted for serotyping 4/15 (26.66% were untaypable. This could be explained that they might belong to those serotypes which are not tested in this study namely A2, A8, A9, A13, A14, A16 and A17. The absence of serotypes A5, A7, A11, A12, T3, and T4 in the present findings could be attributed to the convenience sampling which limited the samples taken for isolation. The predilection site and less number of samples taken from upper respiratory tract could be an explanation and the previous works of Purdy et al (1997) supports this. In this report other serotypes of *M.haemolytica* such as A2, A4, A7, and A11 are a normal constituent of the upper respiratory tract flora in healthy animal. Serotype A1 undergoes explosive growth to become the dominant isolate in cattle subjected to stressful management practice, bacterial and viral respiratory infection, or change in environmental conditions. There was no remarkable difference in the relative frequency of occurence of the serotypes A1 and A6 in this survey. Serotype A1 was by far the most common and the other A6 serotype did not differ significantly in order or prevalence. The percentage of T15 strain seems increased to 100%. The reseason for this is because of the number of *M.haemolytica* isolates 2/2 of which both T15 strain was serotyped. This is inagreement with the works of Fraser et al. (1982), while the percentage of T15 was increased as the reduction or absence of other T serotypes.

The crude prevalence of serotype and untyped *M.haemolytica* (Figure 7) showed that A1, was the predominate serotype from lung isolate and only from one nasal swab and tonsil. While A6 was serotype of only lung neither from nasalswab and tonsil isolate. The two serotypes of T15 were from one lung and one tonsil isolates. The rest untyped were from all specimens. The serotyped and untyped isolates were also repoted in the previous works of Tesfaye (1997) in Ethiopia. In the present study the crude prevalence for untyped one was 26% (95%C.I. 55.10, 7.78). While it was reported as 3.6% untyped M.haemolytica in Ethiopia. This could be attributed to the available reference antisera. The predominant serotypes isolated and serotyped from nasal swab, lung and tonsil from Ethiopia were A1, A8 and A2 from A biotypes and T10,T3 and T4 partially agreed with our result. There was remarkable difference numerically in frequency rates. The *M.haemolytica* serotypes percentage in our study indicated 26.66% for A1Vs 16% of Tesfaye's report (1997). In our result we didn't find serotype T3, T4 and T10 from T biotypes and A8 and A2 from A biotypes.

In the highlands of North Shewa and lowlands of Jijiga zones the large number of sheep indicated that both study zones were potential sheep raising areas in the country. In the highlands sheep are reared for mutton, skin, and wool production, where as in the lowlands they are kept mainly for live export to middleeast countries. However, this untapped resource could not be utilized efficiently because of the prevailing infectious and non-infectious diseases. Further more, the poor management conditions, limited grazing areas are also the major constraints that reduce the potential output of sheep.

Conclusion

Among sheep health problems of the study areas ovine pasteurellosis is a major bacterial disease complicated with different predisposing factors and cause loss of a substantial number of sheep all year round in selected study sites. In the current study an attempt was made to investigate the relationship of the disease with sheep husbandry and identified the causative agents involved in the process of ovine pasteurellosis and the prevalence of the species were estimated.

The PCR result of the overall crude prevalence of 20.27% and 6.75% of *M.haemolytica* and P.multocida isolates in the selected study sites could implicate the exsistence of the field isolates which are not fully covered by the currently available vaccine of ovine pasteurellosis in Ethiopia.

The characterization of *M.haemolytica* biotypes and strains have shown the prominent strains A1 and A6 isolated and serotyped from lung and nasal swab of sheep. This result suggests that there might exsist more field strains if similar survey could be conducted at national level.

The predominant isolates and strains in the current study guides to focus on which candidate vaccinal strains one should concentrate. This study could help to draw feedback information for NVI to include *M.haemolytica* strains of A1 & A6 as a cocktail polyvalent vaccine in addition to the currently produced ovine pasteurellosis killed vaccine of *P.multocida* A.

The prevalence of multiple serotypes of *M.haemolytica* with no cross protection among others that are capable of causing ovine pasteurellosis is one of the reseasons why monovalent vaccines are not effective.

The prevalence of *P.multocida* capsular A & D isolated from lung and swab of sheep confirmed the less involvment of *P.multocida* in the process of the disease.

From the present study it can be concluded that most ovine pasteurellosis cases were due to *M.haemolytica* biotype A strains as compared to *P.multocida* A. Hence, due attention should be given for the quality of ovine pasteurellosis vaccine currently produced and dispatched by NVI.

Although a number of live and killed vaccines have been developed and used, their efficacy in field trials has been variable, ranging from no effect to reduced or even increased morbidity and mortality (Catte *et al.*, 1985; Chengappa *et al.*, 1988; Confer *et al.*, 1988). This observation implies that development of an effective vaccine is possible if the antigens responsible for the protection associated with natural infection can be identified. In this regard, much research has been directed toward the identification of potential vaccine antigens present in in outer membrane proteins (OMPs), capsular polysaccharides (CPSs) and cytotoxic products of *M (P).haemolytica* (Confer *et al.*, 1990).

The result epidemiological study showed the prevalences of pasteurella field isolates in the respective study sites and the possible predisposing factors involved in the induction of ovine pasteurellosis.

The isolated and characterized species indicated that the disease persists and exsists in the healthy flock and diseased flock as commensal and when triggered by risk factors becomes infective to the host with complicated respiratory disease syndrome.

Epidemiological typing studies require the use of both phenotypic and genotypic markers and many methodological approaches have been developed. Biotyping and serotyping have been largely used and limitations of these methods are widely recognized. In line with the currently developed methods this finding also confirmed the exsistence of two biotypes of M.haemolytica (T and A) and on the basis of differences in capsular polysaccharides the exsistence of serotypes A1, A6 and T15 at different frequency rates among the exsisting 17 serotypes of *M.haemolytica*.

M.haemolytica biotype A, serotypes A1 and A6 being the prominent strains are recommended to be the candidate vaccinal serotypes of ovine pasteurellosis to promote the quality of the vaccine currently used in Ethiopia.

The occurence of enviromental and management predisposing factors enhanced the opportunistic *Pasteurella* microorganism to be infective. Due attention should be given to minimaize these risk factors to exploit the untapped potential resource of sheep production in the selected sies in particular and in the country in general.

The serotype A1 and A6 could be given due attention for future polyvalent vaccine production.

Control strategies at national level should be designed to encourage autogenous vaccine production from strains of locally isolated M.haemolytica serotypes. Thus, investigation of serotypes in each outbreak has to be undertaken to include the most homologous antigens for effctive polyvalent vaccine development.

The pathogenecity and immunogenecity of *Pasteurella* and other concurrent pathogens should be given attention for further investigation to reconsider the current immunization strategy against multifactorial respiratory diseases in sheep in Ethiopia.

On the basis of the present study it is worthwhile to monitor continously the prevalence of the various serotypes and to investigate the pathogenicity of new serotype strains inorder to incorporate the appropriate and major serotypes in vaccine production.

Results of the present study support the hypothesis that local field isolates (strains) are different from the current ovine pasteurellosis vaccine (produce of NVI) and suggest further purification and characterization of *M.haemolytica* A1 and A6.

6. REFERENCES

Aarselff, B., Biberstein, E.L., Shreeve, B.J. and Thompson, D.A. (1970): A study on untypable starains of *P.haemolytica J.comp.Pathol.* **80**, 493-498.

