ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

SEROLOGICAL, PATHOLOGICAL AND BACTERIOLOGICAL INVESTIGATION OF MYCOPLASMA INFECTION IN GOATS SLAUGHTERED AT ELFORA EXPORT ABATTOIR, DEBRE ZEIT

BY

DANIEL GIZAW DEMISSIE

JUNE 2006
DEBRE ZEIT, ETHIOPIA

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A thesis submitted to the School of Graduate Studies of Addis Ababa University, Faculty of Veterinary Medicine in partial fulfilment of the requirements for the Degree of Masters in Tropical Veterinary Medicine

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Board of Examiners **Signature**

TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXES

ABBREVIATIONS

CBPP	Contagious bovine pleuropneumonia
CCPP	Contagious caprine pleuropneumonia
c-ELISA	Competitive Enzyme –Linked Immunosorbent Assay
CFT	Complement Fixation Test
CI	Confidence interval
CIRAD-EMVT	Centre de Coopération Internationale en recherché Agronomique pour le Dévelopement - Département d'Elevage et de médecine Vétérinaire
CSA	Central Statistical Authority
IgG	Immuminoglobulin G
IU	International Unit
Mab	monoclonal antibody
MAKePS	Mastitis, Arthritis, Keratitis, Pneumonia and Septicaemia Syndrome
Mccp	*Mycoplasma capricolum* subspecies *capripneumoniae*
Mmc	*M. mycoides* subsp. *capri*
Mcc	*M. capricolum* subsp. *capricolum*
MmmLC	*M. mycoides* subspecies *mycoides Large colony*
MmmSC	*M. mycoides* subsp.*mycoides biotype Small colony*
MoA	Ministry of Agriculture
NVI	National Veterinary Institute
OR	Odds Ratio
OIE	Office International Des Epizooties
PBST	Phosphate buffer saline with Tween -20
PCR	Polymerase Chain Reaction
PPR	Pets des Petits Ruminants
SNNP	Southern Nation Nationalities and Peoples Regional State
Subsp.	Subspecies
SRBC	Sheep Red Blood Cell
TBS	Tris buffer saline

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ABSTRACT

A cross sectional study of contagious caprine pleuropneumonia in goats was conducted from October 2005 to March 2006; to determine seroprevalence, pathology and *Mycoplasma* species involved in pneumonic lungs of goats slaughtered at ELFRA export abattoir. Laboratory work was carried out at National Veterinary Institute (NVI). A total of 704 sera were collected from goats that purchased from Awash (n= 224), Dire Dawa (n =200) and Borana (n =280). Using the Complement Fixation Test (CFT) an overall prevalence of 48.3% (95% CI 47.4 -52.1%) was obtained. The prevalence in Awash, Dire Dawa and Barana were 47.3% (95% CI 41.2- 54.3%), 44.5% (95% CI 38.1-51.9%) and 51.8% (95% CI 46-58%) respectively. There was no significant difference ($p>0.05$) in seroprevalence among three origins, as well as no statistical significance ($p>0.05$) among individual ages of goats. A serial c-ELISA was also conducted on CFT positive sera; accordingly an overall prevalence of 11.8% (95% CI 8.8 - 15.7%) was recorded. Seroprevalence in Awash, Dire Dawa and Borana were 17% (95% CI 11.1 - 25.9%), 5.6% (95% CI 2.4 - 13.2%) and 11.7% (95% CI 7.5 - 18.3) respectively. There was significant difference ($p<0.05$) in individual age of goats using c-ELISA. Further more, there was marginally significant difference ($p<0.05$) among the three origins. Lower seroprevalence was recorded in both tests in goats of Dire Dawa origin. Of the 704 lungs of goat examined for gross pathological lesions 94 (13.4%) had lung lesions of which 33 (14.7%), 18 (9.0%) and 43 (15.5%) were from goats that originated from Awash, Dire Dawa and Borana respectively. The prevalence of lung lesions didn't various significantly ($p>0.05$) among their origins. However, prevalence of lung lesions within individual ages of goats was significantly different ($p<0.01$). Of total 94 pneumonic lungs and 4 thoracic fluids cultured on Hayflik's solid and liquid media, *Mycoplasma* was isolated from 15 (16%) pneumonic lung lesions. The *Mycoplasma* species identified includes: - *M. mycoides* subsp. *mycoides* Large colony *(Mmm*LC*), M. agalactiae, M. capricolum* subsp. *capricolum (Mcc), M. capricolum* subsp. *capripneumoniae (Mccp), M. ovipneumoniae* and *M. arginini.* The isolation of pathogenic *Mycoplasma* species of *Mccp* and *M. agalactiae* from apparently healthy slaughtered goats could be an indication of chronic carrier, which might have spread the organisms throughout their way to the slaughterhouse.

Key words. CCPP prevalence, *Mycoplasma*, pathology, CFT, c-ELISA

1. INTRODUCTION

Goats (*Capra hircus*) are thought to have been the first animals to be domesticated for economic purposes. Goats play a crucial role in food production in developing countries. They can easily be sold in terms of urgent need such as sickness, death or the payment of school (Peacock, 1996). Goats are widely distributed and inhabit all climatic zones, with a higher concentration found in dry than humid areas (Ademosum, 1994).

They are well adapted to hot and dry conditions, and mainly due to the fact that in dry zones there is less opportunity for alternative land use. Goats can survive and produce in harsh environmental conditions and on poor quality fibrous feeds. They have a high reproductive performance and are drought resistance (Smith and Sharman, 1994; Peacock, 1996; FARM Africa, 1996).

In Ethiopia, though, there are about 22 million goats; no detail research work was carried to increase their output. Majority of the population do exist in lowland under pastoral production system (CSA, 2003). Animal health is a very important factor in goat production like in all other livestock systems. High economic losses range from death of more than 50% of flock, to slow and progressive isolated cases of mortalities or, increased morbidity (Peacock, 1996). These subsequently reduce production and are associated with weak veterinary services in low land areas. The production and productivity of the goats depend entirely on the health status of the animal, which in turn is affected by husbandry practices, breeding programs, nutrition and stress, where the veterinary infrastructure is scarce. However, little attention has been given to methods of improving their productivity. Inadequate nutrition, health problems, low genetic potential and traditional production systems are major constraints of goat production in Africa (Ademosum, 1994). As the result a number of goats are easily affected by rampant disease in the tropics (Peacock, 1996).

Seventy *Mycoplasma* species or sub species were found to infect goats and sheep (Gourlay, 1981). Among these the causative agent contagious caprine pleuropneumonia (CCPP) and contagious agalatia are the most pathogenic to goats. Although, mycoplasmal diseases are worldwide in distribution particularly contagious caprine pleuropneumonia (CCPP) mostly occur in African countries (MacOwan, 1976; Harbri *et al*., 1981).

Contagious caprine pleuropneumonia (CCPP) is a disease of major economic importance in Africa and Asia, posing a major constraint to goat production (Nicholas, 2002). The direct losses of the disease result from its high mortality, reduced milk and meat yield and cost of treatment, control, disease diagnosis and surveillance. In addition to this, there are indirect losses due to the imposition of trade restrictions. Contagious caprine pleuropneumonia (CCPP) is characterized by 100% morbidity and 60 to 100% mortality. The severity and pathogenicity of the mycoplasmal diseases depends on the simultaneous infection along with other microorganisms such as *Mannheimia. haemolytica* and *Pasteurella multocida* have also been associated with pleuropneumonia in goats, although experimental evidence of their pathogenicity in this host is inadequate (Radostits *et al*., 1994).

The presence of CCPP in Ethiopia has been suspected since 1983. It was confirmed later in 1990 by isolation and identification of F-38 (Thiaucourt *et al*., 1992). Since then the disease is become endemic in different regions of the country. Repeated outbreaks have been occurred in all regions of the country including Tigray, Afar, Dire Dawa, SNNP, Oromiya, Benishangul-Gumz, and Amhara regional states. It is more prevalent in the arid and semi- arid low land area of Rift Valley, Borana rangelands, South Omo, Afar and other pastoral areas and imposes severe loss in goat population.

Sero-prevalence rate from different authors varies 6% to 77% in different parts of the country. The wide variations may be attributed to agro-ecological variation, production system and over crowded stock. The other factor may be the methods used during the sampling procedure, diagnostic test employed as well as the number of animal examined also affect seroprevalence of the diseases. Furthermore, wide variation in seroprevalence could be associated with other *Mycoplasma* species involved in diseases of CCPP. The type *Mycoplasma* species involved and their significance in contagious caprine pleuropneumonia (CCPP) were not fully studied in Ethiopia.

Therefore this study was planned with the following objectives

- To study seroprevalence of contagious caprine pleuropneumonia (CCPP) in goats slaughtered at ELFORA export abattoir
- To study pathological conditions in lungs of goats infected with *Mycoplasma* species
- To isolate and characterised *Mycoplasma* species from pneumonic lungs of slaughtered goats

2. LITERATURE REVIEW

2.1. Mycoplasma

The mollicutes are members of the order Mycoplasmatales and class mollicutes (soft skin) and they are the smallest of the free-living prokaryotes. Mollicutes is the correct name to use when collectively referring to these members of in this order; however, the trivial name mycoplasma is also used (Levinsohn, 1992; Walker, 1999). The members of the genus *Mycoplasma* (class Mollicutes) are the smallest organisms capable of self-replicating. They possess a relatively small genome of 0.6-1.35 mega bases reflecting their drastically reduced biosynthetic capabilities and parasitic lifestyle (Bölske, 1995). *Mycoplasma* DNA is poor in guanine (G) and cytosine (C) ranging from 18-40%, and rich in adenine (A) and thymine (T). They lack the rigid cell wall present in other eubacteria and have an exceptionally small chromosome with low G+C content. All known *Mycoplasma* are parasites, which usually exhibit a rather strict host and tissue specificity, and many of them are of clinical importance in human and veterinary medicine (Bölske, 1995; Walker, 1999).

Mycoplasma comprises 200 species of the class Mollicutes (Nicholas, 2002). Five distinct groups of Mollicutes were identified by phylogenetic analysis of the 16S rRNA sequences. Spiroplasma group contains *Mccp, which* has been subdivided within the *M. mycoides cluster*. *Mycoplasma*s of the mycoides cluster are pathogens of ruminants and comprise six closely related species that are subdivided into two subgroups based on genetic similarity. The capricolum subgroup includes *M. capricolum* subsp. *capricolum*, *M. capricolum* subsp. *capripneumoniae*, and *Mycoplasma* subsp. bovine group 7 (BG7). The mycoides subgroup consists of *M. mycoides subsp*. *capri*, *M. mycoides* subsp. *mycoides small-colony*, and *M. mycoides subsp*. *mycoides large-colony* (Weisberg *et al.,* 1989).

All six species of the *Mycoides cluster* can cause respiratory, arthrial, genitourinary, or mammary disease, although significant host and strain-related variation in virulence exists. *M. mycoides* subsp. *mycoides* small-colony (*MmmSC*), and *M. capricolum* subsp. *capripneumoniae* (*Mccp*) the etiologies of contagious bovine pleuropneumonia (CBPP) and contagious caprine pleuropneumonia (CCPP) respectively, are the most virulent and typically induce fatal systemic disease in their hosts. Other members of the mycoides cluster, though they are pathogenic, they do not usually cause life-threatening disease

instead establish protracted infections that result in low levels of chronic inflammation (Simecka *et al*., 1992). *M. m. mycoides* large colony (*MmmLC*) *and M. m. capri* (*Mmc*) which cause MAKePS and share immunological and biochemical properties in common (Thiaucourt and Bölske, 1996; Nicholas, 2002).

*Mycoplasma*s (mollicutes) formerly called PPLO (Pleuropneumonia like organisms) are non-sporolating, Gram negative, non-motile bacteria, which do not possess a definite shape for cell. They don't possess cell wall and internal membrane structures except plasma membrane however; many strains possess surfaces structure equivalent to a capsule with the exception of *Acholeplasma*. *Mycoplasmas depend* on supply of intact cholesterol, which they incorporate into the membrane creating sufficient osmotic stability for survival under normal physiological conditions. The *Acholeplassma* synthesizes cholesterol as substitute but will incorporate cholesterol if it is provided. *Mycoplasma* polymorphism is the consequence of missing cell well. *Mycoplasmas* are not only devoid of cell well but the genetic capability to produce one that also renders them completely resistant to B-lactam and other cell wall activity drugs. Due to their small size (0.1- 0.3 nm) and their polymorphism they are able to pass through the usual bacteriological filter (0.1-0.3nm). The cell shapes include spherical, pear shaped spiral and filaments forms. Cell sometimes appears as chain beads as result of a synchronized genomic division. Mollicutes poorly stained Gram-negative stain method although they are classified as Gram negative. The preferred stains are Giemsa Castaned Dienes and Methylene blue (Quinn *et al.*, 1994).

The mollicutes grow slowly and generally require 3 to 6 days of incubation before colonies are apparent. Growth is best at 37^{o} C in atmosphere of increased $CO_{2.}$ Sterols are required by all genera except *Acholeplasma* and *Anaeplasma*. Most genera are facultative anaerobes except *Anaeroplasma* and *Asteroplasmas*, which are obligate anaerobes. Optimum pH for growth ranges from 6.0 for Ureaplasma and 7.5 to other mollicutes. Colony size varies from 0.1mm to1.0 mm. When observed with dissecting microscope, many species exhibit "fried egg" morphology. This umbonate appearance is the result of the central portion of the colony embedding into the agar with peripheral zone of surface growth. Some species produce film and spot, which are composed of cholesterol and phospholipids and seen as a wrinkled film on the media surface (Ojo, 1976; Jones, 1992; Adehana *et al*., 2006).

2.2. Mycoplasma Species in Goats

2.2.1. Mycoplsama mycoides subspecies *capri (Mmc)*

Experimental infection performed by Ojo (1976) showed that strains of *Mmc* could be highly pathogenic producing severe pleuropneumonia in large proportions of the experimental goats. Apart from local oedematous reaction at inoculation site the gross lesions were confined to lungs, pleura and pericardium. The lung involvements were mainly unilateral. It is unclear if *Mmc* has been recovered from other body sites in natural disease of goats but mastitis induced experimentally. It is believed that *Mmc* has a clear tropism for lung involvement (Lefevre *et al.,* 1987b; DaMassa *et al.,* 1992)

2.2.2. Mycoplasma mycoides subspecies *mycoides Large colony (MmmLC)*

Mycoplasma mycoides subsp*ecies mycoides* Large colony (*Mmm*LC) has one of the widest geographically distributed ruminant *Mycoplasmas*, being found on all continents (Bölske *et al.,* 1988). Sever pleuropneumonia similar to that of *Mmc* was produced experimentally by *MmmLC* (Ojo, 1976) which has also been isolated from field cases of pleuropneumonia in goats arthritis in kids mastitis in adult goats are also seen in natural diseases (Lefevre *et al.,* 1987b; DaMassa *et al.,* 1992). The gross lesions seen postpartum can be similar to those observed in *Mccp* infection, but the lung lesions include oedema and thinking of the inter lobular septa. There may be septicaemia accompanied by the enlarged spleen and lesions present in several organs. Microscopically the alveoli contained monocular cells oedema and scattered granulocytes, which also filled the bronchioles (Bölske *et al.,* 1988)

M. mycoides subsp. *mycoides* Large colony and *M. mycoides* subsp. *capri* are antigenically very similar as assessed by numerical analysis of one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein patterns. The nucleotide and deduced amino acid sequences of these two lipoproteins showed a very high degree of similarity between these two *Mycoplasma s*pecies. Analysis of the PCR-amplified *lppA* genes with frequently cutting restriction enzymes showed a certain degree of genetic variability, however, did not cluster the two subspecies (Monnerat *et al*., 1999).

PCR therefore allows a rapid identification of *M. mycoides* subsp. *mycoides* Large colony and *M. mycoides* subsp. *capri* but does not distinguish between these two closely related subspecies (Monnerat *et al*., 1999).

2.2.3. Mycoplasma capricolum subspecies *capricolum (Mcc)*

The natural infection can be characterised by MAKePs syndrome including mastitis, arthritis, keratoconjectivities manifestation in varies organ appear after an initial septicaemic a stage which can be fatal for kids and adult animals in poor condition (Perreau and Bread, 1979). The gross pathology is confined to the joints and lungs. The lungs showed interstitial pneumonia and purulent bronchopneumonia. Inter lobular septa were broadened due to oedema and cellular infiltration mainly by mononuclear cells. The alveoli contained increased number of alveolar macrophages and there was marked peribronichical lymphoid hyperplesia (Bölske *et al.,* 1988). Some alveoli and bronchioles were filled with polymorphonuclar and desquamated epithelial cells. *M. capricolum* subsp. *capricolum* was constantly isolated from pneumonic lungs of goats (Bölske *et al.,* 1988).