Abdulah, K.M., Udoh, E.A.Schewen, P.E. and Mellors, A. (1992): A neutral glycoprotease of the *pasteurella haemolytica* A1 specifically cleaves O-sialoglycoproteins. *Infect.Immun.* **60**,56-62.

Adlam, C. (1989): The structure, function and properties of cellular and extracellular components of *Pasteurella haemolytica*. In: Adlam, C., Rutters, J.M. (Eds.): Pasteurella and Pasteurellosis. London: Academic Press, pp.75-92.

Adusu, T.E., Conlon, P.D.,Schewen, P.E. and Black, W.D. (1994): *Pasteurella haemolytica* leukotoxin induces histamine release from bovine pulmonary mast cell. *Can. J. V. Res.* **58**, 1-5.

Agymang, K., Negussie, A., Voorthuizen, A., Anderson, F.M. (1985): A rapid survey of reproduction in the traditional sector of Debre Berhan, Ethiopian highlands. Wilson P.T. and Bourzat D. (eds.): Small ruminants in African Agriculture. Proceedings of a Conference Held at ILCA, Addis Ababa, Ethiopia. 30 September-4 October 1985, pp. 174-185.

AL-Sultan, I.I. and Aitken, I.D.(1985):The tonsillar carriage of *Pasteurella haemolytica* in lambs. *J. comp. Pathol.* **95**, 193-201.

Alemayehu, Z. and Fletcher, I. (1991): Small Ruminant productivity in the central Ethiopian mixed farming systems. IAR Proceeding. 4 th NLIC. pp 141-147.

Anderson N.V., Youanes Y.D., Vestweber J.G., King, C.A., Klemm R.D., Kennedy G.A. (1991): The effects of stressful exercise on leukocytes in cattle with experimental pneumonic pasteurellosis. *Vet.Res. Commun.* **15** 189-204

Angen, O., Aalbaek, B., Falsen, E., Olsen, J.E., Bisgaard, M. (1997a): Phenotypical relationships among strains classified with the ruminant *Pasteurella haemolytica*-complex using quantitative evaluation of phenotypic data. *Zbl.Bakteriol.***285**, 459-479.

Angen, O., Olsen, J.E., Bisgaard, M (1997b): Furthe studies on the relation ships among strains classified as taxon 15, 18, taxon 20, (*Pasteurella) granulomatis* or the (*Pasteurella*) *haemolytica* complex in ruminants using quantitative evaluation of phenotypic data. *Zbl. Bacterol.* **286,** 317-332.

Angen, O., Caugant, D.A., Olsen, J.E., Bisgaard, M. (1997c): Genotypic relationships among strains classified with the *(Pasteurella) haemolytica* complex as indicated by ribotyping and multilocus enzyme electrophoresis. *Zbl.Bacteriol.* **286**, 333-354.

Angen, O., Mutters, R., Caugant, D.A., Olsen, J.E., Bisgaard, M. (1999): Taxonomic relationships of the Pasteurella haemolytica complex as evaluated by DNA-DNA hybridizations and 16S rRNA squencing with proposal of *Mannheima haemolytica* gen. nov, comb. Nov., *Mannheima granulomatis comb. Nov., Mannheima glucosida* sp. nov., *Mannheima varigena sp. nov. Internationa Journal of Systematic Bacteriology*, **49**,67-86.

Ausubel, F.M.(1994): Current protocols in Molecular Biology. New York, John Wiley & Sons, Inc.

Bahaman, A.R., Nurida, A.B., Sheikh-Omar, A.R., Zamri-Saad, M. (1991): Biotypes and serotypes of *Pasteurella haemolytica* and their importance in the production of vaccines for Pneumonic Pasteurellosis in sheep. *J. Vet. Malaysia*, **3**, 33-35.

Bekele, M. (1996): A preliminary serological survey of small ruminant *Pasteurella haemolytica* sero-types in North and East Shewa. Debrezeit: Addis Ababa University, Faculty of Veterinary Medicine, DVM Thesis.

Bekele, T., Woldeab, W. Lahlou-Kassi, A. and Sherington J. (1992): Factors affecting morbidity and mortality on-farm and on-station in the Ethiopian highland sheep. *Acta Tropica*. **52**, 99-109.

Biberstein, E.L., Gills, M.and Knight,H. (1960): Serological types of *Pasteurella haemolytica* *Cornell Vet.* **50**, 283-300.

Biberstein,E.L., Shreeve, B.J., Thompson, D.A. (1966): Epidemiological studies on *Pasteurella haemolytica* in sheep *J.Comp.Pathol.***76**, 83-94.

Biberstein, E.L., Shreeve, B.J., Thompson, D.A., (1970): Variation in carrier rates of P. haemolytica in sheep flocks. I. Normal flocks. *J. Comp. Pathology* **80**, 499-507.

Biberstein, E.L., (1978): In: Methods Microbiology (eds.T. Bergan and J. R. Norris). Academic Press, London. 10,. 253-269

Bingham, D.P., Moore, R and Richards, A.B. (1990): Comparison of DNA:DNA homology and enzymatic activity between *Pasteurella haemolytica* and related species. *Am.J. Vet. Res.* **51,** 1161-1166.

Biondi M., Zannino L.G., (1977): Psycological stress, neuro immuno modulation, and susceptibility to infectious diseases in animals and man: a review, *Psychother. Psychosom.*66, 3-26.

Brogden, K.A. (1992): Ovine pulmonary surfactant induces killing of *Pasteurella haemolytica*, *Escherichia coli*, and *Klebsiella pneumoniae* by normal serum. *Infec. Immun.* **60**, 5182-5189.

Brogden, K.A., Lehmkuhl, H.D., and Cutlip, R.C. Review article.(1998): *Pasteurella haemolytica* complicated respiratory infections in sheep and goats. *Vet.Res.* **29**(304): 233-254.

Buxton, A. and Fraser, G. (1997): *Pasteurella,* Yersinia *and Francisella.* In: Animal Microbiology, 1st edition. Vol.I. Blackwell Scientific publications. Oxford, London. Pp 121-130.

Carter, G.L., (1984): *Pasteurella, yersinia and Francisella.* In: Diagnostic procedures in Veterinary Bacteriology and Mycology. 4th Ed: Charles Cthomas publishing, U.S.A. PP 111-118.

Carter, G.R. and Chengapa, M.M. (1991): Pasteurella and Francisella. In: Essentials of Veterinary Bacteriology and Mycology. 4th ed. Charles Cthomas publishing, U.S.A. pp. 111-118.

Catt, D.M., Chengappa, M.M., Kadel, W.L. (1985): Preliminary studies with a live streptomycin-dependent *Pasteurella multocida* and *Pasteurella haemolytica* vaccine for the prevention of bovine pneumonic pasteurellosis. *Can.J. Med.*, **49**, 335-371.

Central Veterinary Laboratories(1994): (CVL, Veterinary Laboratories Agency, Weybridge). Volume III.

Chengappa, M.M., McLaughlin, B.G., Kadel, W.l., Herren, C.E., Craft, D.L. (1988): Bovine pneumonic pasteurellosis: Efficacy testing a live vaccine. *Vet.Med.* **8**, 837-840.

Chengappa, M.M., McLaughlin, B.G., Kadel, W.l., Maddux, R.L., and Greer, S.C. (1989): Efficacy of a live Pasteurella multocida vaccine for prevention of experimentally induced bovine pneumonic pasteurellosis. *Vet. Microbiol.* **21,** 147-154.