2.3. Contagious Caprine Pleuropneumonia (CCPP)

2.3.1. History

Contagious caprine pleuropneumonia (CCPP) was first described in 1873 in Algeria by Thomas and known under the local name of "bou frida" because, in the majority of diseased goats, only one lung was affected (Hutcheon, 1889 cited by McMartin *et al*., 1980). Its contagiousness was not initially known because the disease was endemic in most areas under examination, so climatic conditions were thought to be responsible for disease outbreaks. A major outbreak in South Africa in 1881 occurred following the introduction of goats from Turkey (McMartin *et al*., 1980). It is a severe disease of goats caused by *M. capricolum* subsp. *capripneumoniae* (*Mccp*). This organism is closely related to three other *Mycoplasma* species: *MmmLC*, *Mmc*, and *Mcc*. Unlike true CCPP, which is confined to the thoracic cavity, the disease caused by them, is accompanied by prominent lesions in other organs and/or parts of the body besides the thoracic cavity

M. mycoides subsp. *capri* was considered to be the etiological agent of CCPP, before the isolation and identification of strain F38 by MacOwan (1976) and the subsequent demonstration of its causal relationship with F38 strain (MacOwan and Minette, 1976). *Mycoplasma* strain F38 biotype is the only *Mycoplasma* that fulfils Koch's postulates for CCPP and is believed to be the sole cause of CCPP (MacOwan, 1984).

M. mycoides subsp. *mycoides* Large colony has also been isolated from goats with pneumonia. It usually produces septicaemia, polyarthritis, mastitis, encephalitis, conjunctivitis, hepatitis, or pneumonia in goats. Some strains of this agent will also cause pneumonia closely resembling CCPP (Ojo, 1976), but the agent is not highly contagious and is not considered to cause CCPP. *M. capricolum* subsp. *capricolum*, a goat pathogen commonly associated with mastitis and polyarthritis in goats, can also produce pneumonia resembling CCPP but it usually causes severe septicaemia and polyarthritis. This agent is closely related to *Mycoplasma* F-38 biotype but can be differentiated from it using monoclonal antibodies (Rurangirwa *et al*., 1987b).

2.3.2. Etiology

The causative agent of contagious caprine pleuropneumonia (CCPP) is *M. capricolum* subsp. *capripneumoniae* (*Mccp*), which was previously known by the strain name of its type species, F38 biotype (Leach *et al*., 1993).

2.3.3. Clinical signs

The classical disease as caused by *Mccp* is a purely respiratory illness. It is characterised by a fever, of 106^{o}F (41^{o} C), coughing, and a distinct loss of vigour. Affected goats have laboured breathing; later they may grunt or breathe in obvious pain. Frothy nasal discharges and stringy salivation are often seen shortly before death. Acute disease occurred in fully susceptible populations of goats within 7 to 10 days of the onset of clinical signs (Thiaucourt and Bölske, 1996; Mare, 1996).

The clinical signs described for CCPP from different parts of the world have varied enormously. This is not surprising because at least two different *Mycoplasmas* have been regarded as causative agents of the disease. In many field outbreaks, the clinical picture

has probably been further complicated by the presence of viruses and other bacteria (e.g., *Pasteurella* species, PPR) as part of the etiologic agent. A study to correlate clinical signs and early lesions showed that affected goats, died up to a week after contact with affected animals, were free from lung lesions or clinical signs; between two and three weeks after contact, lung lesions were generally small and superficial characterised by hyperemia and oedema with clinical signs being restricted to an infrequent cough; fever was first seen after nearly 4 weeks, which correlated with lung consolidation, the area of which increased as the fever progressed (Wesonga *et al*., 1993).

The disease causes interstitial, fibrinous pleuropneumonia, interlobular oedema and hepatisation of the lung causing high mortality rates of up to 80%. In fully susceptible flocks, morbidity reaches usually 100% and mortality 70 % (McMartin *et al*., 1980). A more chronic form of the disease is often seen in endemic areas and may lead to recovery of a higher percentage of infected animals; many of them become carriers of the *Mycoplasmas*.

M. mycoides subsp. *capri* tends to cause a more generalized infection in which septicaemia is frequently seen. An acute or per acute septicaemia form of the disease involving the reproductive, respiratory and alimentary tracts have been described. In addition, thoracic and reproductive forms of the disease have been attributed to this agent. It is considerably less contagious than *Mccp* induced disease, and the mortality and morbidity rates are also lower (MacOwan, 1976; Wesonga *et al*., 1993).

2.3.4. Pathogeneses

The gross lesions in classical CCPP are confined to the thoracic cavity. Pea-sized yellowish nodules are seen in the lungs in early cases, whereas in more established cases there is marked congestion around the nodules. The lesions may be confined to one lung or both an entire lobe may become solidified. The pulmonary pleurae become thickened, and there may be adhesions to the chest wall (Thiaucourt and Bölske, 1996; Mare, 1996).

In CCPP the lung resembles “somewhat granular looking liver”, which is described as massive hepatisation seen in CBPP lungs. In sharp contrast, *M. mycoides* subsp. *capri* has been reported to cause lesions in a wide variety of organs and to produce lung lesions

closely resembling that seen in CBPP. The generalized lesions described include encephalitis, meningitis, lymphadenitis, splenitis, genitourinary tract inflammations, and intestinal lesions, none of which are a feature of classical CCPP. The lung lesions, which resemble those seen in CBPP, are usually confined to one lung and reflect various stages of fibrinous pneumonia. Extensive pleuritis is usually present, and various stages of hepatisation and marked dilation of interlobular septa is commonly seen. The cardiac and diaphragmatic lobes are the most commonly involved. Some describe this as a mild form of CCPP; others argue that it is not CCPP (MacOwan, 1976; Wesonga *et al*., 1993)**.**

Histological examination of the lung tissues may show acute serofibrinous to chronic fibrino-necrotic pleuropneumonia with infiltrates of serofibrinous fluid and inflammatory cells, mainly neutrophils, in the alveoli, bronchioles, interstitial septae and sub pleural connective tissue. Interlobular oedema is more prominent. Peribronchial and perbronchiolar lymphoid hyperplasia with mononuclear cell infiltration is also present (MacOwan and Minette, 1976; Ojo and Obi, 1996; Wesonga *et al*., 1998).

2.3.5. Epidemiology

Contagious caprine pleuropneumonia is a significant disease of goats characterized by: The disease is readily contagious to susceptible goats; Sheep and cattle are not affected; Local oedematous reactions do not occur in goats and at subcutaneous injection there is no local reaction at the injection site (Hutcheon, 1889 cited by McMartin *et al*., 1980). In Africa where extensive and traditional husbandry is practiced, pathogens spread when animals meet at watering points and grazing areas. Breed and sex appear not to affect the epidemiology of CCPP, but age is an important factor. Though all age groups are susceptible, mortality is higher among young animals than adults (Radostits *et al.*, 1994; Smith and sharman, 1994; Thiaucourt and Bölske, 1996; Wesonga *et al*., 1998; Thiaucourt *et al.*, 2000).

Contagious caprine pleuropneumonia is transmitted by direct contact through inhalation of infective aerosols. Of the two known causative agents, *Mccp* is far more contagious. Outbreaks of the disease often occur after heavy rains after the stress of sudden climatic change (Kusiluka *et al*., 2000).

It is believed that a long-term carrier state may exist. The incubation period can be as short as 6 to 10 days but may be very prolonged (3-4 weeks) under natural conditions (Thiaucourt and Bölske, 1996; Wesonga *et al*., 1998).

In natural infections, susceptible goats acquire the organisms by inhalation of contaminated droplets from infected goats (MacOwan, 1984). The exact distribution of CCPP is not known and there are very few official confirmations of outbreaks. The first reason is that, from a clinical point of view, CCPP can be confused with a number of diseases inducing similar respiratory signs in goats, such as Peste des Petits Ruminants or pasteurellosis. Also, amongst *Mycoplasma* species that induce various syndromes: Mastitis, Arthritis, Keratitis, Pneumonia and Septicaemia (MAKePS) (Thiaucourt and Bölske, 1996). Some peculiar strains may have a specific tropism for the lung. The second reason is that *Mccp* is one of the most fastidious *Mycoplasmas* to be grown in vitro. As a result, isolation trials are often unsuccessful, especially if the conservation of the clinical sample has not been adequate. In addition, other *Mycoplasma* species, such as *M. ovipneumoniae*, may be isolated from CCPP cases, although they are in small number in the sample, simply because they grow faster and more easily. Once isolated, *Mccp* strains may also be difficult to identify as this subspecies belongs to the ‘‘*M. mycoides* cluster’’, which includes six species, or strains (Thiaucourt *et al*., 1992).

The clinical disease has been reported in nearly 40 countries in Africa and Asia, Mccp has only been isolated in 13 countries because few have the facilities for isolating and growing *Mycoplasmas* (Nicholas, 2002). The only African countries where *Mccp* had been isolated are Chad (Lefevre *et al*., 1987a), Eritrea (Houshaymi *et al.,* 2002), Ethiopia (Thiaucourt *et al*., 1992), Kenya (MacOwan and Minette, 1976), Niger and Sudan (Harbi and El-Tahir, 1981), Tanzania (Kusiluka *et al*., 2000), Tunisia (Perreau *et al*., 1984), and Uganda (Bölske *et al*., 1996a) see (Table1). The only reports of suspected CCPP in Europe date back to the 1920s when an outbreak occurred in Greece following the seizure of goats from Turkey. Goncalves (1982) reported a disease in goats in Portugal in 1980, which very closely resembled classical CCPP but from which *MmmLC* was isolated. In 1996 a suspected outbreak of pleuropneumonia clinically resembling CCPP was investigated in herd in England containing some imported goats and which had suffered severe respiratory disease resulting in many deaths. No reports of the isolation of *Mccp* on the American continent although other members of cluster have been described there (Nicholas, 2002).

Incidence of *Mccp* was reported to be 22.2% in sheep and 18.8% in goats (Adehana *et al*., 2006). Ikheloa and his co-workers (2004) recorded a lower incidence of 3.7% in goats.

Table 1. Distribution of CCPP in Africa and Asia

	Confirmed by isolation of Mycoplasma	Clinical disease reported or suspected
Africa	Chad, Eritrea, Ethiopia, Kenya, Níger, Sudan, Tunisia, Uganda. Tanzania	Algeria, Burkina Faso, Benin, Cameroon, Central African Republic, Djibouti, Egypt, Libya, Mali, Nigeria, Somali, Zaire.
Asia	Nepal, Oman, United Arab Emirates, Turkey, Yemen	Afghanistan, Bangladesh, India, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Pakistan, Saudi Arabia, Syria.

Source: (Nicholas, 2002).

2.3.5.1.Contagious caprine pleuropneumonia status in Ethiopia

In Ethiopia CCPP has been suspected to occur for a long period, especially in areas found at the vicinity of endemic areas of Kenya and Sudan. It has been confirmed to be present in Ethiopia since 1980s. CCPP has been reported from almost all regions of Ethiopia including Tigray, Afar, Dire Dawa, SNNP, Oromiya, Benishangul-Gumz and Amhara regional states (Thiaucourt *et al*., 1992; Gezahegn, 1993; Bereket, 1995; Roger and Bereket, 1996; Beyene, 2003; Zenebe, 2004; Yigezu *et al*., 2004; Lisanework, 2005; Solomon, 2005). It is more prevalent in the arid and semi- arid low land area of Rift Valley, Borana rangelands, South Omo, Afar and other pastoral areas of Ethiopia where about 70% of the national goat population are existed. Various authors reported different sero-prevalence rates from different part of the country is presented Table 2.

M. capricolum subsp. *capripneumoniae* induced CCPP-like clinical diseases was not observed in sheep while antibody was quite frequently detected (Mokonnen, 1996; Yigezu *et al*., 2004; Solomon, 2005). There were mixed infection with *MmmLC* and *M. ovipneumoniae*. Sheep are naturally resistant to CCPP diseases. The isolation of *Mccp*, however, proves the role of sheep as reservoir of infection (Yigezu *et al*., 2004).

Table 2. Reported prevalence of CCPP in goats in different parts of Ethiopia (1988-2005)

Area	**Prevalence (%)**	**References**
West Hararghe	50	Mebratu (1988)
East Shoa	51.5	Gezehegn (1993)
Konso, SNNP	35	Bereket (1995)
Arbaminch, SNNP	36	Mokonnen (1996)
Gawane, Afar	33	Roger and Bereket (1996)
Fentale, East Shoa	16	Roger and Bereket, (1996)
Dodota sire, Arsi	8	Roger and Bereket (1996)
Sire Arsi	11	Roger and Bereket (1996)
Borana (Yabelo)	24	Dawit (1996)
East Shoa	17.5	Teshome (1997)
Dire Dawa	6	Beyene (2003)
In different regions of Ethiopia	52.7	Yigezu *et al*. (2004)
Dire Dawa	0.56	Zenebe (2004)
Hashim Nur export abattoir	31	Lisanework (2005)
South Omo and Gamo Gofa, SNNP	16.69	Solomon (2005)

2.3.6. Diagnosis

2.3.6.1. Field diagnosis

A highly contagious disease occurring in goats and characterized by severe respiratory distress, high mortality, and post-mortem lesions of fibrinous pleuropneumonia with pronounced hepatisation and pleural adhesions warrants a field diagnosis of CCPP.

In the field, definite diagnosis of can't be established on clinical signs or on post-mortem examinations alone. In classical acute CCPP, a high mortality and typical early thoracic lesions in goats are highly indicative of *Mccp* infection, but all cases of caprine mycoplasmosis need additional laboratory tests to establish a presumptive diagnosis. It may be difficult to distinguish CCPP from an infection by *MmmLC or MmmSC*, which

have a pulmonary location. In the case of *MmmLC* infection, thickening of the interlobular septa may be evident. These lesions are similar to that observed in the case of CBPP. Sometimes the thickening is absent or inconspicuous and laboratory confirmation is needed. Recently, sequestrum in the lungs of goats infected with *MmmSC* had been described (Kusiluka *et al*., 2000).

From a dead animal that had severe clinical disease; the best specimens to be taken are thoracic fluids, affected lung, swabs of major bronchi, and tracheo-bronchial or mediastinal lymph nodes. Samples should be collected aseptically and if possible, placed in transport medium (heart infusion broth, 20% serum, 10% yeast extract, benzyl penicillin at 250 to 1000 IU/ ml), and kept cool and shipped on wet ice as soon as possible. If transport to the laboratory is delayed more than a few days, samples may be frozen. Blood can be collected for serum (Quinn *et al*., 1994). Diagnosis must be confirmed by isolation of the *Mccp.* The causative agent, once isolated, can be identified by immunofluorescence or by growth or metabolic inhibition tests (Thiaucourt *et al.,* 1992).

2.3.6.2. Serological tests

Several serological tests can be used in the laboratory for the detection of antibodies to the *Mccp*. Antibody detection is not very useful for the detection of a new out break. Most goats die in the acute stage before they have developed antibodies. It is also difficult to rely on serology for the member of *M. mycoides cluster* in goat due to frequent occurrence of cross-reacting of antibodies (Bölske *et al*., 1988). IgM is the first to appear and has little specificity, which gives rise to pronounced cross-reaction and remain in the blood for short duration while IgG is produced lately and lasts much longer than IgM and also more specific than IgM antibody isotypes (Staak *et al.,* 2001).

Serological tests used: - The Complement Fixation Test (CFT) is the most widely used serological test for the diagnosis of CBPP. In CCPP the CFT is the recommended test for trade to detect *Mccp* infection and it has been found to be more specific, though less sensitive than indirect hemagglutination (IHT) test (MacOwan, 1976; O.I.E., 2000). In comparison of blocking ELISA using monoclonal antibody and CFT using *Mccp* and MmmSc antigen, blocking ELISA was found more sensitive to detect CCPP antibody (Sharew *et al.,* 2005). The latex agglutination test uses latex beads sensitised with the

polysaccharide produced by *Mccp* in culture or supernatant in a slide agglutination test. The use of the more defined antigen such as the polysaccharide provides greater sensitivity without cross-reactivity with sera against the other three principal caprine *Mycoplasmas*. Latex agglutination test used to detect early infection while CFT detects serological response (Schaeren and Nicolet, 1982; Rurangirwa *et al*., 1987a; Quinn *et al*., 1994; March *et al.*, 2000; March *et al.*, 2002). Litamoi *et al.* (1989) found the slide agglutination test was more sensitive than CFT.

The direct and indirect fluorescent antibody tests are one of the most effective, simple and rapid serological methods of identification for most *Mycoplasma* species. Several forms have been described; the most commonly used one is the indirect fluorescent antibody (IFA) test, which is applied to unfixed colonies on agar (Rosendal and Black, 1972). Imuunobinding on membrane filtration (MF dot) and dot blot on nitrocellulose paper are used to identify species of *Mycoplasma*. They are rapid ready standardised and allow running many samples at time (Poumarat *et al.*, 1991; Poumarat *et al*., 1992).

Competition enzyme- linked immunosorbent assay (c-ELISA): is a newly developed test that permits the specific detection of antibodies in animals, which have been affected by CCPP. This test is based on the use of a monoclonal antibody (Mab) that compete with goat antibodies to bind to the antigen that is coated on the plates. The specificity of the test depends on the epitope, which is recognized by the Mab. Analysis of sera from field cases has shown that sero-conversion did not occur whatever test was used. In the case of c-ELISA, the percentage of positive animals in affected herds varies between 30% and 60%. Hence tests are used to test at herd level than individual level. All current tests for the detection of *Mccp* with the exception of the PCR; show cross-reactivity with Bovine sero-group7 including a monoclonal antibody based ELISA (Thiaucourt *et al.*, 1994).

2.3.6.3. Isolation and biochemical characterization

Wild strains should be passed and preferably cloned, three times before identification is attempted. Colony morphology and growth characteristics may be of some help for the differentiation of *Mycoplasmas*. Biochemical characterisation and growth inhibition with known anti sera are the main tools to identify the species of *Mycoplasma*, although, more modern techniques like PCR and restriction enzyme analysis could reveal the confirmative

diagnosis (Bölske *et al*., 1996b). Biochemical tests cannot identify an isolate unequivocally, which at present can only be done by serological or genetic means. Intraspecies variation in some biochemical reactions is often considerable, but some tests perform a useful function both as a preliminary screening system and in providing supportive data for serological findings. In some studies the *M. mycoides* strains were subjected to biochemical studies using sensitivity to digitonin, phosphatase activity, protein digestion, arginine, 2,3,5-tetrazolium chlorides, and glucose. All the strains were found to be digitonin sensitive, indicating that they require cholesterol for growth (Freundt *et al.,* 1974; Bölske, 1995; Nicholas, 2002).

Glucose fermentation, arginine hydrolysis and film and spot fermentation tests are performed routinely in isolation and cultivation procedures. The member of the *M. mycoides* cluster are typically glucose fermenting and unable to hydrolysis arginine. However, certain strains of *Mccp* appear not to metabolise sugars and depend up on organic acid as energy source (Abu-Groun *et al.,* 1994). The detection of the glucose fermentation by the pH change in broth medium generally takes two to three days, thus in concentration of biochemical tests for glucose fermentation and arginine hydrolysis, almost all *M. mycoides* cluster strains appear similar. Glucose breakdown is indicated by acid (yellow) changes, and arginine hydrolysis by alkaline (red) changes in broth media, using phenol red as indicator (Cottew, 1979).

Arginine use cannot be assessed on conventional medium for isolation and culture, as the media for testing the arginine diaminase pathway should contain high concentrations of arginine and no glucose. Arginine hydrolysis pathway involves three enzymes reaction; arginine is converted by arginine diaminase into citrulline and ammonia and this is followed by conversion of citrulline plus phosphate ion into orthinine carbamyltrasfferrase (= orthine transcarbamylase). Finally a phosphate group is transferred from carbamyle phosphate to adenosine diphosphate to form adenosine triphosphate with the concomitant release of carbon dioxide and ammonia by carbamate kinase (Sjostrom *et al*., 1986). Arginine hydrolysise detected by this method in most strain of the *M. capricolum* subsp. *capricolum* not in any other strains in the *M. mycoides* cluster (Tully *et al.*, 1979; Jones, 1992)

Film and spots describes an apparent wrinkling of the agar surface due to deposition of an iridescent film of lipid, together with the development of black spots within the medium in the vicinity of ageing colonies. This phenomenon produced by three *Mycoplasmas* of small ruminants, is demonstrated on agar media containing 20% or more serum, preferably of horse or pig origin. Supplementation of the medium with 10% egg yolk emulsion improves the sensitivity of the test. The remaining biochemical tests require specific media or reagents. The test for tetrazolium reduction provides corroborative evidence of the *Mycoplasma* nature of *M. agalactiae* isolates, as this organism is neither glycolytic nor arginine hydrolysing (Cottew, 1979).

Serum digestion distinguishes members of ruminant *Mycoplasmas* species belong to *M. mycoides* cluster with exception of *MmmSC* type can be distinguished from other *Mycoplasmas* by their proteolytic activities utilized in the test of liquefaction of insipisissated horse serum. This feature is measured by the serum digestion test or casein digestion test (Cottew and Yeast, 1978) is the most important for the practical differentiation of *MmmSC* and *MmmLC* types of *M. mycoides* subspecies *mycoides* in diagnostic work. Phosphatase production separates *Mccp* from other members of this cluster. Digitonin sensitivity distinguishes members of the order *Mycoplasmatales* from those of the order *Acholplasmatales* (Bölske, 1999; Nicholas, 2002).

Table 3. Biochemical reactions of the *Mycoplasma* of goats and sheep

Strain	Glucose fermentation	Arginine hydrolysis	Tetrazolium aero/ana	Film& spots formation	Phosphatase activity	Casein digestion	Degitonin sensitivity
Mccp	+/-	-	-/+ W/+v	-	-	+	+
MmmLC	+	-	+(+)	_	-	+	+
Mmc	+	_	+(+)	-	-	+	+
Mcc	+	+	+(+)	-	+	+	+
M.ovipneumoniae	+	-	W (+)	-	-	-	+
M.arginini	-	+	-(+)	-	-	-	+
M.agalactiae	-	-	+(+)	+	+	-	+
M. putrefaceiens.	+	-	V	+	+	-	+
M. conjuctivae	+	-	-(+)	-	-	-	+

Source: (Nicholas, 2002) V = variable, + = positive, - = negative, w = weak, aero = aerobic, ana =anaerobic

2.3.6.4. Nucleic acid recognition methods

Diagnostic systems based on Polymerase Chain Reaction (PCR) have been developed for the rapid detection, identification and differentiation of members of the *M. mycoides* cluster and the specific identification of *Mccp*. Polymerase Chain Reaction is used to amplify a conserved segment of the 16S rRNA gene of the mycoides cluster. Ribosomal RNA offers the likelihood of a finer identification of the strain circulating in a region, further more, H2 of determining the geographic origin of the strain. This tool can make use of full contribution to understanding the epidemiology of the CCPP (Lorenzon *et al*., 2002). The PCR product is analysed by restriction enzyme cleavage for the identification of the *Mccp* amplicon. The test is used directly on clinical materials such as lung tissue and pleural fluid. A DNA probe that differentiates *Mccp* from others was developed. However, isolation of *Mccp* remains the confirmatory test (Taylor *et al*., 1992; Bashiruddin *et al*., 1994; Bölske *et al*., 1996b; Stakenborg *et al.*, 2005).

2.3.7. Control

2.3.7.1. Treatment

Successful treatment varies with affected site and time course of disease. Commonly employed antibiotic includes tetracyclines, tylosin, spiramycin, erythromycin, and tiamulin fumarate. In early treatment the prognosis is good (Walker, 1999). The prognosis for recovery with prompt treatment is approximately 87% and then animals recovered from clinical diseases may remain carrier (Onoviran, 1974; El Hassan *et al*., 1984). However, Rurangirwa *et al*. (1981) in their study indicated that a single dose of 20, 30, 40 or 50-mg/kg body weight of the dihydrostreptomycin sulphate led to the recovery of the treated goats. The recovered goats did not transmit CCPP to susceptible goats housed with them for 2 months. Chakarabarti *et al*. (2001) had found tylosin with Bidanzen could bring recovery of 83.5% and tylosin with Bromhexine yielded 91.6% response. They concluded that Bidanzen and Bromhexine could be used against mycoplasma pneumonia as supportive treatment for better and quick recovery. Bereket (1995) reported that after injection of oxytetracycline and streptopenicillin to infected goats for three consecutive days bring recovery with minimal pathological lesion. Treatment is ineffective after long period of infection (Wesonga *et al*., 1993).

2.3.7.2. Vaccination

Vaccine inactivated with saponin that protects goats against CCPP for approximately a year has been developed in Kenya. A single immunisation with the optimum formulation of 0.15 mg of *Mycoplasma* in saponin gives a protective immune response in goats that lasted for longer than one year (Rurangirwa *et al*., 1987c). It was demonstrated that using sonicated *Mccp* antigens in Freund's complete and/or incomplete adjuvant could induce protective immunity in goats. The immunity was present for at least six months (Rurangirwa *et al*., 1984).

National Veterinary Institute presently manufacturing inactivated vaccine adjuvated with saponin from *Mccp* strain but the current vaccine supply is found to be far below the requirements of the country. The annual average production capacity of the institute in thousands while the national demand is in million (Gelagay personnel communication).

3. MATERIALS AND METHODS

3.1. Study Site

The study was conducted at ELFORA export abattoir, which is located in Debre Zeit and is engaged in export of red meat of goats and mutton. Debre Zeit is located at a distance of about 45km South East of the capital, Addis Ababa.

Goats slaughtered at the abattoir were purchased from Eastern and South Eastern part of the country as far as 800Km mainly from Borana, East Shoa, Arsi, Bale, Awash, Dire Dawa, East and West Hararghe, Somali region, and Afar. Awash, Dire Dawa and Borana were purposely selected based on existing diseases prevalence and availability goats slaughtered through out the study periods. ELFORA has holding station at each site that purchased goats from local market of the surrounding areas. Goats were marked with respective identification marks of the sites.

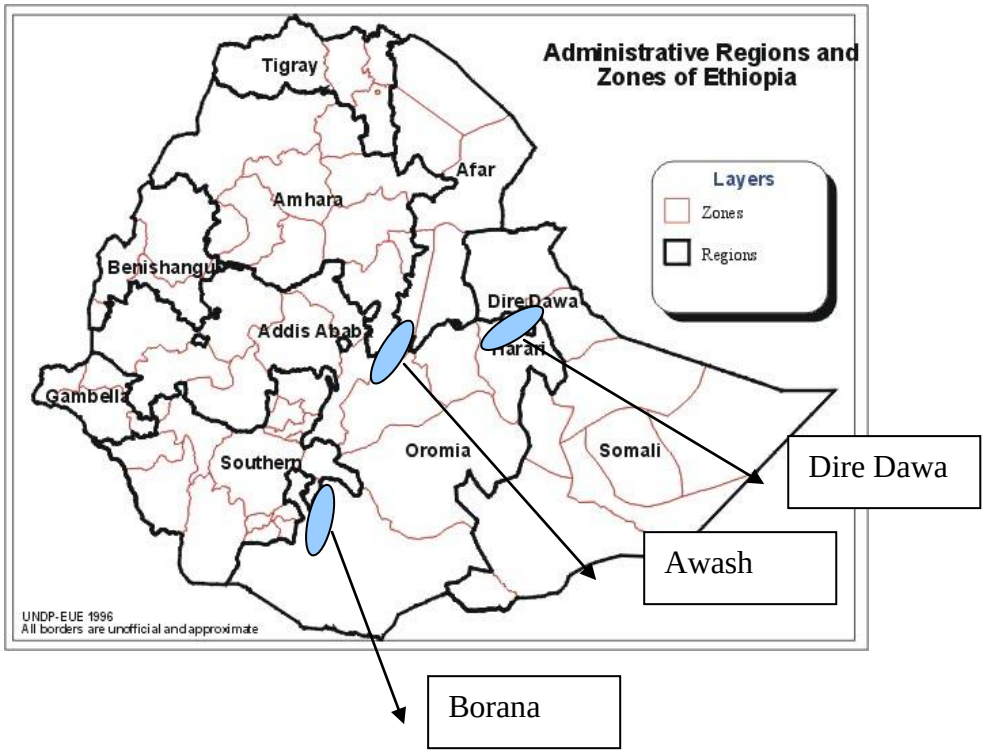

Figure 1 Map of location of origin of goats for study

3.2. Study Population

The animals were local breed of goats that originated from Borana, Dire dewa (West Hararghe, East Hararghe and Somali region) and Awash (East Shoa, Arsi and Afar). Only male goats are slaughtered. Over 100,000 goats are slaughtered annually in the export abattoir.

3.3. Sample Size Determination

Sample size for serum collection was determined using the formula given by (Thrusfield, 1995) for simple random sampling method.

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

(Where n = sample size, P_{exp} = expected prevalence, d = absolute precision). At 95% confidence interval level with expected seroprevalence 31% (Lisanework, 2005) and an absolute precision of 5% a sample size was 329. To increase precision the sample was (329 x 2 = 658) additional 46 goats were sampled. A total of 704 male goats were used for study. Samples for culture were determined based on the capacity to process in the six-month study periods.

3.4. Study Methodology

Cross-sectional study design was under taken to find out the prevalence of CCPP and involvement of *Mycoplasma* species in CCPP like lung lesions and thoracic fluids of goats slaughtered at ELFORA export abattoir, Debre Zeit.

A repeated systematic simple random sampling technique was used to determine the unit that included in samples for study. First one day of the week was selected followed by animal selection. The numbers of goats in lairage were counted and divided to the number of sample intended for the day. Then every n^{th} goat was selected until sample for day completed.

Goats were identified for their area of origin, age, sex then ear tagged at same time blood was collected. They were kept in separate pen for the next morning to conduct post mortem examination on lung.

The origins of goats were determined by mark made by respective marketing site of export abattoir at each site (Awash, Dire Dawa and Borana). The goats were transported from the source to the abattoir by truck. The age of goats were determined by their dentations according to Mike (1996) as indicated in Annex 1.

3.5. Samples Collection

3.5.1. Serum samples

Five to ten millilitre of blood was collected from jugular vein using sterile vacutainer tubes and needles. Then allowed to stand in slant position for 6 to 8 hours. The serum was separated and transferred to sterile tubes. Labelled with specific numbers corresponding to other data in the record book and stored at -20ºC until they were evaluated by CFT and c-ELISA.

3.5.2. Gross pathological examination

The goats from which blood samples were collected were separated in one pen and examined. Each of lungs was thoroughly examined for any gross pathological lesions and findings were recorded in respective record sheet of blood collection. Part of lung, extent of lesion and whether fibrosis or adhesions with thoracic fluids exist were recorded.

3.5.3. Tissue sampling for *Mycoplasma* isolations

About $3cm^2$ sections of hepatised lung were excised from the interface between consolidated and unconsolidated areas then kept in sterile screw cupped glass tube and transported with icebox to the laboratory for *Mycoplasma* isolation. The tissue samples were labelled. Isolations were conducted at National Veterinary Institute (NVI), Bacteriology section.

3.5.4. Thoracic fluids

About 10ml pleural fluid was collected in sterile screw capped bottle using sterile syringe and needles during post mortem examination. The samples were transported immediately to the laboratory and cultured in both Hayflick's broth and solid media for *Mycoplasma* isolation. Parts of the samples were stored at -20ºC until evaluated by dot blot using *Mccp* antigen.

3.6. Sample Processing

3.6.1. Cultivation of samples

Modified Hayflick's medium was used to cultivate *Mycoplasma* species. The media was prepared as described by Jones and woods (1988) as shown in Annex 2.

Shortly lung tissue samples were chopped with scissors and smashed in 1ml sterile saline solution, using a mortar and pestle and 0.5ml of the suspension was diluted into broth an equal amount the suspension was dispensed on solid medium, and broth culture was incubated at 37ºC where as solid medium incubated at 37ºC with 5% CO_2 humidified atmosphere.

The clotted pleuritic fluids were shacked gently and 0.5 ml delivered into 4ml mycoplasma medium. The mixture was agitated gently and cultured on Hayflik's medium after three blind serial passages were done, 0.1ml of the sample was simultaneously dispensed on solid media. Broth cultures were incubated at 37ºC, while agar plates were kept in an incubator at 37ºC of 5% CO_2 with humidified atmosphere.