Clinkenbeard K.D., Clarke C.R., Confer A.Q., Morton R.J., Monsier D.A., Panciera R.J. (1992): Role of *P.haemolytica* leukotoxin in virulence and immunity in shipping fever pneumonia. *Compendium on continuing education for the practicing veterinarian.* 14, 1249-1260.

Confer, A.W., Panciera, R.J., Corstvet, R.E., Rummage, J.A. and Fulton, R.W., 1984. Bovine pneumonic pasteurellosis: effect of culture age of *Pasteurella haemolytica* used as a live vaccine. *Am. J. Vet. Res.* **45,** 2543-2545.

Confer, A.W., Lessley, B.A., Panciera R.J., Fulton, R.W. and Kreps, J.A. (1985): Serum antibodies to antigenes derived from saline extracts of *Pasteurella haemolytica*: correlation with resistance to experimental bovine pneumonic pasteurellosis. *Vet. Immunol. Immunopathol.* **10,** 265-278.

Confer, A.W., Panciera, R.J., Gentry, M.J. and Fulton, R.W. (1986): Immunologic response and resistance to experimentally induced pneumonic pasteurellosis immunized with various dosages of lyophilized *Pasteurella haemolytica. Am. J. Vet.Res.* **47,** 1853-1857.

Confer, A.W., Panciera, R.J., Gentry, M.J. and Fulton, R.W. (1987): Immunologic response to *Pasteurella haemolytica* and resistance against experimental bovine pneumonic pasteurellosis induced by bacterins in oil adjuvants. *Am. J. Vet.Res.* **48,** 163-168.

Confer, A.W., Panciera, R.J., Moiser, D.A. (1988): Bovine pneumonic pasteurellosis: Immunity to *Pasteurella haemolytica. J.Am.Vet. Med. Assn.,.* **193,** 1308-1316.

Confer, A.W., Panciera, R.J., Clinken beard , K.D. (1990): Molecular aspects of virulence of *Pasteurella haemolytica. Can.J. Vet. Res.* **54,** S48-52.

Confer, A.W. (1993): Immunogenes of pasteureklla. *Veterinary Microbiology.* **37,** 353-368.

Conlon, J.A.R., Shewen, P.E. and Lo, R.Y.C., (1991): Efficacy of recombinant leukotoxin in protection against pneumonic challenge with live *Pasteurella haemolytica A1. Infect.Immun.* **59,** 589-591.

Conlon, J.A.R. and Shewen, P.E. (1993): Clinical and serological evaluation of a *Pasteurella haemolytica* A1 capsular polysaccharide vaccine. *Vaccine.* **11.** 767-772.

Craven, R.C., Confer, A.W. and Gentry, M.J.(1991): Cloning and expression a 30 kDa surface antigen of *Pasteurella haemolytica. Vet. Microbiol.* **27,** 767-772.

Czuprynski,C.J. Hamilton, H.L., Noel, E.J.(1987): Ingestion and killing of *P.haemolytica* A1 by bovine neutrophills in vitro. *Vet. Microbiol.* **14**,61-74.

De Alwis M.C.L. (1993): Pasteurellosis in production animals. In: ACIAR proceeding. Watson and Ferguson Pty Ltd., Brisbane, Australia. **43**, 11-22.

DeAngelis, P.L., Jing,W., Richard R. Drake, Ann Mary Achyuthan. (1998): Identification and Molecular cloning of a unique Hyaluronan synthase from *Pasteurella multocida. J.Biol.Chem.* **273,** 8454-8458.

Donachie, W., Gilmour, N.J.L., Mould, D.L., Poxton, I.R.(1984): Comparison of cell surface antigen extracts from two serotypes of *P.haemolytica. J.Gen.Microbiol.* **130**, 1209-1216.

FAO (1983): World statistical compendium for raw hides and skins, leather and leather footwear 1961-1982. Revised and updated edition. Food and Agricultural Organization of United Nations, Rome: pp. 1-14.

FAO (1987): FAO production yearbook. Vol. 41. Rome, Italy.

FAO (1993): Agrostat data, Statistical Division. Vol. 39. Rome, Italy.

Frank, G.H, (1989): pasteurellosis of cattle in: Adlam., Rutter, J.M, (Eds), Pasteurella and pasteurellosis. Academics press New York. Pp. 126-197.

Fennerr, F.J., Gibbs, E.P., Murphy, F.A., Rott, R., Studdert, M.J., White, D.O. (1993): Paramyxoviridae. In: Veterinary Virology, 2nd edition. London, Academic press, Inc, pp. 477-478.

Feseha, G-Ab. and Gebre-Negus, T.M. (1977): Epidemiology of D.filaria in the areas around Debre Zeit, Modjo and notes in its prevalence at Alemaya. Report to Ministry of Agriculture of Ethiopia.

Fodor, L., Varga, Hajitos, I., Donachie, W., Gilmour, N.J. (1987): New serotype of *Pasteurella haemolytica* isolated in Hungry. *Vet. Rec.* **121,** 155-155.

Fraser, J., Laird, S., Gilmour, N.J.L. (1982a): A new sero var (biovar T) *of Pasteurella haemolytica. Res. Vet. Sci.* **32**, 127-128.

Fraser, J., N.J.L. Gilmour, S.Laird, and W. Angus. (1982b): Prevalence of *Pasteurella haemolytica* serotypes isolated from ovine Pasteurellosis in Britain. *Vet.Rec.* **110**, 560-561.

Frederiksen, W. (1973): In contributions to microbiology and immunology (ed. S. Win bald), Karger Basel NewYork, **2**,170-176.

Gelagay .A. (1996): Epidemiological and serological investigations on ovine multifactorial respiratory diseases and vaccine trials on the highlands of North Shoa. Debrezeit: Addis Ababa University, Faculty of Veterinary Medicine, DVM Thesis.

Gilmour, N.J.L. (1978): Pasteurellosis in sheep. *Vet. Rec.* **102**, 100-102.

Gilmour, N.J.L., Martin, W.B., Sharp, J.M., Wells, P.W., Thompson, D.A. (1979): The development of vaccines against Pneumonic Pasteurellosis in Sheep. *Vet.Rec.* **104**, 15.

Gilmour, N.J.L., Martin, W.B., Sharp, J.M., Wells, P.W., Thompson, D.A., Wells, P.W., Donachie, W. (1983): Experimental immunization of lambs against Pneumonic Pasteurellosis. Res. Vet. Sci. **35**(1), 80-86.

Gilmour N. J. L. and Gilmour J.S. (1989): Pasteurellosis of sheep. Pasteurella and Pasteurellosis., Adam. C., and Rutter J. M. Eds, pp.223-262.

Gilmour N.J.L., Angus, K.W., Gilmour, J.S., (1991): Pasteurellosis. In: Martin, W.B., Aitken, I.D. (eds.), Diseases of sheep, London: Blackwell Scientific Publications, pp. 133-138.

Gilmour, N.J.L. (1993): Pasteurellosis: The disease. In: Patten, B,E., Spencer, T.L., Johanson, R.B., Hoffmann, D., Lehane, L., (eds.), Pasteurellosis in Production Animals. ACIAR proceeding no. **43**, pp. 238-239.

Graber, M. (1975): Helminths and Helminthiasis of Domestic and Wild animals in Ethiopia.

Gonzalez C.T. and Maheswaran S.K. (1993): The role of induced virulence factors produced by *P.haemolytica* in the pathogenesis of Bovine Pneumonic Pasteurellosis. *British Veterinary Journal*. **149**, 183-193.

Gryseels, G. (1988): Role of livestock on mixed smallholder farmers in the Ethiopian highlands. Wageningen: Agricultural University, Dissertation.