Growth of mycoplasma was monitored for several days. Broth cultures were examined daily for colour change or turbidity, to compare growth of *Mycoplasma* species in cultivated medium and in inoculum free controls. Cultures suspected of contaminations, those showing turbidity within two days, were passed through a 0.45µm membrane filter before sub culturing. Passages were made after 3-10 days of incubation, whether there were colour change or not (i.e. blind subculture).

The rate of growth of *Mccp* organism was compared only with that of *M. ovipneumoniae.* All other members of the mycoides clusters grew within 3 and 4 days producing bigger colonies (1 to 3mm in diameter). The colonies of *Mccp* have observed after 4 to 5 days incubation and would be seen by close observation with a binocular microscope. *M. ovipneumoniae* was suspected when the colonies lack the classic "fried egg" appearance and didn't stick to the agar surface. Solid media were examined every day using a stereomicroscope with 25 to 50x magnification. Blocks bearing colonies were excised with sterile scalpel blade and transferred to new broth and/ or agar medium by pushing the block over the medium to make subculture. After third passage transfer of a single colony, which had specific morphological structure was done to make cloning.

Fifteen isolates of *Mycoplasma* samples were sent to CIRAD–EMVT, France for further analysis. The isolates lyophilised in five millilitre bottles. The lyophilised culture were packed and sent according to the procedure of the CIRAD-EMVT, France.

3.6.2. Immunobinding on Nitrocellulose paper (Dot- blot)

The procedure of immunoblotting for *Mccp* detection from thoracic fluids and lung tissue were adapted from Poumarat *et al*. (1992). Materials required are given in Annex 3. Shortly, five micro liters of the thoracic fluids and tissue suspension were poured on the tip of the nitrocellulose (NC) paper. The papers was incubated for 10 minutes at 37^0C and washed with TBS for 3 minutes. Then it was immersed into 200µl blocking buffer per well and kept for 30 minutes with slow agitation. Then 200µl monoclonal antibody in aliquots was transferred to each well and incubated at room temperature for another 30 minutes, with slow agitation. After washing three times in 250µl of TBS, 200µl (peroxidase labelled goats anti rabbit and anti mice IgG conjugate) was added per well. The paper was again kept in it for 30 minutes with slow agitation. The washing steps were repeated three times as above. Finally 150µl of developing solution (3% of H_2O_2 and chromogen) was transferred per well and incubated for two minutes, rinsed in distilled water and visually observed. A red dot on the tip of the NC paper was considered as positive reaction and ranked according to the colour intensity, (+++) strong, (++) average, (+) weak, and (-) negative or no reaction.

3.6.3. Biochemical characterisation of *Mycoplasma* isolates

The biochemical identification media were prepared according to Cottew (1979) as indicated in Annex 4. This technique was applied after the *Mycoplasma* isolates were cloned through consecutive subculturing. For each samples two millilitres of the biochemical identification media were dispensed in four test tubes. After agitation of the broth culture, 0.1ml of the suspension were transferred into the four test tubes. For tetrazolium reduction test the samples were inoculated deep into the media aerobic conditions and over laid by paraffin in anaerobic to identify the multiplication. Serum digestion tube was seeded in slant position and followed for digestion of serum. The inoculated media were incubated and checked daily for colour changes. The test results are shown in Table 4. Phosphatase activity was determined by dropping sodium hydroxide after growths of culture were seen.

Table 4. Interpretation of the biochemical reactions of *Mycoplasma* species

S/n	Test	First colour	Positive reaction	Negative reaction
1	Glucose fermentation	Pink red	Yellow	First colour
2	Arginine hydrolysis	Red	Deep red	"
3	Phosphotase activity	Yellow	* Red	"
4	Tetrazolium reduction			
	Aerobic	Yellow	Pink on the surface	"
	Anaerobic	"	Pink on the bottom	"

*When NaOH is added.

3.6.4. Serological tests

3.6.4.1. Complement Fixation Test (CFT)

The OIE standard test procedure of the test (O.I.E, 2000) was adapted. Test sera including positive and negative control were decomplemented in hot water bath at 60^0 C for 30 minutes. Series of two fold dilutions of test sera were pipetted 25µl per well. The first two columns were left for control positive and negative sera, complement and sheep red blood cells (SRBC) and 25µl (1:20) antigen was added into each well, except in rows reserved serum anti complementary activity (antigen preparation, Annex 5).

It was agitated and incubated for 30 minutes. 25µl of titrated complement were added into the wells of test sera. It was incubated at 37^0C under constant agitation on orbital shaker for 30 minutes. 2% one-day-old SRBC was prepared by washing three times with VCM and centrifuged at 2500 rpm for 5 minutes for each washing. An equal volume of diluted Amboceptor (SRBS antisera) (1:1000) was added to sensitise the SRBC, which brings hemolytic system to 1% instead of 2%. 25µl sensitised SRBC (indicator) was pipetted into each well and the plates were sealed to avoid evaporation and then incubated at 37^0C for 30 minutes with constant agitation. The plates were examined for sedimentation and haemolysis. Then the plates were kept at refrigerator at $+4^0$C over night in order to allow non-lysed cells to settle (for interpretation Annex 6).

Table 5. Plat lay out for CFT

	1	2	3	4	5	6	7	8	9	10	11	12
A	NS	PS	1*	2*	3*	4*	5*	6*	7*	8*	9*	10*
B	NS	PS	1	2	3	4	5	6	7	8	9	10
C	NS	PS	11	12	13	14	15	16	17	18	19	20
D	NS	PS	11	12	13	14	15	16	17	18	19	20
E	HS	Cc	21	22	23	24	25	26	27	28	29	30
F	HS	Cc	21	22	23	24	25	26	27	28	29	30
G	HS	Cc	31	32	33	34	35	36	37	38	39	40
H	HS	Cc	31	32	33	34	35	36	37	38	39	40

* Serum anticomplementary HS haemolytic control

NS negative serum control Cc complement control

PS positive serum control

3.6.4.2. Competitive ELISA (c-ELISA)

The serological kit was provided by CIRAD-EMVT, France and the test was done according to guidelines of manufacturers. All wells of a micro plate were charged with 50µl of PBS. Each well was coated with 50µl of antigen (*Mccp* lysed with 1% SDS at 60^o C for 30 minutes) at dilution of 1:100. It was incubated in moist chamber incubation at 37^0C over night. Free antigens were removed by washing it 2 times with PBST for three minutes.

100µl of dilution buffer was dispensed into each well of pre-plates. 11µl of the three control samples CP++ in B1, B2, C1, and C2 and CP+ in D1, D2, E1, E2 and negative control in H1and H2. 11µl test serums were dispensed in all other wells (A3 to H12). *M. capricolum* subsp. *capripneumoniae* specific monoclonal antibody diluted in 1:250 in dilution buffer and 110µl was dispensed to each well except A1 and A2 but in these two wells only 110µl of dilution buffer was added, this after called conjugate control. Test sera with monoclonal antibody in pre-plate with 96 wells were incubated for an hour at 37^0C in constant shaker. The plates were emptied manually and washed two times and dried on towels. Conjugate was diluted in 1:50 in PBS and 100µl was dispensed in to each well. The plates were sealed and incubated for thirty minutes at 37^0C under constant agitation. The content of plates were emptied manually and washed three times with washing solution. 100µl of revelation solution 3 or substrate was dispensed in to each well then plates were incubated under shelter of light with constant agitation at 37^0C for 30 minutes. 100µl of stop solution was added into each well and shacked well until coloured solution was homogenized. The photometer was first blanked on air and then the plates were read at optical densities of 450 nm (Annex 7).

Table 6. Plate lay out for competitive ELISA for *M. capricolum* subsp. *capripneumoniae*

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cc	Cc	1	2	3	4	5	6	7	8	9	10
B	Cp++	Cp++	11	12	13	14	15	16	17	18	19	20
C	Cp++	Cp++	21	22	23	24	25	26	27	28	29	30
D	Cp+	Cp+	31	32	33	34	35	36	37	38	39	40
E	Cp+	Cp+	41	42	43	44	45	46	47	48	49	50
G	Mab	Mab	51	52	53	54	55	56	57	58	59	60
F	Mab	Mab	61	62	63	64	65	66	67	68	69	70
H	CN	CN	71	72	73	74	75	76	77	78	79	80

Cc = conjugate control Cp++ = strong positive control, Cp+ = weak positive control

Mab = monoclonal antibody control, CN = negative control

1= Sample n^{o}1, 2 = Sample n^o 2, 3 =….

Percentage of inhibition of each serum was calculated as fellows

Percent inhibition (PI) =100x[(OD cm-OD test)/(OD cm- OD Cc)]

Where = OD Mab = optical densities mean of Mab, OD_{Test}= optical densities of test serum, OD Cc= optical densities of conjugate control.

4.7. Data Analysis

The data were recorded in Microsoft excel spreadsheet, and descriptive values and 95% confidence interval was analysed using Winepiscope 2.0 (Thrusfield *et al.,* 2001). The risk factor for CCPP seropositivity or lung lesions was analysed using biivariate and multivariate logistic regression in STATA 7.0 software (STATA Corporation, 2001). A probability value of less than 0.05 was considered as significant.

4. RESULTS

4.1. Seroprevalence

4.1.1. Seroprevalence of CCPP using CFT

A total of 704 sera were collected from goat that originated from three different areas namely Awash, Dire Dawa and Borana. All samples were tested using CFT for the serum antibody against CCPP infection with *Mccp* antigen. The results are given in Table 7. The overall prevalence was 48.3% (95% CI = 47.4 – 52.1). The seroprevalence using CFT was highest in Borana (51.8%) and lowest in Dire Dawa (44.5%). There was no significant difference in seroprevalence by area of origin ($p>0.05$).

Table 7. Prevalence of CCPP using CFT in goats slaughtered at ELFORA export abattoir by area of their origin

Origin of goats	Sample tested	Positive sample	Seroprevalence (%)	95 % CI
Awash	224	106	47.3	41.2 -54.3
Dire Dawa	200	89	44.5	38.1 -51.9
Borana	280	145	51.8	46.3 -58.0
Total	**704**	**340**	**48.3**	**47.4 - 52.1**

Pearson χ^2 (2) = 2.60 P = 0.27

Relatively higher seroprevalence (51.7%) was observed at 2 years of age; however the difference in seroprevalence between the difference ages was not significant (P>0.05).

Table 8. Prevalence of CCPP using CFT in goats slaughtered at ELFORA export abattoir by individual ages

Age estimated in year	Samples tested	Positive samples	Prevalence (%)	95 % CI
1	134	61	45.52	37.8 - 54.8
2	236	122	51.69	45.7 - 58.5
3	128	58	45.31	37.5 - 54.8
4	111	56	50.45	42.0 - 60.7
5	95	43	45.26	36.3 - 56.5
Total	**704**	**340**	**48.3**	**47.4 - 52.1**

Pearson χ^2 (4) = 2.52 p = 0.64

Ages of goats were categorized as indicated in Table 9. Statistically there was no significant difference (P>0.05) between different age categories.

Table 9. Seroprevalence of CCPP in goats slaughtered at ELFORA export abattoir using CFT against age category

Category of age in years	Sample tested	Samples positive	Seroprevalence %	95%CI
1 (1 ≤ 2)	370	183	49.46	44.6 – 54.8
2 (2 ≤ 4)	239	114	47.70	41.8 – 54.5
3(> 4)	95	43	45.26	36.3 – 56.5
Total	**704**	**340**	**48.3**	**47.4 - 52.1**

Pearson χ^2 (2) = 0.58 p = 0.747

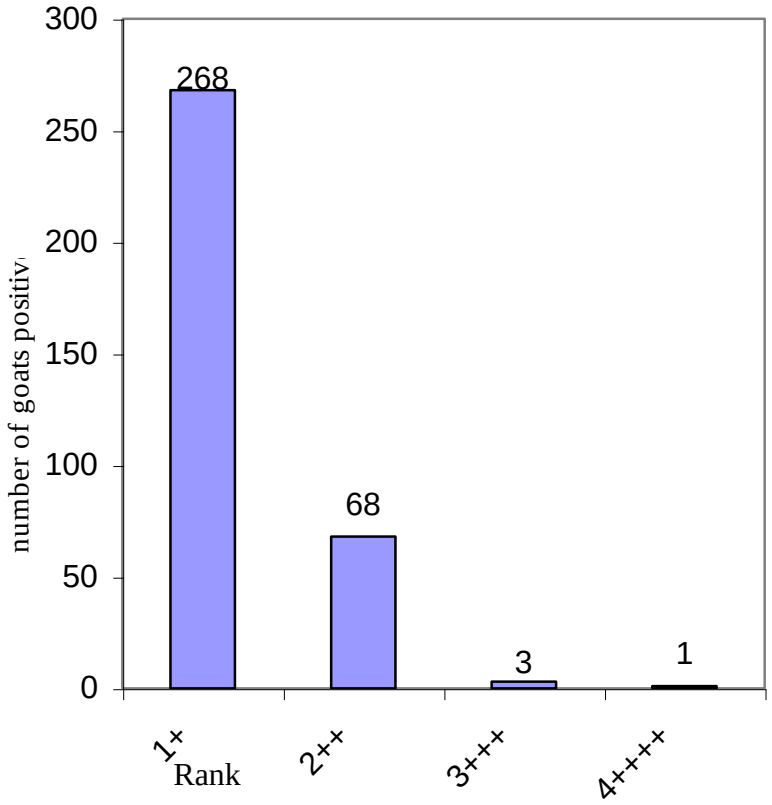

Figure 2 Rank of CFT positive goats sera

Of the total 94 pneumonic lung lesions only 50 (53.2%) were positive by CFT detection for *Mycoplasma* antibodies. while the rest were 44 (56.8%) pneumonic lungs were negative. Eventhough, characteristic of CCPP- like lesions were observed become negative by CFT antibody detection. There was positive correlation between CFT and Lung lesion (r = 0.0385).

4.1.2. Seroprevalence using competitive ELISA

Those sera that were positive in CFT were subjected to *Mccp* specific monoclonal antibody based c-ELISA. There were four categories of the test result based on the percentage of inhibition (PI), Negative, Doubtful, Weak and Strong positive corresponding to the percent inhibition of less than 20%, 20-25%, 25-55% and above respectively. Weak and strong test sera were considered as positive while the doubtful once were left from the statistical analysis. 340 goats sera that were positive in CFT subjected for c-ELISA.

The prevalence using c-ELISA was 11.8% (n = 40) with 95% CI (8.8 – 15.7%). While those in the doubtful ranges (11) samples were considered as negative and were not used for statistical analysis.

There was significance difference ($p<0.05$) between areas of goats' origin. Low prevalence was observed in goats that originated from Dire Dawa (Table 10). Using biviariate logistic regression c-ELISA and origin. The goats that originated from Awash compared with goats that originated from other origin variation occur with goats of Dire Dawa origin (OR =0.291 P = 0.019 and 95% CI = 0.10 - 0 .82). Goats of Dire Dawa origin when compared with goats that originated from Awash and Borana significant variation occurred with goats of Awash (OR = 3.43 P = 0.019 and 95% CI = 1.22 – 9.67). However, there was no significant variation between goats that originated from Borana compared with goats from Awash (OR = 0 .65 P = 0.237 and 95% CI 0 .317 - 1.328).

Table 10. Seroprevalence of CCPP in goats slaughtered at ELFORA export abattoir using c-ELISA by their area of origin

Origin of goats	Samples tested	Samples positive	Prevalence (%)	95% CI
Awash	106	18	17	11.1-25.9
Dire Dawa	89	5	5.6	2.4-13.2
Borana	145	17	11.7	7.5-18.3
Total	**340**	**40**	**11.8**	**8.8-15.7**

Pearson $\chi^2(2) = 6.02$ $p = 0.0493$.

There was significant difference ($p<0.05$) in seroprevalence in individual ages of goats using competitive ELISA. It was higher in goats of a year old (24.6%) and five years old (11.6%) as shown in Table 11.Using univariate logistic c-ELISA and individual ages of goats, goats in age one year significantly vary with goats in age two and three years. Goats in age one year were 4 times more exposed to the CCPP infection than goats in years two (OR =0.24 p = 0.002) and three (OR = 0.29 p = 0.025) however there were no difference with goats age four years (OR = 0.368 p = 0.057) and age five years (OR = 0. 40 p = 0.106). No significant variation goats in age four and five years with goats in other ages.

Table 11. Seroprevalence of CCPP in individual ages of goats using c-ELISA

Age estimated in year	Samples tested	Samples positive	Prevalence (%)	95 % CI
1	61	15	24.6	15.8-38.2
2	122	9	7.4	3.9-13.8
3	58	5	8.6	3.7-19.9
4	56	6	10.7	5.0-22.8
5	43	5	11.6	5.1-26.5
Total	**340**	**40**	**11.8**	**8.8-15.7**

Pearson χ^2 (4) = 12.54 p = 0.014

There was no significant difference between age categories (p>0.05). The highest (13.11%) was observed in age category one and followed by age category three (11.63%). The variation between age categories was statistically insignificant (p>0.05) as indicated in Table 12.

Table 12. Seroprevalence of CCPP using c-ELISA in goats slaughtered at ELFORA export abattoir by age category.

Age category (years)	Sample tested	**Samples positive**	Seropervalence (%)	95%CI
1 (1 ≤ 2)	183	24	13.11	9.0 – 19.0
2 (2 ≤ 4)	114	11	9.65	5.5 –16.9
3 (> 4)	43	5	11.63	5.0 – 26.5
Total	**340**	**40**	**11.8**	**8.8 –15.7**

Pearson χ^2 (2) = 0.81 p = 0.666

4.2. Gross Pathological Examination of Lungs

There were high significant differences in prevalence of lung lesions at different ages of goats (Pearson χ^2 = 46.76, p = 0.000). The higher prevalence of lung lesions was observed in goats of 4 years old (27%). Using logistic regression and goats in a years old taken as

reference and compared with other goats significant variation in likely hood of being affected by pneumonic lung lesion occurred with all other goats. Goats at age four years were 24 (OR = 24.44 p = 0.000 and 95% CI 5.6- 105.02) times more likely to be affected than in goats in age one year followed by goats in age three (OR = 16.01 P = 0.000 and 95% CI 3.70 – 69.19), five (OR = 15.42 p = 0.000 and 95 % CI 34.48 – 68.29) and two (OR = 5.77 p = 0.020 and 95% CI 1.32 – 25.21) with likely hood affection of 16,15 and 5.8 respectively.

When goats in age two years old were taken as reference, goats in age four years were (OR = 4.23 p = 0.000) 4.23 times likely hood of developing lung lesions followed by goats in age three (OR = 2.77 p = 0.002) and five years (OR = 2.67 p =0.006) with likelihood of lesions development of 2.77 and 2.66 respectively.

When goats in age three were taken as reference and compared with goats in other ages significant variation occurred with goats of age one (OR = 0.062 p = 0.000) and age two years (OR = 0.360 p = 0.002). However, there was no variation in development of lung lesion between goats in age four years (OR = 1.52 p = 0.171) and age five years (OR = 0.96 p = 0.913). Accordingly, goats in age one and two years were less developed lung lesions than goats in age three

Table 13. Prevalence of lung lesions in individual ages of goats slaughtered at ELFORA export abattoir

Age estimated in year	No. lung Examined	Lesions observed	Prevalence (%)	95%CI
1	134	2	1.5	0.4 – 5.9
2	236	19	8.1	5.2 – 12.4
3	128	25	19.5	13.7 – 27.8
4	111	30	27.0	19.9- 36.7
5	95	18	18.9	12.5 – 28.7
Total	**704**	**94**	**13.4**	**11.1 – 16.1**

Pearson χ^2 (4) = 46.76 p = 0.000

Table 14. Prevalence of lung lesions in goats slaughtered at ELFORA export abattoir by their area of origin

Origin of goats	Goats examined	Lesions observed	Prevalence (%)	95% CI
Awash	224	33	14.7	10.8 – 20.8
Dire Dawa	200	18	9.0	5.8 – 14.0
Borana	280	43	15.4	11.7 – 20.2
Total	**704**	**94**	**13.4**	**11.1 – 16.1**

Pearson χ^2 (2) = 4.62 P = 0.095

There was no significant difference in prevalence of lung lesions between areas of goats' origin ($p>0.05$), however, low prevalence was observed in goats that originated from regions of Dire Dawa than others. Univariate logistic analysis of lung lesion by origin (OR = 1.16 p = 0.27 and 95% CI 0.89- 1.52) of goats and age (OR = 1.66 p = 0.000 95% CI 1.40 –1.97) revealed there was significant difference among age of animals in likely hood of development of lung lesions. However, no significant variation was observed within areas of goats' origin.

Of the total 704 lungs examined pneumonic lesions were detected in 94 (13.4%). Among 94 lung lesions observed 81 (86.17%) were unilaterally affected while the rest 13 (13.83%) were bilaterally involved. Among the lesions 80 (85.11%) of them were on the apical lobe, while in 12 (12.77%) were on lower (ventral) part of lung. Only 2 (2.12%) lesions were observed on generalized part of the lung. Focal lesions were observed in 40 (40.43%), whereas it was superficial in distribution in 56 (59.57%). There was fibrin formation in 26 (27.7%) of the lesions. 14 (14.89%) of them developed adhesions with surrounding tissue and thoracic cavity and in 4 (4.26%) of the lungs sever adhesion with thoracic fluid was observed (Table 15).

Table 15. Gross pathological findings of pneumonic lungs in goats slaughtered at ELFORA export abattoir by their area of origin

Origin	No. Examined	Part of lung affected		Position of lung involved			Distribution of Lung lesion		Fibrosis and thoracic fluid		
		Unilateral	Bilateral	Apical	Lower	Both	Focal	Extensive	Fibrosis	Adhesion	Thoracic fluids
Awash	224	27	6	26	6	1	20	13	16	8	1
Dire Dawa	200	18	0	17	1	0	5	13	2	1	0
Borana	280	36	7	37	5	1	13	30	8	5	3
Total	**704**	**81**	**13**	**80**	**12**	**2**	**38**	**56**	**26**	**14**	**4**
Total lung lesions	**94**	**94**		**94**			**94**		**94**		
Percentage	**13.4**	**86.17**	**13.83**	**85.11**	**12.77**	**2.12**	**40.43**	**59.57**	**27.7**	**14.89**	**4.26**

4.3. *Mycoplasma* Isolation

Of the ninety-four lung tissues and four thoracic fluids cultured, *Mycoplasma* species were isolated from 15 samples. In broth culture growth were detected by the extent of turbidity. On solid media growth was daily examined under stereomicroscope at magnification of 25 –50X and compared with growth on liquid media.

Table16. *Mycoplasma* culture results from pneumonic lungs and thoracic fluids in both liquids and solid media.

	Pneumonic lungs		Thoracic fluids	
Culture	Broth	Plates	Broth	Plates
Contamination	25	25	0	0
No growth	54	54	4	4
Mycoplasma growth	15	15	0	0
Total	**94**	**94**	**4**	**4**

Some of the colonies of isolates had characteristic 'fried egg' appearance with central nipples shaped spot while others had pinpoint appearance (Figure2 and 3 respectively), still others revealed film and spot and wrinkling of the media (Figure1).

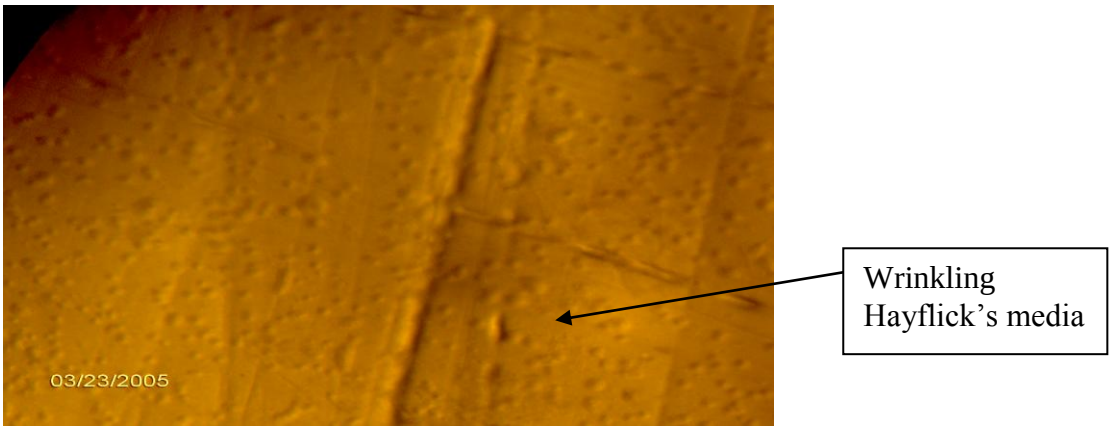

Figure 3. Wrinkling formation of the media on growth of *M. agalactiae* isolated from pneumonic lungs of goats

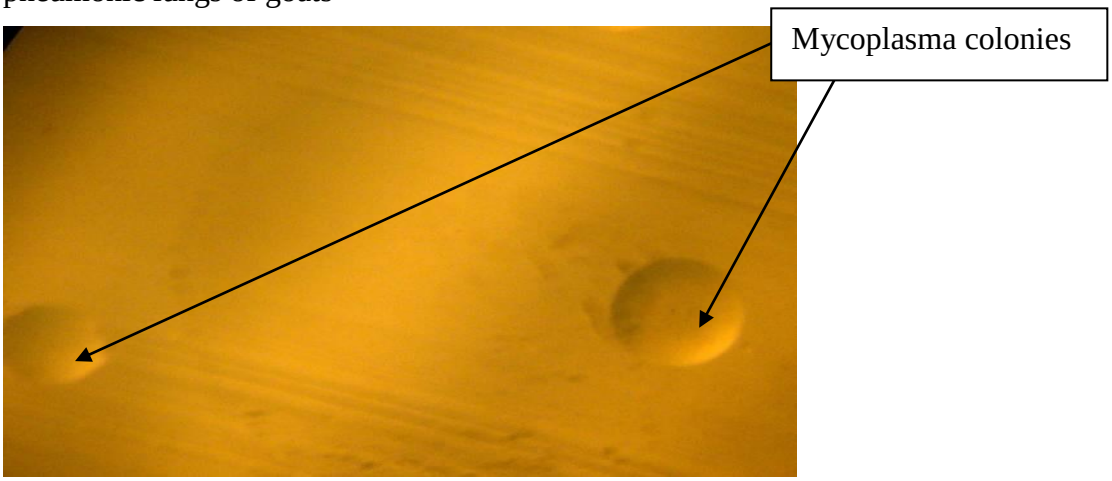

Figure 4.Typical *Mycoplasma* colonies on Hayflick's media after 3 passage of 72 hours of incubation

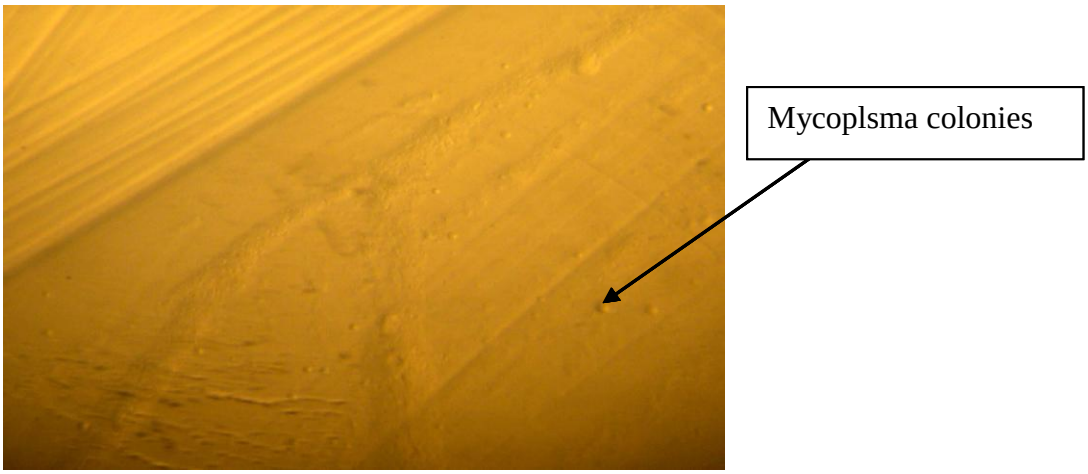

Figure 5. *M. capricolum* subsp. *capripneumoniae* showing *pinpoint* colonies

The distributions of the different *Mycoplasma* species isolated are given in Table 17. Based on biochemical characterisation the majority (5) of isolates were *MmmLC* followed by *Mccp* (3) and *M. agalactiae* (3).

M. agalactiae was isolated from three pneumonic lung lesions. The colonies showed film and spot and wrinkling of the media and biochemically characterized didn't reducing glucose and were negative in hydrolysis of arginine. Two of the isolates of *M. agalactiae* were confirmed by PCR (CIRAD-EMVT, France).

M. ovipneumoniae as it was usually mixed with *Mccp* culture six of them were found in isolated culture by PCR at (CIRAD-EMVT, France), however, these isolates were positive on nitrocellulose paper (dot blot) for *Mccp*. *M. ovipneumoniae* in proceeding subculture were overgrown our original samples of *Mccp*.

M. ariginini and *M. capricolum* subsp. *capricolum* (*Mcc*) were identified only based on biochemical tests by hydrolysis of arginine while *Mcc* ferment glucose and phosphatase positive *M. arginini* samples were glucose and phosphatase negative. *M. mycoides* subsp. *mycoides* Large colony *(MmmLC)* was identified by biochemical characterization as indicated in (Table17). Due to lack of hyper immune sera for each isolates, further identifications were not performed. From isolates suspected of *Mccp* in growth and dot blot and sent to CIRAD-EMVT one isolate of *M. arginini* was confirmed by PCR analysis.

M. capricolum subsp. *capripneumoniae (Mccp)* three of isolates were biochemically characterized (Table 17) was also positive for *Mccp* antigen by dot blot but other four isolates which didn't give clear picture in biochemical test further tested by dot blot using monoclonal antibody of *Mccp*.

Isolation of *Mycoplasma* species from thoracic fluids was unsuccessful. Theses thoracic fluids revealed typical characteristics of CCPP with adhesion, straw colour and full of fibrin that clotted immediately on stand. Even though, these cultures were observed for ten days and were blindly sub cultured followed for another ten days, no growth occurred. However, to detect the presence of *Mccp* antigen immunobiniding on nitrocellulose paper (dot blot) were done and positive trace of red dot observed on tips of nitrocellulose paper.

Table 17. Biochemical characterization of *Mycoplasma* species isolated from pneumonic lungs of goats slaughtered at ELFORA export abattoir.

Mycoplasma isolation	No. isolate	Glucose fermentation	Arginine hydrolysis	Phosphatase activity	Film & spot	Tetrazolium reduction Aerobic/ana.	Serum digistion
Mccp	3	+	-	-	-	+/+	+
Mcc	2	+	+	+	-	+/+	+
MmmLC	5	+	-	-	-	+/+	+
M.arginini	2	-	+	-	-	-/+	-
M.agalactiae	3	-	-	+	+	+/+	-
Total	**15**						

Table 18. Isolation of *Mycoplasm* species from goats slaughtered at ELFORA export abattoir by areas of their origin

Origin of goats	Sample cultured	No isolated	Prevalence (%)	95% CI
Awash	33	4	12.12	4.8 – 30.4
Dire Dawa	18	5	27.78	13.2 – 58.5
Borana	43	6	13.95	6.6 – 29.3
Total	**94**	**15**	**15.96**	**10.0 - 25.4**

Fisher's exact df (2) = 0.34

There was no significant difference (P > 0.05) in prevalence of *Mycoplasma* species in goats by their area of origin. The isolation rate was higher in Dire Dawa though; few numbers of pneumonic lung lesions were encountered.

Table 19. Prevalence of *Mycoplama* species from goats slaughtered at ELFORA export abattoir by ages

Age estimated in years	Sample Cultured	No isolated	Prevalence (%)	95% CI
1	2	0	0	-
2	19	1	5.26	0.8 –35.5
3	25	2	8.00	2.1 –30.2
4	30	4	13.33	5.4- 33.2
5	18	3	16.66	5.7 –46.8
Total	**94**	**15**	**15.95**	**10.0 –25.4**

Fisher's exact df (4) = 0.14

There was no significant difference (p>0.05) in prevalence of *Mycoplasma* species in individual ages.

4.4. Immunobinding on Nitrocellulose Paper (Dot blot)

Samples that were characterized by growth being late and with dark centre and biochemical test suggestive of the *Mccp* and thoracic fluid were used in dot blot with monoclonal antibody of *Mccp* to confirm whether the isolates or thoracic fluids contain *Mccp* antigen. Four thoracic fluids and seven isolates including biochemically characterised *Mccp* were found positive for dot blot, while one isolate from lung tissue even though its growth characteristics being a sign of *Mccp* was found negative.