Hajitos, I., Harrach, R.,Szigeti, G., Fodor, L., Malik, G. and Varga, J. (1983): Pasteurellosis of sheep. *Acta.Vet. Hung*. **31**, 181-188.

Heddleston, K.L. (1976): Pasteurellosis in laboratory animals. Experimental pasteurellosis .Comparative studies on pasteurella multocida. *Am.J.Vet.Res*. **37**, 745-747

Hunt, M.L., Adler, B., Townsend, K.M. (2000): The molecular biology of *Pasteurella multocida.. Journal of veterinary microbiology*. **73**, 3-23.

Hussen, A.M. , Elsawi Mohammed, O. (1984a): A serological survey of sheep sera for antibodies to *P.haemolytica* serotypes in Sudan. *Rev. Elv. Med. Vet. Pays trop*. **37** (4): 418-421.

Hussen, A.M. , Elsawi Mohammed, O. (1984b): Serology and mycoplasma. *Research in Veterinary Science*. **47**,84-89.

ILCA (1989): Annual Report. Addis Ababa, Ethiopia, pp. 46-51.

Jakab, G.J.(1982): Viral-bacterial interactions in pulmonary infection. *Adv.Vet. Sci. Com. Med*. **26**, 155-172.

Jamaludin, R. (1993): Country report: Malaysia. In: Patten, B.E., Spencer, T.L., Johanson, R.B., Hoffmann, D., Lehane, L., (eds.), Pasteurellosis in Production Animals. ACIAR Proceeding no. **43**, pp. 238-239.

Jaworski, M.D., Hunter, D.L., Ward, A.C.S. (1998): Biovariants of isolates of Pasteurella from domestic and wild ruminants. *J. Vet. Diagn. Invest.* **10**, 49-55.

Jones G:E:, Donachie W., Gilmour J.S. and Rae G. (1987): Attempt to prevent the effect of experimental chronic pneumonia in sheep by vaccination against *Pasteurella haemolytica*. *Br.Vet.J.* **142**(2), 189-194.

Kassahun, A. (1985): The status of sheep and goat research and development in Ethiopia. (eds.) Institute of Agricultural researches proceeding. Beyene, K and Lambourne, L.J. pp. 49-61.

Lee, C.W., Lo, R.Y.C., Shewen, P.E. and Mellors, A. (1996): The detection of the siyloglycoprotease gene and assay for sialoglycoprotease activity among isolates of *Pasteurella haemolytica* A1 strains, serotypes A13, A14, T15 and T16. FEMS *Microbiol Lett* **121**, 199-206.

MacDonald, J.T., Maheswaran, S.K., Opuda-Asibo, J., Townsend, E.L., Theis, E.S.(1983): Susceptibility of *P.haemolytica* to the bactericidal effects of serum, nasal secretions,and bronchoalveolar washings from cattle. *Microbiol.* **51**, 319-330.

Magwood, S.E., Barnum, D.A., Thompson, R.G. (1969): Nasal bacterial flora of calves in healthy and pneumonia prone herds. *Can. J. comp. Med.* **33**, 237-243.

Mekonnen, T. (2000): An Epidemiological study on ovine pasteurellosis in Arsi, Southeast. Debrezeit: Addis Ababa University, Faculty of Veterinary Medicine, DVM Thesis.

Merchant, I.A. Packer, R.A. (1983): Genus Pasteurella. Veterinary Bacteriology and Virology, 7th ed. London: CBS publisher and distributor. PP 341-343.

Ministry of Agriculture (1985): Sheep production project, Main Report. Australian agricultural consulting and Management Company Limited, Addis Ababa, Ethiopia.

Mohamad, M., Quirie, M., Donachie, W. (1993): Prevalence of *Pasteurella haemolytica* serotypes isolated from ovine and caprine pasteurellosis in Malaysia. Proc. 16th Malaysian Society of Animal Production Conf. PP. 126-127.

Moiser, D.A., Confer, A.W., Panciera, R.J. (1989): The evolution of vaccines for bovine Pneumonic Pasteurellosis. *Res. Vet. Sci.* **47**, 1-10.

Moiser, D.A. (1993): Prevention and control of Pasteurellosis. In: Patten, B.E (eds.), Pasteurellosis in Production Animals, ACIAR proceeding no. 43, pp. 121-134.

Mutters, R., Bisgaard, M., pohl, S. (1986): Taxonomic relationship of selected biogroups of Pasteurella haemolytica as revealed by DNA: DNA hybridization. *Acta. Pathol. Microbiol. Immunol. Scand. Sect. B.* **94,** 195-202

Mutters, R., Mannheim, W., Bisgaard, M. (1989): Taxonomy of the group. In: Pasteurella and Pasteurellosis. Adlam, C., Rutter, J.M. Pp. (eds.). London: Academic Press. Pp. 3-30.

Mwangota, A.U., Mohammed, S.I., Thompson, R.G. (1978): Serological types *of P. haemolytica* in Kenya. *Cornell. Vet.* **68**, 84-93.

OIE (1992): Diagnosis of Pasteurellosis. In: Manual of Standard for Diagnostic tests and Vaccines.

Pass, D.A., Thompson, R.G., (1971): Wide distribution of *Pasteurella haemolytica* type 1 over the nasal mucosa of cattle. *Can. J. Comp. Med.* **35**, 181-186.

Pegram, R.G., Roeder, P.L., Scott, J.M. (1979): Two new sero types of *Pasteurella haemolytica* from sheep in Ethiopia. *Trop. Anim. Health. Prod.* **11**, 29-30.

Personal communication with Ato Gifawosen Abebe, former Jijiga Range land Development Unit (JIRDU) project coordinator.

Petersen, S.K. (1990) The complete nucleotide sequence of the *Pasteurella multocida* toxin gene and evidence for a transcriptional repressor, TxaR. Mol. Microbiol. 4, 821-830.

Progress reports (1997): Annual report of Animal health reseaerch division, Sheno Agricultural Research Center, Unpublished.

Purdy, C.W., Cooley, J.D., Straus, D.C. (1998): Cross-protection studies with three serotypes of Pasteurella haemolytica in a goat model. *Cur. Microbiol.* **36**, 207-211.

Purdy C.W., Rualeigh RH. Collins JK (1997): Serotyping and enzyme characterization of *Pasteurella haemolytica* and *Pasteurella multocida* isolates recovered from pneumonic lungs of stressed feeder calves. *Cur. Microbiol.* **34**, 44-249.

Qunin, P.J., Cater, M.E., Markey, B. and Carter, G.R. (1994): Pasteurella species. In: Clinical Veterinary Microbiology. Wolfe Publishing. Mosby, London, England pp 254-260.

Radostits, O.M., Blood, D.C. and GAY, C.C. (1989): Disease caused by Pasteurella species. In: Veterinary Medicine: A textbook of Cattle, Sheep, Pigs, goats, and Horses. 7th edition. London: Bailliere Tindail. Pp. 782.

Radostits, O.M., Blood, D.C. and GAY, C.C. (1994): Disease caused by Pasteurella species. In: Veterinary Medicine: A textbook of Cattle, Sheep, Pigs, goats, and Horses. 8th edition. London: Bailliere Tindail. pp. 747-787.

Rimler, R.B. (1993): Laboratory techniques for Pasteurella typing and diagnosis of infection. In: Pasteurellosis in production animals. ACIAR proceeding. Brisbane, Watson and Ferguson Pty Ltd. **43**, 136-200.

Roger, F. (1996): Pathologie des Petits Ruminants en Ethiopie. Rapport annuel D'activite'. Rapport CIRAD-EMVET N0. 96007.

Sabri, M.Y., Zamri-Saad, M., Mutalib, A.R., Israf, D.A., Muniandy, N. (2000): Efficacy of an outer membrane protein of *Pasteurella haemolytica* A2, A7 or A9- enriched vaccine against intratracheal challenge exposure in sheep. *J. Vet.Mic.* **73**, 13-23.

Schewen, P., Wilkie, B.N. (1988): Defining the role that *P.haemolytica* plays in shipping fever. In: Veterinary Medicine: Practical solution to diagnostic and therapeutic problems. Pp.1053-1058.

Shewen, P.E. and Wilkie B.N. (1988a): Vaccination of calves with leukotoxin culture supernatant from *P.haemolytica*. *Can. J. Vet. Res.*, **52,** 30-36.

Shewen, P.E, Sharp, A and Wilkie, B.N. (1988b): Efficacy testing a *P.haemolytica* extract vaccine. *Vet. Med.*, **83,** 1078-1083.

Shreeve, B. J., Thompson, D.A., (1970): Studies on the carriage of *Pasteurella haemolytica* in Lambs. *J. Comp. Pathol.* **80**, 107-112.

Sharma, S.A., Olchowy, W.J., Breider, M.A. (1992): Alveolar macrophage and neutrophil interactions in *Pasteurella haemolytica*-induced endothelial cell injury. *J.Infec. Dis.* **165**, 651-657.

Smith, G.R. (1961): The characterstics of two types of *Pasteurella haemolytica* associated with different pathological conditions in sheep. *J.Pathol.Bacteriol.* **81**, 431-440.

Smth, G.R. and Phillips, J.E. (1990): Pasteurella and Actinobacillus. In: Principles of Bacteriology, Virology and Immunology. Vol.2. Topley and Wilsons (ed). pp 383-397.

Sneath, P.H.A., and M. Stevens (1990): *Actinobacillus rossi sp.nov., nom. rev. Pasteurella betti sp.nov., Int. J. Syst. Bacteriol.* **40**, 141-153.

Stamp J.T., Watt, J.A.A. and Thomlinson, J.R. (1955). *Pasteurella haemolytica* septicemia of lambs.*J. Comp. Pathol.* **65**, 183-196.

Sutherland, A.D.(1985): Effects of *Pasteurella haemolytica* cytotoxin on ovine peripheral blood leukocytes and lymphocytes obtained from gastric lymph. *Vet. Microbiol.* **10,** 431-438.

Swada, T., Rimler, R.B. and Rhodes, K.R. (1982): *J.Clin.Microbiol.* **15**, 752-756.

Tesfaye, S. (1997): Serological and bacteriological investigation of *Pasteurella haemolytica* serotypes in sheep in the highlands of Wollo (Northeast Ethiopia). Debrezeit: Addis Ababa University, Faculty of Veterinary Medicine, DVM Thesis.

Thompson, D:A.J. fraser, Gilmour, N.J.L. (1977): Serotypes of *Pasteurella haemolytica* in ovine Pasteurellosis. *Res. Vet. Sci.* **22,** 130-131.

Thrusfield, M. (1995): Veterinary Epidemiology. London: Second edition. Blackwell Science Ltd. p. 185-186.

Townsend, K.M., Frost, A.J., Frost, , C.W., Lee, Papadimitriou, J.M., Dawkins,H.J. (1998): development of PCR assays for species- and type-specific identification of *Pasteurella multocida isolates. J. Clin. Microbiol.* **36,** 1096-1100.

Townsend E.L., Maheswaran S.K., Lininger J.R., Ames T.R. (1987): Detection of immunoglobulin G to P.haemolytica polysaccharide by ELISA. *J. Clinical Microbiology* **25**, 242-247.

Veterinary Investigation Diagnosis Analysis (1995): Veterinary Investigation Surveillance Report.

Weaver, R.E., and Hollis, D.G. (1980): Manual of Clinical Microbiology, 3rd ed. (Eds. E.H. Lennette, A. Ballows, W.J. Hauseler and J.P. Truant), pp. 242-262, Amer. Soc. Microbiol., Washington, D.C.

Whiteley, L.O., S.K. Maheswaran, D.J.., Wiess, T.R. Ames, and M.S. Kannan. (1992): *Pasteurella haemolytica* A1 and bovine respiratory disease: Pathogenesis. *J.Vet. Int. Med.* **6**, 11-22.

Woolcock, J.B. (1993): The biology of *P.haemolytica* and *P.multocida*. In: Pasteurellosis in Production Animals. ACIAR Proceedings. **43**, 25-34.

Younan, M., Wallmann, J. (1989): A new serovar of *Pasteurella haemolytica* from sheep in Syria. *Trop. Anim. Health Prod.* **21**, 87-87.

Younan, M., Fodor, L. (1995): Characterization of a new pasteurella haemolytica serotype (A17). *Res. Vet. Sci.* **58**, 98.

Zamri-Saad, M., Norizah, A., Sheikh-Omar, A.R. (1994): Detection of immunogenic components of several serotypes of *Pasteurella haemolytica*. Proceeding 17th Malaysian Microbiology Symposium. pp. 25-27.

Zeng, H., K., Pandher, G.L., Murry. (1999): Molecular cloning of the pasteurella haemolytica PomA surface domains. *Infection and Immunity*. **67,** 4968-73.

7. LIST OF ANNEX

7.1.Questionnaire for the assessment of predisposing factors of ovine pasteurellosis in sheep flock.

OwnersName_____ Date_____Region_____Zone_____

District/Woreda_____ Village/ PA_____ Flock identity_____

1. Family income sources (rank 1-4)

Crop farming_____animal products_____

animal sale_____others_____

2. Livestock ownership

2.1. Which animal species do you own? Sheep___ goats ____ cattle_____.

Camels_____ horses_____ donkeys_____ poultry_____ others _____

2.2. How many sheep do you own? (In number)

Male _____Female_____

2.3. How many sheep did you buy and with how many Birr for the last one year? (Price/animal)

Young male in No.___ Price____young female in No. ____ price_____

Old male in No_____price_____old femaleinNo___ price _____

2.4. How many sheep did you sell and for how many Birr? (Price/animal)_____

Young male in No _____price_____Young female in No ____price_____

Old male in No _____price _____Old female in No -price_____

2.5. When do you sell your sheep?

At times of food scarcity _____ at times of religious and New- year festivals and home-consumption

3. Environmental and Managemental factors:

3.1. Is there a sheep market in the nearby? Yes* No*.

3.2. If no how long does it take to transport to market? In hours_____ in days_____

3.3. 3. What is the means of transportation to the market? Yes No

By truck_____migrating the flock to the market_____

3.4. Please indicate during which period your sheep flocks are manifesting pneumonic signs like coughing, fever, nasal discharge.