Table 20. Results of dot blot test using known monoclonal antibody against *Mccp* antigen

Sample number	Sample code	Intensity
Control	Positive control	++
	Negative control	-
Samples	Thoracic fluid 78	+
	Thoracic fluid 580	+
	Thoracic fluid 640	+
	Thoracic fluid 650	+
	Isolate 166	+
	Isolate 182	+
	Isolate 311	-
	Isolate 363	+
	Isolate 381	+
	Isolate 526	+
	Isolate 558	+
	Isolate 564	+

5. DISCUSSION

Of total 704 goats sera tested using CFT the overall seroprevalence was 48.3% (n = 340) in goats slaughtered in ELFORA export abattoir. Seroprevalence in goats that originated from Awash, Dire Dawa and Borana were 47.3% (n= 106), 44.5% (n= 89) and 51.8% (n =145) respectively. Despite previous studies by Gezaghen (1993) and Mebratu (1988) who based on samples obtained from field, they reported seroprevalence of 51.8% and 50% respectively. The present finding is in line with their findings.

In the same way in other countries MacOwan and Minett (1976) obtained 53.1% seroprevalence by CFT in outbreak occurred in Kenya. Sharew *et al.* (2005) reported prevalence of 77% using CFT from field samples of CCPP endemic areas in Ethiopia. Higher prevalence in this study was not in harmony with study of Solomon (2005) who reported lower prevalence of 16.69% in South Omo and Gamo Gofa Zone of SNNP, Ethiopia using similar test.

On the other hand, other authors reported in their studies using B-ELISA tests 35% in Konso reported by Bereket (1995), 36% in Arbaminich by Mekonnen (1996), 31% at Hashim Nur export abattoir by Lisanework (2005), 24% in Borana (Yabelo) by Dawit (1996), 33% in Afar by Roger and Bereket (1996), 17.5% at Air force abattoir, Debre Zeit by Teshome (1997), 1.7% by Zenebe (2004) and 6% in Dire Dawa administrative region. Their findings were not in agreement to the present study. The higher prevalence of CCPP using CFT in this study most likely due to cross-reactivity between members of *M. mycoides* cluster which infected goats such as *MmmLC, Mcc, MmmSC* (Bölske *et al*., 1988; Thiaucourt *et al.,* 1994; Kusiluka *et al.,* 2000; Sharew *et al.,* 2005). Higher prevalence in using CFT in this study indicated that recent infection because CFT fixes IgM, which produced immediately after infection.

Of the total 340 goats sera that were CFT positive were retested using c-ELISA and resulted in prevalence of 11.8% (n = 40). Competitive ELISA using monoclonal antibody (Mab) specific to *M. capricolum* subsp. *capripneumoniae,* which binds specifically to the antigen that is coated on the plates, permits the specific detection of antibodies in animals, which had been affected by CCPP (Thiaucourt *et al.*, 1994).

In this study higher prevalence using CFT were recorded in goats that originated from Borana 51.8% and followed by Awash and Dire Dawa 47.3% and 44.5% respectively. There was no significant difference in seroprevalence among the areas of origin ($p>0.05$).

Using c-ELISA test the prevalence was 17% in Awash, 11.7% in Borana and 5.6 % in Dire Dawa. The difference between areas of origin was marginally significant ($P< 0.05$) using c-ELISA. Using univariate logistic regression c-ELISA and origin, those goats originated from Awash compared with goats originated from Dire Dawa and Barana variation occur with goats of Dire Dawa origin (OR = 0.291 P. = 0.019 and 95% CI = 0.10 - 0 .82). Goats of Dire Dawa origin when compared with goats originated from Awash and Borana significant variation occurred with goats of Awash (OR = 3.43 P. = 0.019 and 95% CI = 1.22 – 9.67). How ever, there was no significant variation between goats originated from Borana compared with goats from Awash. This could be due to agro ecological or management difference between theses areas. Those goats from Dire Dawa region had less likely hood of exposure to the CCPP infection than goats of Awash. Goats originated from Awash 3.43 times likely hood of being affected by CCPP than goats Dire Dawa. This was in harmony with the work of Solomon (2005) who indicated the difference in seroprevalence of CCPP between mixed farming system and pastoral agro-ecological zones were different. Previously, Teshome (1997) reported that seroprevalence was higher in goats in the low land (Kola) than midland altitude (Weyena Dega). In Dire Dawa areas by Beyene (2003) reported 6% prevalence, a low seroprevalence of 0.56% was reported by Zenebe (2004). Furthermore, reports of MoA (2004) demonstrated fewer numbers of outbreaks in Dire Dawa relativity to other regions. The detection of antibodies in goats that originated from three areas, which is geographically far apart indicates that CCPP is widely distributed in goat rearing areas of Ethiopia. Stress factor due to transport of goats from one market to another and over crowded flock could increase risk of distribution of the diseases (Thiaucourt and Bölske, 1996). Since there is no restricted trade route for animal movement in the country the mixing of goats from different areas facilitate transmissions of infection. Mycoplasma infection like other infectious agents need close contact with the source of infection and susceptible host (McMartin *et al*., 1980).

The distribution of seropositivity using c-ELISA among different age groups was significant ($p<0.05$). Higher seroprevalence was recorded in a year old goats (24.6%) then increases as age increases from 7.4% to 11.6%. However, other studies indicate that age

had no effect on prevalence of CCPP (Gezahegn, 1993; Dawit, 1997; Mekonnen, 1996; Beyene, 2003; Zenebe, 2004; Lisanework, 2005). Additionally, studies done in other countries indicated that all ages of goats are equally susceptible to the disease (Nicholas, 2002; Lefevre *et al.*, 1987b; MacOwan and Mintte, 1976). Variation in age in this study could be due to the inclusion of a few numbers of older ages sampled, relative to younger animals. Movement of goat especially younger ages for their high demanded for export purpose could expose them to infection. Even though, goats of all ages are equally susceptible for CCPP infection chance of being infected increased as they staid longer in the environment. Likewise, Solomon (2005) reported high seropositivity in older goats.

Of 704 goats examined at post mortem, lung lesions were observed in 94 (13.4%). Most of the lesions 81(81.7%) were unilateral and mainly on the apical lobe 80 (85.11%). Mekonnen (1996) in clinically sick goats and Lisanework (2005) in goats slaughtered at Hashim Nur export abattoir reported that most lesions were found on one side of the lung mainly on the right side. This study support the earlier observation of MacOwan and Mintte (1976) and McMartin *et al.* (1980) who observed that CCPP lesions confined to the thoracic cavity. The gross lesion-compressing palm coloured mostly in apical lobe as well as cardiac diaphragmatic lobes with nodules formations, fibrin formation and adhesion to the heart and thoracic cavity with straw-coloured fluids were indicatives of those CCCP (Thiaucourt and Bölske, 1996; Wesonga *et al.*, 1993). Some of lesions observed were not firm and consolidation was superficial and hyperaemic, these could be due to recent infection (Wesonga *et al.*, 1993).

Even though, clinical history of these goats were unknown the presence of the fibrin and adhesion without thoracic fluids in 26 (27.7%) animals in this study indicates the existence of chronicity. Bereket (1995) observed that goats survived first clinical disease naturally or after antibiotic treatments showed minimal lesions at post mortem. Wesonga and his co-workers (1993) in their studies of relationship between clinical signs and post mortem lesions demonstrated, goats that passed acute stage would show minor lesions at post mortem.The prevalence of CCPP like lung lesions in 13.4% in this study is in agreement with similar studies carried out by Lisanework (2005) who reported prevalence of 12% in goats slaughtered at Hashim Nur export abattoir, Debre Zeit. On other hand Yener *et al.* (2001) reported 4.9% of pneumonic lungs in goats slaughtered at bitlis slaughterhouse in Turkey, which lower than present study.

There was significant difference in prevalence of lung lesions between individual ages (P<0.01). Goats older than four years were 24 times more likely to be affected than at one-year-old goats. The odds ratios of goats in other ages 2, 3, 5 years old compared with goats in age one year were 5.78, 16.2 and 15.4 respectively. The logistic estimation of odds ratio within age groups was (OR= 1.64 p = 0.000 and 95% CI = 1.38 –1.94). When goats in age two years old were taken as reference, goats in age four years were (OR = 4.23 p = 0.000) 4.23 times likely hood of developing lung lesions followed by goats in age three (OR = 2.77 p = 0.002) and five years (OR = 2.67 p = 0.006) with likelihood of lesions development of 2.77 and 2.66 respectively. When goats in age three were taken as reference and compared with goats in other ages significant variation occurred with goats of age one (OR = 0.062 p = 0.000) and age two years (OR = 0.360 p = 0.002). However, there was no variation in development of lung lesion between goats in age four years (OR = 1.52 p = 0.171) and age five years (OR = 0.96 p = 0.913). Accordingly, goats in age one and two years were less developed lung lesions than goats in age three. This indicated that as goats stayed in the environment and become older repeatedly exposed to the etiologic agent and other infectious disease such as pasteurollosis and PPR.

The observation of thoracic fluids in this study in apparently healthy goats slaughtered at ELFORA export abattoir was in agreement with observation of Dawit (1996) in Yabello (Borana) back yard slaughtered goats. In this study lower prevalence of lung lesions was recorded in goats from Dire Dawa (9%) through the difference was not statistically significant (p>0.05). More ever, report of other workers showed low seroprevalence of CCPP in this part of region coincides with low prevalence of lung lesions in goats originated from this region (Beyene, 2003; Zenebe, 2004).

Of 94 pneumonic lungs and 4 thoracic fluids cultured 15(16%) samples showed growth of mycoplasma organisms. The following *Mycoplasma* species were identified from pneumonic lungs of goats. They were *M. agalactiae, M. mycoides* subsp. *mycoides* Large colony (*MmmLC*), *M. capricolum* subsp. *capriciolum* (*Mcc*), *M. arginini, M. ovipneumoniae* and main pathogenic agent *M. capricolum* subsp. *capripneumonie (Mccp). M. ovipneumoniae* and *M. arginini* are considered opportunistic pathogen (Bölske, 1995). They outgrow *Mccp* culture even if they exist in small number in the original samples (Thiaucourt *et al.*, 1992). In this study some strain were characterised based on the growth morphology and biochemical characteristics due to lake of specific hyper-immune sera for

specific identification. *M. capricolum* subsp. *capriciolum (Mcc),* and *M. arginini* hydrolysed arginine while *Mcc* is the only mycoplasma in the 'mycoides cluster', which hydrolysed arginine and positive for phasphatase reaction (Cottew, 1979; Jone, 1992; Nicholas, 2002). *M. arginini* hydrolysed arginine but not glucose.

The cultures from thoracic fluids were found negative for any *Mycoplasma* species but gave positive traces for *Mccp* antigen on dot blot. Thoracic fluids from goats in CCPP were supposed to be the best samples for isolation of *M. capricolum* subsp. *capripneumoniae* (Thiaucourt *et al*., 1992; OIE, 2000). The failure to isolate *Mycoplasma* species from these thoracic fluids might be due to previous exposure of the animals to antimicrobials. Since *Mccp* is also most fastidious *Mycoplasma* species usually overgrown by other fast growing ones. In experimental studies March *et al*. (2002) were unsuccessful to re isolate *Mccp* by culture and PCR in *Mccp* infected goats. Similarly MacOwan and Minette (1978) failed to isolate *Mccp* from experimentally infected goats that showed gross pathologic lesion in lungs. This is due to fastidious nature of this *Mycoplasma* species.

The *Mycoplasma* species from pneumonic lungs of goats in this study showed that *Mycoplasma* is the important organisms in the pathology of goats' pneumonia and their association in contagious caprine pleuropneumonia (CCPP). In similar studies the *Mycoplasma* species were isolated from thoracic fluids and pneumonic lungs of goats slaughtered at backyard slaughtered by Dawit (1996), Teshome (1997), Lisanework (2005) and Ikheloa *et al.* (2004). Yener *et al*. (2001) reported 28.6% *Mycoplasma* infection in Bitles slaughterhouse in Turkey. Goats examined in our study were only male; Mohanty *et al.* (2002) observed severe death of male than female goats due to *Mycoplasma* of *MmmLC* in India. In the field these *Mycoplasma* species were isolated from sick goats in CCPP endemic areas of the country by various authors: - From East Shoa by Thiaucourt *et al*. (1992); Fentale, East Shoa by Gezahegn (1993); Konso by Berakat (1995); Arbaminich by Mekonnen (1996); from different areas of the country by Yigazu *et al*. (2004) and South Omo and Gamo Gofa by Solomon (2005).

6. CONCLUSION AND RECOMMENDATIONS

The detection of *M. capricolum* subsp. *capripneumoniae* antibodies by CFT and c-ELISA from goats that originated from Awash, Dire Dawa and Borana indicated that CCPP was widely distributed in goats rearing areas of South and South Eastern Ethiopia. However, there was variation in seroprevalence between goats that originated from these areas. Seroprevalence was lowest in goats of Dire Dawa than Awash and Borana. Bacteriological isolations and pathological examination of lung lesions findings indicate that *M. capricolum* subsp. *capripneumoniae* (*Mccp*) and other *Mycoplasma* species occur concurrently and they are major health problems in goats for export and treating goat-rearing areas of the country. Infection other than *M. capricolum* subsp. *capripneumoniae* (*Mccp*) could thus causes cross reactivity in immunological tests, CFT shows higher seroprevalence compared with c-ELISA. The isolations of different *Mycoplasma* species from apparently healthy animals were the indication of chronic carrier state in goats. Gross pathological findings of the pneumonic lung lesions in slaughtered goats and condemnation of these organs indicate that high lose. Based on the above conclusions and findings the following recommendations are forwarded.

- Studies so far done in the country on caprine mycoplasmosis mainly focused on the seroprevalence of contagious caprine pleuropneumonia it needs further study to be conducted to assess epidemiological picture of pathogenic *Mycoplasma* species involved in contagious caprine pleuropneumonia of goats.

- Mix up of infected and susceptible animals from different areas facilitates the distribution of CCPP. Therefore, there must be identified trade rout for animal movement, which is enforced by legislation and policy.

- Slaughtered animals should be identified to trace back possible endemic foci of contagious caprine pleuropneumonia.

- Since vaccination is cost effective to control contagious caprine pleuropneumonia annual vaccination program should be encouraged.

7. REFERENCES

Abu-Groun, E.A.M., Taylor, R.R., Varsan, H., Wadher, B.J., Leach, R.H., Miles R.J. (1994): Biochemical diversity within the '*Mycoplasma mycoides* cluster' *Microbiology*. **140:** pp 2033-2042.

Adehana, R. K., Ajuwape, A.T.P., Adetosoye, A.I., and Alakac, O. O. (2006): Characterization of Mycoplasmas isolated from pneumonic lungs of sheep and goats. *Small Rumin. Res*. **63:** 44 - 49.

Ademosum, A.A. (1994): Constraints and Prospects for Small Ruminant Research and Development in Africa. In: Lebbie, B., Rey and Irungu B. (ed.): Proc. of the 2nd Biennial Conference of the African Small Ruminants Research Network S.H.B. AICC, Arusha, Tanzania, pp 1-9.

Bashiruddin, J. B., Taylor, T. K., Gould, A.R. (1994): A PCR-based test for the specific identification of *M. mycoides* subsp. *mycoides small colony. J. Vet. Diagn. Investig.* **6**: 428-434.

Bereket, Z. (1995): Contagious caprine pleuropneumonia (CCPP) prevalence in Konso, Southern Ethiopia and study on effect of antibiotic treatment. DVM, Thesis. FVM, AAU. pp 62.

Beyene, N. (2003): Epidemiology and sero-surveillance of Contagious Caprine Pleuropneumonia (CCPP) in Dire Dawa, Administration Region. DVM, Thesis. FVM, AAU. pp 21

Bölske, G. (1995): Respiratory Mycoplasmoses in Goats: Especially with Regard to Diagnosis of Contagious Caprine Pleuropneumonia. Ph.D, Dissertation. Uppsala, Sweden. pp 65.

Bölske, G., Johanson, K.E, Heinonen, R., Panullaga, P.A., Twinamasiko, E. (1996a): Contagious Caprine Pleuropneumonia (CCPP) in Uganda and isolation of Mycoplasma *Mccp* from goats and sheep. *Vet. Rec*. **337**: 594.