During winter _____

Inclement weather_____

During dry season _____

Feed and water shortage_____-__

During animal transportation to market_____

When the animal is already sick and infected by another disease_____

4. Mode of animal husbandry (0/1)

Settled_____semi-pastoralist_____pastoralist_____

If yes: Intensive_____

Semi-intensive_____

Extensive_____

5.Purpose of sheep production

Income generation/Market value_____

Home consumption_____

Manure/Fuel_____ Hides_____

5.1. Does the housing allow:

adequate ventilation (0/1)

No ventilation_____ (0/1)

5.2. Is housing: traditional___ (0/1) (earth floor, over crowded in the same pen)

Modern (separate pen with optimum stocking rate, concrete floor)____Mixture_____

6.What disease affect your sheep flock

Pneumonic pasteurellosis (coughing, nasal discharge and fever)

- Internal parasites_____
- External parasites_____
- Sheep pox_____
- Abortion_____
- Bloat_____
- Anthrax_____
- Sudden death_____
- Others_____
-

6.1. To which cause(s) was/were the deaths you indicated above attributed?

- Pneumonic pasteurellosis_____
- Internal parasites_____

- External parasites_____
- Sheep pox_____
- Abortion_____
- Bloat_____
- Anthrax_____
- Sudden death_____
- Others_____
- 6.2. Have you vaccinated your sheep flock?_____ (0/1)

6.3.. Is there Veterinary clinic in the nearby? _____(0/1)

6.4.Do you take your animals to veterinary clinic? ____(0/1)

6.5.. What is the main illness/symptoms?

Nasal discharge_____coughing_____ Fever_____diarrhea___

7.Breeding practice:

7.1. How many ewes have you bred to the ram?_____

7.2. How many ewes did conceive?_____

7.3. How many lambs were born from the ewes conceived?

7.4. Were there twining?___ (0/1)

7.5. Triplets_____0/1)

5.5. How many of them did survive?_____

5.6. What do you do the male lambs?_____

Slaughter_____fatten and sell _____sell with out fattening_____ use for breeding_____ Castrate to control breeding_____

No=0; Yes=1

7.2. Sample collection record sheet

Collection date:_____

Sample No_____

Investigator(s):_____

_____

Region:_____

Zone:_____

District/ Wereda_____

Village/PA_____

Flock size_____

Flock No_____

Owners name_____Sheep No_____

Health status of sheep at sampling time:

A/healthy_____Sick_____If sick indicate symptoms/syndromes

_____

Sampletype: blood swab_____Tissue/organ_____Sheep breed_____ age_____ sex_____

7.3 Media preparation, biochemical characterization and Test

Media preparation

Blood Agar (Oxoid)

Composition: Special peptone (Oxoid 172) 23.0 g, starch 1.0g, Nacl 5.0g, Agar No.1 (Oxoid L11) 10.0g and 5% sheep blood.

Preparation: 39gram of the tryptose blood agar base was suspended in 1 litre of distilled water. It was then brought to boil to disolve completely and auto claved at 121 °C for 15 minutes. Finally, sterile sheep blood (5%) was added after cooled to 50°C and dispensed 15 ml amount to petridisch to solidity for use.

MacConkey Agar (oxoid)

Composition: pepton (oxoid 137) 20.0g/l, lactine-10.0g/l, Bile salt (Oxid L55) 50g/l, Nacl 5.0g/l, Neutral red 0.075g/l, agar(oxoidL13) 12.og/l. The PH adjusted to 7.4.

Preparation: 52 gram of the base medium was suspended in one litre of distilled water and was allowed to dissolve by boiling. It was autoclaved at 121°C for 15 minutes. It was then cooled, dispensed in to petridish in 15 ml amount, solidified and was ready for use.

Christensen's Urea Medium (Oxide)

Composition: Peptone (oxide 137) 1.0 g/l, dextrose 1.0g/l, Nacl 5.0g/l, disodium phosphate 1.2 g, KH2PO4 8.0g/l, phenol red 0.012g, agarNo3 (oxid 113) 15.0g/l The Ph was adjusted at 6.8.

Preparation: 2.4gm of the base medium was suspended in 95 ml of distilled water and then was dissolved compeletly by boiling . It was then autoclaved at 121 °C for 20 minutes. There after 5ml of sterile 40% urea solution was added aseptically after cooled to 50°C . The well mixed preparation was distributed in to sterile tubes and solidified in slope position.

Triple Sugar Iron (TSI)Agar (Oxid)

Composition: Lab-lemeco (oxid 137) 3.0g/l, yeast extract (oxoid) 3.0g/l, peptone (oxoide L13) 20.0g/l, Nacl 5.0g/l, Lactose 10g/l, dextrose 1.0g/l, fericcitrate 0.3g/l, sodium thiosulphate 0.3g/l, phenol 4.5 and agarNo3 (oxoid)12.0g/l.

Preparation: 65 gm perlitre of distilled water was dissolved completely by boiling and autoclaved at 12°C for 15 minutes after the Ph was adjusted to 7.4. It was dispended in the test tubes in amount which could give about 1 inch long in slope position.

Biochemical Tests:

Catalase test: A drop of 3% H2O2 was mixed with a loopful of bacterial colony on a slide, then observed for the production of gas bubbles which is an indication of a positive reaction.

Oxidase test: A loopful of colony was smeared over a filter paper. Then a drop of 1% N,N,N,N-tetramethyl-phenylenediamine dihydrochloride was poured over the smear and examined for color development. The production of a pink color of the colonies was an indication of the positive reaction or otherwise, negative.

Kovac's Indole test

One ml of ethyl ether was added to a 48 hour pure culture in peptone water, shaked well and allowed to stand until the ether rises to the surface. About 0.5 ml of Kovac's reagent was added gently down the sides of the tubes, so that it formed arediving between the media and the solvent for positive reaction.

Carbohydrate fermentation test

15 gm phenolved broth base was suspended in 1 litre of distilled water. It was made, dispensed to final containers in 5ml amount and autoclaved at 115°C for 5-10 minutes. The sugar media were heavily inoculated with a 24 hours pure pasteurella culture using an inoculating loop, incubated at 37°C and read after 24 hours. Fermentation was indicated by the development of yellow colour.

Reagents

Phosphate Buffered Saline(PBS)