Bölske, G., Mattsson, J. G., Bascunana, C. R., Bergstrom, K., Wesonga, H.O., Johansson, K.E. (1996b): Diagnosis of Contagious Caprine Pleuropneumonia by detection and identification of *M. capricolum* subsp. *capripneumoniae* by PCR and restriction enzyme analysis. *J. Clin. Microbiol*. **34**: 785–791.

Bölske, G., Msami, H., Humlesnjo, N. E., Erno, H., Jonsson, L. (1988): *Mycoplasma capricolum* in outbreak of polyarthritis and pneumonia in goats. *Acta Vet. Scand*. **29:** 331-338.

Chakarabart, A., Mondal, D., Guha, C. (2001): Therapeutic trials in goat kids with mycoplasma pneumonia. *Ind. Vet. J*. **78**: 152-153.

Cottew G. S. (1979): Caprine-Ovine *Mycoplasmas*. In: The Mycoplasma ÌÌ, Human and Animal *Mycoplasmas*. Jully, G.J. and WhiteComb, R.F. (Editors). Academic press, New York San Francisco London, pp 104 - 132.

Cottew, G.S. and Yeats, F.R. (1978): Subdivision of Mycoplasma mycoides Sub mycoides from cattle and goats in to two types. *Aust. Vet. J.* **54:** 293-296.

CSA (2003): Statistical Report on Livestock and Farm implements. In: Federal Democratic Republic of Ethiopia, Central Agricultural Census Commission Ethiopian Agricultural Sample Enumeration, 2001/2002.

DaMassa, A.J., Wakenel, P.S., Brooks, D.L. (1992): Mycoplasma of goats and sheep. *J. Vet. Diagn. Invest*. **4:** 101-113

Dawit, K. (1996): Serological and Microbiological Studies in Major Respiratory Mycoplasmoses of goat in Yabello (Range land of Borana/Ethiopia). DVM, Thesis, FVM, AAU. pp 58.

El Hassan, S.M., Harbi, M.S., Abubakr, M.I. (1984): Treatment of contagious caprine pleuropneumonia. *Vet. Res. Commun*. **8:** 65- 67.

FARM-Africa (1996): Goat types of Ethiopia and Eritrea Physical description and Management systems. Published jointly by FARM-Africa, London, UK and ILRI. Nairobi, Kenya pp.76.

Freundt, E.A., Erno, H., Lanke, R. M. (1974): Identification of mycoplasma. In: Methods in microbiology Vol.**13**, Bergan and Norris (eds.) Academic press, London, pp 377-434.

Gezahegn, M. (1993): Preliminary study of Contagious Caprine Pleuropneumonia (CCPP) in selected site of East Shoa. DVM,Thesis. FVM, AAU. pp 62.

Goncalves, M. R. (1982): Un caso de pleuroneumonía contagiosa caprina. *Rep. Trab INV, XIV*: 85-92.

Gourlay, R.N. (1981): Mycoplasmosis in cattle, sheep and goats. *Isr. J. Med. Sci*. **17:**531-536.

Harbi, M. and El-Tahir, M. S. (1981): Mycoplasma strain F38 and contagious caprine pleuropneumonia in The Sudan. *Vet. Rec.***108**: 26.

Houshaymi, B., Tekleghiorghis, T., Wilsmore, A.J., Miles, R.J., Nicholas, R.A.J. (2002): Investigation of Contagious pleuropneumonia in Eritrea. *Trop. Anim. Hlth. Prod*. **34:** 383-389.

Ikheloa, J. O, Ajuwape, A.T.P., Adetosoye, A. I. (2004): Biochemical characterization and serological identification of *Mycoplasmas* isolated from pneumonic lungs of goats slaughtered in abattoirs in Northern Nigeria, *Small Rumin. Res.* **52**: 93–97.

Jones, G. E. (1992): Contagious caprine pleuropneumonia (CCPP). In: O.I.E. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.3rd ed. O.I.E. Paris France: pp 376-392.

Jones, G. E. and Woods, A.R. (1988): Microbiological and serological studies on Caprine pneumonia in Oman. *Res. Vet. Sci*. **44**. *125-131*.

Kusiluka, L.J.M., Semuguruka, W.D., Kazwala, R.R., Ojeniy, B., Fris, N.F.S. (2000): Demonstration of *Mycoplasma capricolum* subsp. *capripneumoniae* and *Mycoplasma Mycoides*, subsp. *mycoides*, Small Colony Type in Outbreaks of Caprine Pleuropneumonia in Eastern Tanzania. *Acta Vet. Scand*. **41**: 311-319.

Leach, R.H., Erno, H., Macowan, K.J. (1993): Proposal for designation of F38-Type caprine *Mycoplasmas* as *Mycoplasma capricoloum* subsp. *capripneumoniae* subsp. nov. and consequent obligatory delegation of strains currently classified as *M. capricolum* (Tulley, Barile, Edward, Theodore, and Erno, 1974) to an additional new subspecies, *M. capricolum* subsp. *capricolum.* nov. *Int. J. Syst. Bact*. **43**: 603-605.

Lefevre, P. C., Breard, A., Alfourk, I., Buron, S. (1987a): *Mycoplasma* species F38 isolated in Chad. *Vet. Rec.***121:** 575-576.

Lefevre, P.C., Jones, G.E, Ojo, M.O. (1987b): Pulmonary mycoplasmosis of small ruminant. *Rev. Sci. Tech. Off. Int. Epiz* **6:**759-799

Levinsohn, S. (1992): Mycoplasma in Veterinary Medicine part I. The role of mycoplasma in animal diseases. *Isr. J. Med*. Sci. **47**:1-6.

Lisanework, E. (2005): A study of contagious caprine pleuropneumonia (CCPP) in goats at Hashim Nur export abattoir, Debre Zeit. DVM, Thesis. pp 34.

Litamoi, J.K., Lijiodi, F.K., Nandoka, F. (1989): Contagious caprine pleuropneumonia: some observations in a field vaccination trial using inactivated Mycoplasma strain F38**.***Trop. Anim. Hlth. Prod*. **21:**146-150

Lorenzon, S, Wesonga, H.O., Yigesu, L., Tekleghiorgis, T., Maikano, Y., Angaya, M., Hendrikx, P., Thiaucourt, F. (2002): Genetic evolution of *Mycoplasma capricolum* subsp. *capripneumoniae* strains and molecular epidemiology of contagious caprine pleuropneumonia by sequencing of locus H2. *Vet. Microbiol*. **85:** 111-123.

MacOwan, K .J. (1976): A mycoplasma from chronic caprine pleuropneumonia in Kenya. *Trop. Anim. Hlth. Prod*. **8**:28-36.

MacOwan, K. J. (1984): Role of *Mycoplasma strain* F38 in contagious caprine pleuropneumonia. *Isr. J. Med. Sic*. **20:** 979-981.

MacOwan, K.J. and Minette, J. E. (1976): A mycoplasma from acute contagious caprine pleuropneumonia in Kenya. *Trop. Anim. Hlth. Prod*. **8:** 91-95.

MacOwan, K.J. and Minette, J. E. (1978): The effect of high passage of Mycoplasma strain F38 on course of Contagious Caprine Pleuropneumonia (CCPP). *Trop. Anim. Hlth. Prod*. 10: 31-35.

March, J. B., Gammack, C., Nicholas, R. A. J. (2000): Rapid Detection of Contagious Caprine Pleuropneumonia Using a *Mycoplasma capricolum* subsp. *capripneumoniae* Capsular Polysaccharide-Specific Antigen Detection Latex Agglutination Test. *J. Clin. Microbiol.* **38:** 4152 -4159.

March, J.B., Harrison, J.C., Borch, S.M. (2002): Humeral immune response following experimental infection of goats with *Mycoplasma capricolum* subsp. c*apripneumoniae. Vet.Microbiol.* **84**: 29-45.

Mare, C. J. (1996): Contagious caprine pleuropneumonia. In: The Gray Book. Part IV: Foreign Animal Diseases.

McMartin, D.A., MacOwan, K.J., Swift, L. L. (1980): A century of classical contagious pleuropneumonia: from original description to aetiology. *Brit. Vet. J.* **136**: 507-515.

Mebratu, GY. (1988): The incidence of Contagious Caprine Pleuropneumonia (CCPP) in Ethiopia In: IAR proceeding, 2nd National Livestock Improvement Conference, 24-26 February, 1988. Addis Ababa. 270-273.

Mekonnen, L. (1996): Contagious Caprine Pleuropneumonia (CCPP): Study On Economic Significance and Vaccine Trial. DVM, Thesis. FVM, AAU. pp 66.

Mike, S. (1996): Goats: In the Tropical Agriculturalist Macmillan, TCTA. pp 79-83.

MoA (2004): Monthly Disease Outbreak report: In: National Animal Health Department Reports. Addis Ababa, Ethiopia.

Mohanty, B.C., Kar, B.C., Mishera, P., Roy, P.K. Mishera, R., Bal, M.K. (2002): Kid mortality in Orissa. *Indian J. Anim. Sci.* **72:** 546-548.

Monnerat, P.M., Thiaucourt, F. Jose, B. P., Nicolet, J., Frey, J. (1999): Genetic and Serological Analysis of Lipoprotein lppA in *M. mycoides* subsp. *mycoides* LC and Mycoplasma *mycoides subsp*. c*apri. Clin. Diagn. Lab. Immunol.* **6:** 24–230.

Nicholas, R. J. A. (2002): Improvements in the diagnosis and control of diseases of small ruminants caused by mycoplasmas. *Small Rumin. Res*. **45:** 145–149.

O.I.E. (2000): Contagious Caprine Pleuropneumonia. In: Office International Des Epizooties (O.I.E.). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.3rd ed. O.I.E. Paris France: pp 503-513.

Ojo, M. O. (1976): Caprine Pneumonia IV: Pathogencity of *Mycoplasma mycoides* subsp. *Capri* and caprine strains of *M. mycoides* subsp. *mycoides* for goats. *J. Comp. Path*. **86**: 519-529.

Ojo, M.O. and Obi, T. U. (1996): Respiratory diseases of goat. *Trop. Vet*. **14**: 85–99.

Onoviran, O. (1974): The comparative efficacy of some antibiotics used to treat experimentally induced mycoplasma infection. *Vet. Rec*. **94**: 418-420.

Peacock, C. (1996): Improving Goat Production in the Tropics. A Manual for Development Workers. Published jointly by FARM-Africa and Oxfam (UK and Ireland), Oxfam, UK, pp 387.

Perreau, P., Breard, A., Goff, C.L. (1984): Experimental infection of goats with type F38 mycoplasma strains (CCPP*). Annales de Microbiologie*, **135:** 119-12.

Poumarat, F., Longchambon, D., Martel, J.L. (1992): Application of dot immunobinding on membrane filtration (MF-dot) to study of relationship within "*M. mycoides cluster*" and within "glucose and arginine negative cluster" of ruminant mycoplasmas. *Vet. Microbiol.* **32:** 375-390.

Poumarat, F., Perrin, B., longchambon, D. (1991): Identification of Ruminant Mycoplasmas by Dot- Immunobinding on Membrane Filtration (MF dot). *Vet. Microbiol.* **29:** 29-338.

Quinn, P. J., Carter, M.E., Markey, B., Carter, G. R. (1994): Mollicutes. In: Veterinary Clinical Micro biology. Wolfe publishing Yearbook, Europe Limited, Spain. Pp 320-326.

Radostits, O.M., Blood, D.C., and Gay, C.C. (1994): Disease caused by Mycoplasma In: Veterinary Medicine. Textbook of the diseases of Cattle, Sheep, Pigs, Goats and Horses. 8th.ed. Blaire Tindall. pp 910-913.

Roger, F. and Bereket. Z. (1996): Contagiouse Caprine Pleuropnuemia (CCPP) in Ethiopia. Laboratory investigation and field studies 1994/95 preliminary reports CIRAD-EMVT. No. 96006.

Rosendal, S., and Black, F.T. (1972): Direct and indirect immunofluorescence of unfixed and fixed mycoplasma colonies. *Acta. Pathologica .et Microbiologica Scand.* **80:** 615-622.

Rurangirwa, F. R., McGuire, T. C., Musoke, A. J., Kibor, A. (1987b): Differentiation of F-38 mycoplasmas causing contagious caprine pleuropneumonia with a growth-inhibiting monoclonal antibody. *Inf. Immun*. **55**: 3219-3220.

Rurangirwa, F. R., McGuire, T.C., Kibor, A., Chema, S. (1987a): A latex agglutination test for field diagnosis of contagious caprine pleuropneumonia. *Vet. Rec*.**121:** 191-193.

Rurangirwa, F.R, Masiga, W.N., Muthomi, E.K. (1984): Immunization of goats against contagious caprine pleuropneumonia using sonicated antigens of F-38 strain of Mycoplasma. *Res. Vet. Sci*. **36: 174**-176.

Rurangirwa, F.R., Masiga, W.N., Muriu, D.N., Muthomi, F., Mulira, G., Kagummba,M., Nandokha, E. (1981): Treatment of contagious caprine pleuropneumonia. *Trop. Anim. Hlth. Prod*. **13**: 177-82.

Rurangirwa, F.R., McGuire, T.C., Kibor,A., Chema., S.(1987c): An inactivated vaccine for contagious caprine pleuropneumonia. *Vet. Rec* **121**: 397-400.

Schaeren, W. and Nicolet, J. (1982): Micro-ELISA for detecting contagious agalactia in goats. *Schweiz. Arch. Tierheilkd*. **124:** 163-177.

Sharew, A.D., staak, C., Thiaucourt, F., Roger, F. (2005): A serological investigation into Contagious Caprine Pleuropneumonia (CCPP) in Ethiopia. *Trop. Anim. Hlth. prod*. **37**: 11-19.

Simecka, J. W., Davis, J. K., Davidson, M. K., Ross, S. E., Stadtlander, C. T. K. H., Cassell, G. H. (1992): Mycoplasma diseases of animals. American Society for Microbiology, Washington, DC.

Sjostrom, K.E Chen, K.C.S. and Kenny, G.E. (1986): Detection and product of the origin dihydrolyslase pathway in both fermentative and non-fermentative Mycoplasma species by thin layer chromatography. *Int. J. Syst. Bactriol*. **36:** 60-65.

Smith, M. C. and Sherman, D. M. (1994): Contagious Caprine Pleuropneumonia In: Goat Medicine. Williams &Wilkins, Mary land. pp 84-89.

Solomon, M. (2005): Epidemiological survey of Contagious Caprine Pleuropneumonia (CCPP) in South Omo and Gamo Gofa Zone Southern Ethiopia. MSc, Thesis. FVM, AAU. pp 101.

Staak, C., Salchow, F., Denzin, N. (2001): Complement Fixation Test and Competitive ELISA. In: Practical Serology from the basics to the testing: Urban & Vogel Medien und Medizinverlagsgesell-schaft mbH & Co. KG, Munchen Germany. pp 41-56.

Stakenborg, T., Vicca, J., Butaye, P., Maes, D., De Baere, T., Verhelst, R., Peeters, J., de Kruif, A., Haesebrouck, F., Vaneechoutte, M. (2005): Evaluation of amplified rDNA restriction analysis (ARDRA) for the identification of *Mycoplasma* species. *BMC. Infect. Dis*. **5:** 46.

Stata corp. (2001): Stata statistical soft ware. Releases 7.0, Lake way, Drive College Station, Texas

Taylor, T. K., Bashiruddin, J. B., Gould, A.R. (1992): Relationships between members of the *Mycoplasma mycoides* cluster as shown by DNA probes and sequence analysis. *Int. J. Syst. Bacterio.* **42**: 593-601.

Teshome, F. (1997): Epidemiological survey of PPR and CCPP in selected area of Ethiopia. DVM, Thesis. FVM, AAU. pp 69.

Thiaucourt, F. and Bölske, G. (1996): Contagious Caprine Pleuropneumpnia and other pulmonary Mycoplasmosis of sheep and goats. *Rev. Sci. Tech. Off. Int. Epiz*. **15**: 397–1414.

Thiaucourt, F., Breard, A., Lefevre, P. C., and Mebratu, G.Y. (1992): Contagious Caprine Pleuropneumonia in Ethiopia. *Vet. Rec*. **131**: 585.

Thiaucourt, F., Johansson, K. E., Smith, D. G., Wesonga, H.O., Yigezu, L.M. (2000): Contagious caprine pleuropneumonia recent advances.7th International conference on goats, France.15-21. May 2000.