Nacl	8.00g

Kcl	0.20g
Na2HPO42H	1.13g
Cacl2	0.10g
MgSO4. 7H2O	0.76g
Distilled water	1000ml

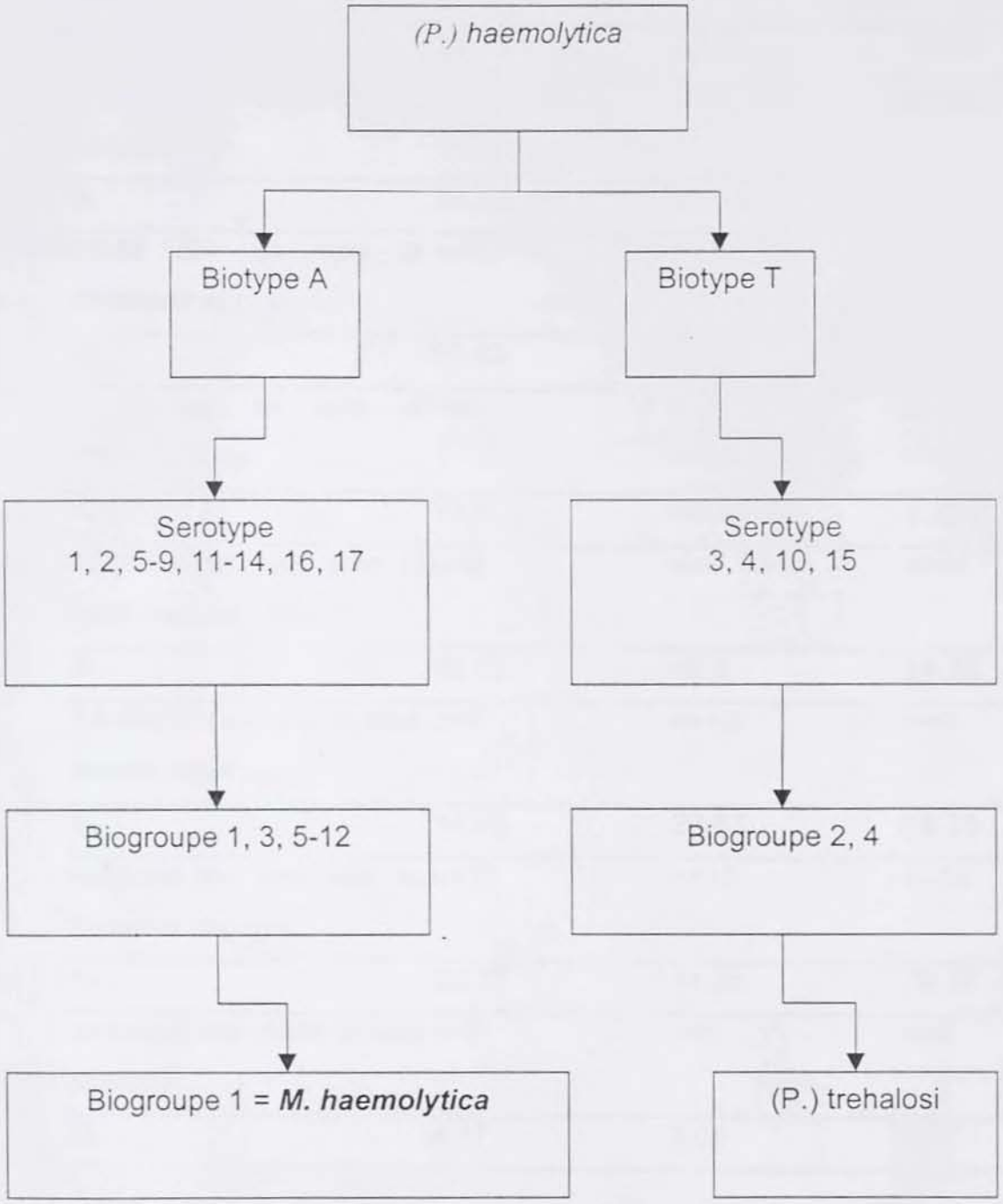
Adjust PH to 7.4, Autoclave at 120°C for 30 minutes

Alsever's solution

Dextrose	20.5
Nacl	4.18g
Trisodium citrate	8gm
Distilled water	1000ml

7.4. Reclassification of Mannheimia haemolytica

Mannheimia haemolytica



7.5. Response frequency and percentage of questionnaire survey

Questionnaire survey

Observations	com.leader response		flock owner response		Shepherd response	
	Frequency	%	frequency	%	frequency	%
Livestock as farm income source	n=16		n=16		n=16	
%	33.33		33.33		33.33	
ownership of shoat among Livestock	n=16		n=6		n=16	
%	33.33		33.33		33.33	
70-80 birr as sale of YMshp/unsupple.	n=10		n=11		n=12	
%	20.83		22.92		25	
65-75 birr as sale of Yfshp/unsupp.	n=6		n=5		n=4	
%	12.5		10.42		8.33	
150-200 birr as sale of adult male shp/supp.	n=9		n=6		n=7	
%	18.75		12.5		14.58	
75-100 birr as sale of adult female shp/supp	n=7		n=10		n=9	
%	14.58		20.83		18.75	
religious and new year as seasona shp mrkt	n=14		n=15		n=14	
%	29.17		31.25		29.17	
seasonal shp markt at food scarcity	n=2		n=1		n=2	
%	4.17		2.08		4.17	
long distance to shpmarkt	n=13		n=12		n=13	
%	27.08		25		27	

in the near by to shp markt	n=3	n=4	n=3
%	6.25	8.33	6.25
on hoof transporting sheep flock to markt	n=16	n=15	n=16
%	33	31.25	33
by truck transporting sheep flock to markt	n=0	n=1	n=0
%	0	2.08	0
weather effect of sheep pnumonia	n=15	n=14	n=15
%	31.25	29.17	31.25
transport&nutrition,otherinf ection effectof pnm	n=1	n=2	n=1
%	2.08	4.17	2.08
settled sheep husbandry	n=8	n=8	
%	16.67	16.67	16.67
semi-pastoralist sheep husbandry	n=4	n=4	n=4
%	8.33	8.33	8.33
pastoralist sheep husbandry	n=4	n=	n=4
%	8.33	8.33	8.33
sheep as income generation	n=15	n=13	n=15
%	31.25	27.08	31.25
sheep as home consumption & manure use	n=1	n=3	n=1
%	2.08	6.25	2.08
housing with adequate ventillation	n=10	n=14	n=11
%	20.83	29.17	22.92

housing with in adequate ventillation	n=6	n=2	n=5
%	12.5	4.17	10.42
traditional housing with no concrete floor	n=16	n=16	n=15
%	33.33	33.33	31.25
housing with concrete floor/modern	n=0	n=0	n=1
%	0	0	2.08
cough,N.discharge&diarrhea as major clinics	n=15	n=14	n=15
%	31.25	29.17	31.25
iching,bloat,abortion&suddendeath as clinics	n=1	n=2	n=1
%	2.08	4.17	2.08
cough,N.discharge&diarrhea as caseof death	n=14	n=13	n=16
%	29.17	27.08	33.33
itching,bloat,abortion,suddendeath as casdeath	n=2	n=3	n=0
%	4.17	6.25	0
vet.clinic visit by flock owners	n=14	n=14	n=5
%	29.17	29.17	31.25
vet clinics with no visit by farmers	n=2	n=2	n=1
%	4.17	4.17	2.08
sheep flock with regular vaccination	n=15	n=15	n=16
%	31.25	31.25	33.33
sheep flock with no regular vaccination	n=1	n=1	n==0

%	2.08	2.08	0
all of the served ewes concieved	xx	n=39	n=9
%	xx	81.25	18.75
half of the served ewes concieved	xx	xx	xx
%	xx	xx	xx
unknown/not specified breeding	n=8	n=8	n=8
%	16.67	16.67	16.67
specified breeding	n=8	n=8	n=8
%	16.67	16.67	16.67
ewes with twining	n=16	n=16	n=16
%	33.33	33.33	33.33
ewes with no twining	xx	xx	xx
%	xx	xx	xx
fatten and sell as male sheep purpose	n=12	n=13	n=14
%	25	27.08	29.17
male sheep for breeding purpose	4	n=3	n=2
%	6.33	6.25	4.17
young female as replacer	n=16	n=16	n=16
%	33.33	33.33	33.33
young female for sale & slaughter purpose	xx	xx	xx
%	xx	xx	xx

7.6. Coding of questionnaire survey

Farm/Village: 1= Jijiga Zuria I; 2= jijiga Zuria II; 3= Jijiga Zuria III; 4= Jijiga Zuria V

Farm/Village: 5= Baligacas;6= Awber; 7= Ejista; 8= Harshin; 9= Keyit; 10= Dalati; 11= Faji; 12= Atakilt; 13= Sariti one; 14= Sariti two; 15= Chaki; 16= Kottu

Respondent: 1= Community leader; 2= Flock owner; 3= Shepherd

Zone: 1= North shewa; 2= Jijiga

Fisource: 1= Live animal sale& animal product; 2= Crop farming; 3= others

Lownshp: 1= shoats; 2= cattle; 3= donkeyand horse; 4= others

Flocksize: 1= 2-10; 2= 11-50; 3= 51-150; 4= >150

ShYslprc: 1= 70 birr Mlamb+ 70 birr yfemale; 2= 70 birr Mlamb+65 birr Y female

Shoslprc:1= 150birr old male+ 65 birr old female;2=200 birr old male+65 birr old female