Thiaucourt, F., Libeau, G., LeGoff, C., Lefevre,P.C.(1994): The use of monoclonal antibody in diagnosis of contagious caprine pleuropneumonia. *Vet. Microbol*. **41**: 191-203.

Thrusfeild, M., Ortega, C. DeBlas, I., Noordhuizen, J.P., Frankena, K. (2001): Winepiscope2.0: Improved epidemiological software for veterinary medicine. *Vet. Rec*.**148:** 567-572

Thrusfield, M. (1995): Veterinary Epidemiology. 2nd ed. Blackwell Science Ltd. London. pp 179-187.

Tully, T.G., Barile, M.F., Edwards D.G.F.F, Theodor, T.S Erno, H. (1974): Characterization of some caprine mycoplasma with prosalls for new species *Mycoplasma capricolum* and *M. putrefciens J. Gen. Microbiol*. **85:** 102-120.

Walker, R. L. (1999): Mollictus: In: Hirsh D C., Y C, Zee. Veterinary microbiology: Black well Science Int pp 65-172.

Weisberg, W. G., Tully, J. G., Rose, D. L., Petzel, J. P., Yaizu, H. O., Yang, D. (1989): A phylogenetic analysis of the mycoplasmas: basis for their classification. *J. Bacteriol*. **171:** 6455–6467.

Wesonga, H .O. Lindberg, R., Litamoi, J. K., Bölske, G. (1998): Lat lesions of experimental Contagious Caprine Pleuropneumonia caused by *Mycoplasma capricolum* subsp. *capripneumoniae. J. Vet. Med.* **45:** 105-114.

Wesonga, H.O., Litamoi, J. K. Kagumba, M., Wakhusama, E. (1993): Relationship between clinical signs and early lesions of contagious caprine pleuropneumonia caused by *Mycoplasma. Strain F38*. *Small Rumin. Res.***10**: 45-54.

Yener, Z., Gurturk, K., Gulbahear, Y., Solmaz, H. (2001): Pathological and bacteriological studies on pneumonia in goats slaughtered at Bitlis slaughterhouse. *Veteriner Bilimeleri Dergisi* **17:**12-20

Yigezu, L.M., Tariku, S., Aylet, G., Roger, F. (2004): Respiratory mycoplasmoses in Small Ruminant. *Ethiop. Vet. J.* **8**: 67-74.

Zenebe, Z. (2004): Epidemiology and sero-prevalnce of Contagious Caprine Pleuropneumonia (CCPP) in and around Dire Dawa, Administration Region. DVM, Thesis. FVM, AAU. pp 1 –21

8. ANNEXES

Annex 1 Determining age of goats according to Mike (1996).

	Age group	Teeth condition
1	Kid under one year	Eight sharp incisors
2	Yearling (1-2) year	Central pair of baby teeth replaced by permanent
3	Young adult (3-4) years	4 permanent teeth
4	Adult (3-5) years	8 permanent teeth
5	Older adult greater than 5 years	Worn teeth and some missing

Annex 2 Preparations of Hayflick's medium

The media comprises the basal component which contain brain heart infusion 37gm/ml (w/v), Difico Bacto Neoptone 2.5gm(w/v), Difco Bacto casitone 2.5gm, D (+)- glucose amhydrose 2gm and 1%(w/v) agar for slid media, where as for broth culture without agar supplement component include horse serum 200ml (NVI, produced) yeast extract (25%) 100ml,penicillin 200,000 IU and DNA 0.2%, Glucose (50%), Thallium acetate 50 unit and pH 7.8

Annex 3 Immunobinding on Nitro cellulose paper (Dot blot)

Materials: -Test samples of pleural fluids and isolate; Nitrocellulose paper cut in small sizes (0.3 cm x 1.5cm), which can easily fit in micro plate wells, Micro plates 96 wells (U-bottom), micropipettes and tips. Incubator with an agitator, Tris Buffer saline (TBS) pH 7.4, 0.05M tris Sodium citrates Solution, 0.2M NaCl (pH adjusted to 7.4), Blocking buffer solution.10% horse serum in TBS with 0.05% Tween-20 (polyoxyethylene sorbitanolaurat). Monoclonal antibodies (Mab 1: 5,000), Mouse hyper immune serum against *Mccp,* Conjugates: peroxidase labelled goats anti rabbit (1:200) and anti mouse (1:500) IgG, diluted in blocking solution, Developing Solution (Substrate): TBS containing 0.5% w/v tetra hydrochloride, 3, 3'- diaminobezidine (DAB), and 0.1% H_2O_2 (30%) (Sigma chemical CO) and reference *Mycoplasma* strains of *Mccp* (F38).

Procedure: The procedure of Poumarat *et al.* (1992) was followed for antigen detection of *Mccp* in thoracic fluids and lung tissue isolates.

1. Nitrocellulose paper was cut in small sizes (0.3 cm x 1.5cm), which can easily fit in micro plate wells, Micro plates 96 wells (U-bottom),

2. Five micro litters of the thoracic fluids and lung tissue isolate were poured on the tip of the nitrocellulose (NC) paper.

3. The papers was incubated for 10 minutes at 37^0C and washed with TBS for 3 minutes.

4. Then NC was immersed into blocking buffer 200µl per well kept for 30 minutes with slow agitation.

5. The paper was then transferred to Mab, 200µl aliquots per well and kept for another 30 minutes with slow agitation. It washed three times in TBS, 250µl per well (3 minutes per washings).

6. 200µl conjugate was added per well and the paper was kept in it for 30 minute with slow agitation. The washing steps were repeated as procedure 5, three times in TBS.

7. 150µl of developing solution was transferred per well and incubated for two minutes. Finally, rinsing in distilled water that stopped the reaction.

8. Appearance of red dot on the tip of the NC paper was considered as positive reaction and ranked according to the colour intensity, (+++) strong, (++) average, (+) weak, and (-) negative or no reaction.

Annex 4 Preparation of biochemical test media

1. Glucose hydrolysis

Heart infusion broth Difco…….180ml

Horse serum. ...……40ml

Yeast extract 25%.....20ml

Penicillin G.……..…….200,000IU

Thallium acetate……50IU

DNA (solution of 0.2%)……..2.6ml

Glucose (solution of 50%)…….2ml (1g)

Red phenol 0.1%.....5ml

pH of the medium adjusted to be 7.8

2. Arginine hydrolysis

Heart infusion broth Difco…….180ml

Horse serum……..……..40ml

Yeast extract 25%.....20ml

Penicillin G …….200,000IU

Thallium acetate……50IU

DNA (solution of 0.2%)…….2.6ml

30% arginine……..……8.5ml (2.5g)

Red phenol 0.1%.....5ml

pH of the medium adjusted to be 7.3

3. Tetrazolium reduction

Heart infusion broth Difco…….180ml

Horse serum…….40ml

Yeast extract 25%.....20ml

Penicillin G …….200,000IU

Thallium acetate……50IU

DNA (solution of 0.2%)……...2.6ml

2% chlorine tri phenyl tetrazolium ……5.ml

Red phenol 0.1%.....5ml

pH of the medium adjusted to be 7.5

4. Phosphate activity

Heart infusion broth Difco…….180ml
Horse serum diphosphatized at 60^0C……...20ml
Yeast extract 25%.....5ml
DNA (solution of 0.2%)……...2.6ml
Penicillin G …….200,000IU
Thallium acetate……50IU
Glucose (solution of 50%)……2ml (1g)
Red phenol 0.1%.....5ml

pH of the medium adjusted to be 7.8

Annex 5 *M. capricolum* subsp. *capripneumoniae* antigen preparation

Mycoplasma biotype F38, isolated characterised by an immunopexidase test and protein electrophoresis, and persevered as lyophilised pure culture (Thiaucourt *et al.*, 1992) was obtained from CIRAD-EMVT and used as sources of antigen. A portion of freeze dried material was inoculated into 3ml of modified Newing's tryptose broth growth medium and incubated at 37^0C for further five days subculturing was done for second time into 100ml of growth medium with incubation of at 37^0C for another five days. The culture was then centrifuged at 7000g for 15 minutes at 4^0C and sediment was washed three times using distilled water. The packed organisms were resuspended in 1ml-distilled water, diluted 1:60 and ultrasonicated by ultrasonicator for 3minute at lower power in a container of iced water. The suspension was centrifuged at 1250g for 30 minutes to remove any debris (OIE, 2000). The suspension was then stored at 20^0 C in aliquots of 10ml in screw cupped glass bottle until it was used.

Antigen titration: To determine range of suitable antigen concentration working dilution stock of certain concentration positive and negative control serum was used. Check board titration was used to combine range of antibody concentration, which produce clearly positive reaction margin was considered the working dilution of that could react optimum reaction of test sera. According to antigen check board titration *Mccp* antigen working dilution was **1:20** for CFT.

Annex 6. Complement Fixation Test (CFT)

Materials: Test sera of goats, U- bottomed micro plates, multi channel micropipettes and tips, Guinea pig complement and complement diluent's, *Mccp* (F38) antigen, Veronal buffer solution in Calcium and Magnesium (VCM) pH 7.2, water bath, incubator with agitator, Alseiver's solution, male sheep red blood cells (SRBC), Amboceptor (rabbit anti sheep red blood cells), trough, syringe, arranged test sera, positive and negative control sera in sets and sheet of plate lay out for records.

Procedure: The OIE standard test procedure of O.I.E (2000) was adopted.

1. Test sera including positive and negative control were decomplemented in hot water bath at 60^0 C for 30 minutes.
2. Series of two fold dilutions of test sera pipetted 25µl per well. The first two columns were left for control of the positive and negative serum, Complement and Sheep Red blood cells (SRBC) that was hemolytic system.
3. 25µl (1:20) antigen was added into each well, except in one column (with 1:20 diluted sera) to check serum anti complementary activity. It was agitated and incubated for 30 minutes.
4. 25µl of titrated complement were added in to the wells of test sera. It was incubated at 37^0 C under constant agitation on orbital shaker for 30 minutes.
5. 2% one-day-old SRBC was prepared before by washing three times with VCM and centrifuged at 2500 rpm for 5 minutes for each washing. An equal volume of diluted Amboceptor (1:1000) was added to sensitise the red corpuscles, which brings hemolytic system to 1% instead of 2%.
6. 25µl sensitised SRBC (indicator) was pipetted into each well and the plates were sealed to avoid evaporation then incubated at 37^0C for 30 minutes with constant agitation.
7. The plates were examined for sedimentation and hemolysis. Then the plates were kept at refrigerator at +4 ^{0} C over night in order to allow non-lysed cells to settle.

Interpretation: - Positive reactions in sedimentation of SRBC and its haemolysis indicate negative reaction. The results were ranked according to the extent of SRBC sedimentation haemolysis (-), weak (+), moderate (++) and strong (+++). When the sedimentation in test serum and control serum for anti complementary (with out *Mccp* antigen) were equal the test serum was taken as negative.

Annex 7 Competitive ELISA

Materials: - Arranged test sera, U-bottom multi channel plates, micropipettes and tips, Mccp antigen provided with kit, Monoclonal antibodies (Mab) (OIE/EMVT) Phosphate buffer saline (PBS) pH 7.4,Washing buffer (PBST), PBS with 0.05% Tween-20, Blocking buffer (PBST) containing 10% horse serum used for diluting the sera, monoclonal antibodies and the conjugate, Conjugate: rabbit anti-mouse immunoglobilin G (IgG) labelled with peroxidase, Substrate (1mm ABTS solution 40.05M citrate buffer pH 5.0 $+H_2O_2$), Reference sera, strong and weak positive control, Incubator with an agitator, photometer reader, Sheet of plate lay out, trough, micro plate sealer, distilled water, vortex and orbital shaker.

Procedure: The serological kit was provided by CIRAD-EMVT (France) and the test was done according to guidelines of manufacturers.

1. All wells of a micro plate were charged with 50µl of carbonate buffer (PBS). 50µl of antigen was added to each well at dilution of 1:100. It was incubated in a moist chamber at 37^0C (incubator) over night. Free antigens were removed by washing it 2 times with PBST for three minute.
2. 100µl of dilution buffer was dispensed in to each well of pre-plates. 11µl of the three control samples CP++ in B1, B2, C1, and C2 and CP+ in D1, D2, E1, E2 and negative control in H1, H2.
3. 11µl test serums were dispensed in all other wells (A3 to H12). Monoclonal antibody diluted in 1:250 in dilution buffer and 110µl of this was dispensed to each well except A1 and A2 but in these two wells only 110µl of dilution buffer was added, which hears after called conjugate control. Test sera with monoclonal antibody in pre-plate with 96 wells were incubated for an hour at 37^0C in constant shaker.
4. The plates were emptied manually and washed two times and dried on towels.
5. Diluted conjugate in 1:50 and 100µl of it was dispensed in to each well. The plates were sealed and incubated for thirty minute at 37^0C under constant agitation.
6. The content of plates were emptied manually and washed three times with washing solution.

7. 100µl of revelation solution 3 or substrate was dispensed in to each well then plates were incubated under shelter of light with constant agitation at 37^0C for 30 minutes.
8. 100µl of stop solution was added in to each well and shacked well until coloured solution was homogenized. The photometer was first blanked on air and then the plates were read at optical densities of 450 nm.

Annex 8. Contagious caprine pleuropneumonia outbreak report from 1998-2004

Year	No of outbreak	Cases	Deaths	Slaughtered
1998	36	771	453	13
1999	22	530	172	10
2000	17	346	222	18
2001	29	5863	800	77
2002	55	12383	2546	853
2003	42	2447	980	162
2004	5	271	102	94
Total	**206**	**22611**	**5275**	**1227**

Source: (MoA, 2004): Monthly diseases outbreak report

Annex 9. Monthly contagious caprine pleuropneumonia outbreak reports in different regions of Ethiopia from 1998- 2004

Region	No of outbreak	Cases	Deaths	Slaughtered
SNNP	88	14358	2967	976
Afar	43	6741	1301	107
Amhara	14	282	142	21
Oromia	48	618	140	10
Benishangul	3	14	310	2
Dire Dawa	10	576	413	111

Source: (MoA, 2004)

Annex 10. Data collection sheet

S/code	Origin of goats	Age	Presence Of lung lesion	Unilateral / /Bilateral	Apical /lower/ both	Focal /extensive	Fibrosis	Adhesion	Thoracic fluids
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
.									
.									
704									

9. CURRICULUM VITAE

1. Personal data

Name	Daniel Gizaw Demissie
Date of birth	December 5, 1973
Places of birth	Oromia, East Hararghe, Kurfachale, Dekeba,
Nationality	Ethiopian
Martial status	Married
Children	One
Religion	Christian
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Educational Background:	Primary school, Wakjira 1981-1985 Junior secondary school Kurfachale, 1986-1987 Senior secondary school Girawa, 1988-1991 Achievement: ESLCE Higher education Addis Ababa University, Faculty of Veterinary Medicine Spet, 1991- July, 1996 Achievement: Doctor of Veterinary Medicine (DVM)
Work experience:	October, 1996 to August, 2004 District Vet team leader at Ministry of Agriculture September, 2005 to June, 2006 study at Addis Ababa University, Faculty of Veterinary Medicine.

Paper writing:

1. Fetal and Neonatal Immunity in and principles of immunization. Seminar paper (1995) FVM, AAU.
2. Assessment of cow-calf nutritional status in smallholder dairy farms in Debre Zeit (1996). DVM, Thesis. FVM, AAU.
3. A review on small ruminant mycoplasmoses. Seminar on current topics (2005) FVM, AAU
4. Serological, pathological and bacteriological investigation of *Mycoplasma* infection in goats slaughtered at ELFORA export abattoir, Debre Zeit. MSc Thesis (2006)

Additional Training:

1. Computer literacy: Word processing, spread sheet, Microsoft and data management (11/11/2003 - 08 /05/2004 G.C)
2. Training course on Integrated Laboratory Diagnostic Techniques. Bale Zonal Agricultural Development office and Assela Regional Veterinary Laboratory (11-15 /9/1989 E.C).

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10. SIGNED DECLARATION SHEET

I, the under singed, declare that the thesis is my original work has not been presented for a degree in any other University, and that all sources of material used for the thesis have been duly acknowledged.

Name Daniel Gizaw Demissie

Signature _____

Date of submission _____

The thesis has been submitted for examination with our approval as university advisers

Dr. Ademe Zerihun _____

Dr. Berhe Gebreegziabher _____