Sspmrkt: 1= at times of religious, new year festival and home consumption; 2=at time of food scarcity

Distmarkt: 1= long distance; 2= nearby

Trptmrkt: 1= on hoof; 2= by truck

Shpnumn: 1= during rainy and inclement weather; 2= during dry season ,transportation to market, feed&water shortage and concurrent infection

Shphusdry: 1= settled; 2= semi-pawstoralist;3= pastoralist

Eishprodn: 1= income generation;2= home consumption,manure,fueland hides

Hosalow: 1= adequate ventillation; 2= no ventillation

Housing: 1= traditional; 2= modern

Dsesafct: 1= coughing and nasal discharge due to pneumonic pasteurellosis,diarrhea due to internal parasites; 2= external parasites, sudden death,anthrax, sheep pox, abortion and bloat; 3= others

Casdeath: 1= coughing and nasal discharge due to pneumonic pasteurellosis/ diarrhea internalparasites; 2=external parasites,sudden death,anthrax,bloat and abortion

Vaccted: 1= yes; 2= no

Vetclnc: 1= yes; 2= no

EweconcR: 1= half of the served ewe;2= allof theserved ewe

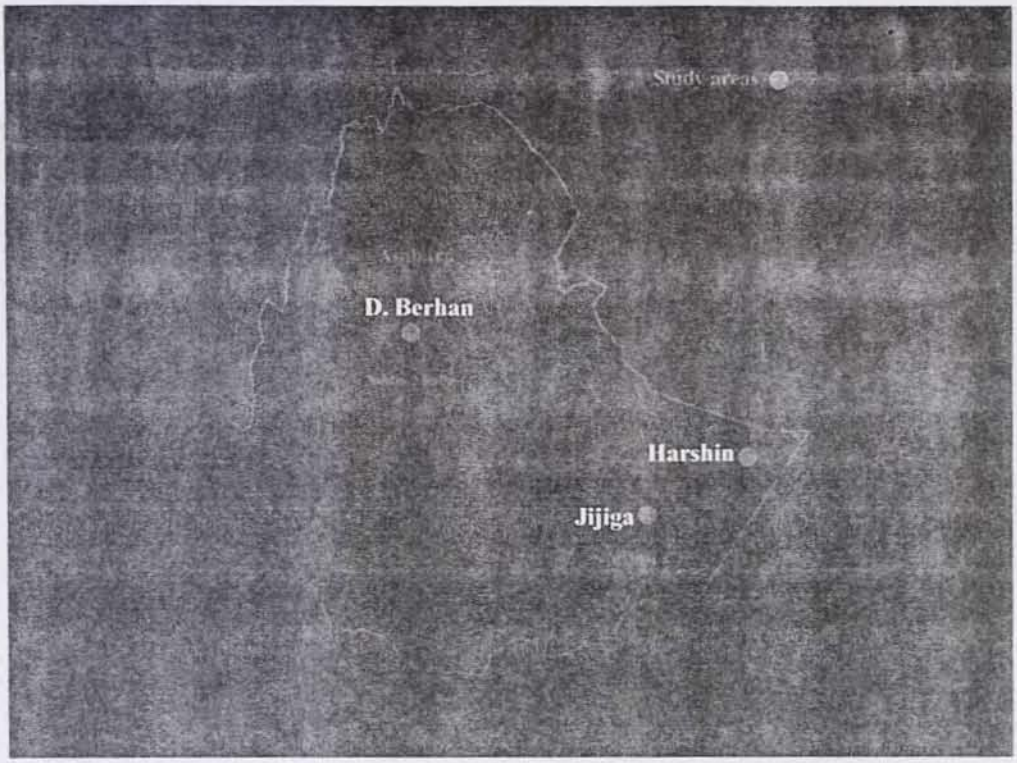
Breeding: 1= unknown/not specified; 2= specified

Twing: 1=yes; 2=no

Mlpurp: 1= fatten and sell, sell without fattening and slaughter 2= breeding

Yfempurp: 1= used for replacement of breeding stock 2= sale and slaughter

7.7. Map of the selected study sites in Ethiopia



7.8 Clinical picture of Sheep Pasteurellosis

Mucoppurulent nasal and ocular discharge

8.CURRICULUM VITAE

1. Full name — Asefa Deressa Hundera
2. Date of Birth — April,1960
3. Place of Birth — Horro
4. Present Research Centre — Sheno/ DebreBerhan
5. Division — Animal health
6. Department — Animal science
7. Occupation — Associate Researcher I
8. Professional Qualification — Doctor of Veterinary Medicine
9. Educational back ground:

Duration	Institution	certified	Field of study
1968-1979	Shambu Junior & Secondary School	Ethiopian School Leaving Certificate	Science
1980-1981	Awassa College of Agriculture	Diploma	Animal Science
1988	Hohenheim University	certificate	Animal Nutrition
1990-1994	Kharkov Zooveterinary Institute	D.V.M.	Veterinary Medicine
2000-2001	Freie Üniversität Berlin, Germany and Addis Ababa University, Ethiopia	Msc in Tropical Veterinary Medicine	Veterinary Epidemiology

10. Experience :

Responsibility	Duration	Service year	Field of work
Technical assistant	July,1981-August,1988	7	Animal Nutrition Laboratory Holotta Research Center
Research Officer II	August,1994-June,1996	2	Animal Health Division Sheno/Debreberhan Research Center
Assistant Researcher II	July,1996- July,1998	2	Same as above
Associate ResearcherI	August,1998-todate	4	Same as above

11. Publication:

Animal Health Research Division progress report for the period April 1996-March 1997; April 1997-March,1998;April,1998-March 1999.

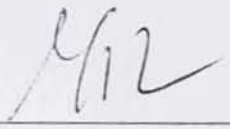
11.2. Technical Papers

1. **Asefa Deressa** and Sissay Lemma (1998): Preliminary investigation on the seasonal occurrence of ovine helminths around Sheno: Proceedings of 5th Annual conference of Ethiopian Society of Animal Production. PP. 128-137.
2. **Asefa Deressa** (1997): Sheep health management issues in North Shewa. Proceedings of the 11th Annual Conference of Ethiopian Veterinary Association. PP. 1-14.
3. Sissay Lemma, Solomon Gizaw, **Asefa Deressa** and Yimam Hassen (1998): Sheep and Goat research in the Amhara region PP. 203-233. In: Beyene Seboka and Abera Deressa (eds.) Agricultural Research and Technology Transfer Attempts and Achievements in Northern Ethiopia. Proceedings of the Fourth Technology Generation, Transfer and Gap Analysis Workshop. 18-21 March 1997, Bahir-Dar, Ethiopia.
4. **Asefa Deressa** (1998): Economic importance of ovine helminth parasites at Sheno. Proceedings of the 12th Annual Conference of Ethiopian Veterinary Association PP. 43-51.
5. **Asefa Deressa** (1998): Some indications of plants that have anthelminitic properties. Ethiopian Veterinary Association News Letter. Vol. 5, NO. 3, pp. 4-5.

Declaration sheet

I, the undersigned, declare that the thesis is my original work and has not been presented for degree in any University.

Name Asefa Deressa Hundera

Signature 

Date of submission 31/01/2002

Epidemiology and strain characterization of ovine pasteurellosis in selected sites of Amhara and Somali Regions, Ethiopia.

Asefa Deressa