SERO-PREVALENCE AND PARTICIPATORY STUDY OF CONTAGIOUS CAPRINE PLUEROPNEUMONIA IN GULINA WEREDA, AFAR NATIONAL REGIONAL STATE, ETHIOPIA

A thesis submitted to School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Masters of Veterinary Science in Tropical Veterinary Microbiology.

BY

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LIST OF ABBREVIATIONS

ANRS	Afar National Regional State
CBPP	Contagious Bovine PleuroPneumonia
CCPP	Contagious Caprine PleuroPneumonia
c-ELISA	Competitive Enzyme Linked Immuno Sorbent Assay
CFT	Complement Fixation Test
CI	Confidence Interval
CIRAD-EMDT	Center de coopration Internationale en Recherché Agonomique Pour le. Devlopment Department d' Elevage et de Medecine Vetrinaire
CSA	Central Stastical Authority
IgG	Immunoglobin G
IgM	Immunoglobin M
IHT	Immuno Hemmaglutination Test
IU	International Unit
Mab	Monoclonal antibody
MAKePS	Mastitis, Arthritis, Keratitis, Pneumonia and Septicemia Syndrom
Mcc	*Mycoplasma capricolum* subspecies *capricolum*
Mccp	*Mycoplasma capricolum* subspecies *capripneumoia*

Mmc	*Mycoplasma mycoides* subspecies *Capri*
MmmLC	*Mycoplasma mycoides* subspecies *mycoides* Large Colony
MmmSC	*Mycoplasma mycoides* subspecies *mycoides* Small Colony
OIE	Office Internationale des Epizooties
OR	Odds Ratio
PA	Pastoralist Association
PCR	Polymerase Chain Reaction
PPR	Pets des Petites Ruminants
SNNP	Southern Nation Nationalities and Peoples Regional State
SRBC	Sheep Red Blood Cell
Subsp.	Subspecies
PDS	Participatory Disease Search

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ABSTRACT

A cross sectional study of Contagious Caprine Pleuro pneumonia (CCPP) in goats was conducted from October 2007 to April 2008 to determine sero prevalence in Gulina district of Afar National Regional State. A total of 446 sera were collected from goats. Complement Fixation Test (CFT) was carried out at National Veterinary Institute. The Participatory approaches are holistic in nature where by all aspects of the phenomenon questions are studied by the use of multiple methods in a process referred to as triangulation. Camels had the highest proportion (44.6%) followed by 30.6% of goats to the relative importance of livestock species to pastoralists. The possible source of CCPP is watering followed by grazing (31.9%) due to goats gathered from different sites for the search of feed and water. The main control tactic for CCPP is Isolation of the affected goat(s). Among the cases in the area 34.7% were pasteurellosis followed by CCPP. Significant numbers of goat were lost due to CCPP (32.6%) next to PPR (38.7%). Drought (65%) is a major challenge to their livestock. The overall sero prevalence was 42.8% (n = 191). The age group sero prevalences were 42.4, 36.8 and 49% in age group less than 1, 1-3 years and >3 years older respectively. Sex specific sero prevalence observed was 40.9% in males and 43.7% in females. Because of few male goats compared to females in each flock, the contribution of males to the total sero positivity was only 32.9%. High significance association was found between Flock size 51-150 and greater than 150 (OR=25.6, P=0.000) to CFT test. Goats of less than 1 year were found to be safer (OR=0.974, P=0.324).

Keywords: Contagious Caprine Pleuro pneumonia, Goats, Seroprevalence, Risk factors, Participatory approach, Gulina wereda, Afar region.

1. INTRODUCTION

Goats (*Capra hircus*) are thought to have been the first animals to be domesticated for economic purposes. They play a crucial role in food production in developing countries. They can easily be sold in terms of urgent needs such as sickness, death or the payment of school (Peacock, 1996). Goats are widely distributed and inhabit all climatic zones, with the 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 poor quality fibrous feeds. They have high reproductive performance and drought resistance (FARM Africa, 1996; Peacock, 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 local and under the pastoral production system (CSA, 2008). Animal health is very important factor in goat's production like in all other livestock systems. High economic losses ranging from death of more than 50% of the flock, to slow and progressive isolated cases of mortalities or, increases morbidity (Peacock, 1996). Inadequate nutrition, health problems, low genetic potential and traditional production systems are major constraints of goat production in Africa (Ademosum, 1994).

Contagious Caprine Pleuro pneumonia (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 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. CCPP is characterized by 100% morbidity and 60-100% mortality. The severity and the pathogenecity of the Mycoplasma disease depends on the simultaneous infection along with other micro organisms such as *Mannheimia hemolytica* and *Pasteurella multocida* have also been

associated with pleuropeumonia in goats, although experimental evidence of the pathogenecity in this host is inadequate(Radostits, *et al.,* 2002).

The presence of CCPP in Ethiopia has been suspected since 1983. It was confirmed later in 1990 by isolation and identification of F-38 (Thiacourt *et al.,* 1992). Since then the disease is becoming endemic in the different regions of the country. It is more prevalent in the arid and semi-arid lowland area of the Rift Valley, Boran range lands, South Omo, Afar and other pastoral areas and imposes severe losses in goat population (Gezahgn, 2006).

Sero prevalence rate from different authors varies from 6% to 77% in different parts of the countries. The wide variation 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 the sero prevalence of the diseases.

Therefore, with the assumption of no regular vaccination so far for CCPP, lack of stability in the area, significant number of goat population with recurrent drought; this study was planned with the following objective:

- To Study Sero prevalence of Contagious Caprine Pleuro pneumonia of goats with participatory approach in Gulina wereda.

2. LITERATURE REVIEW

2.1. Mycoplasma

2.1.1 Taxonomy and Morphology

The Mollicutes are member of the Order Mycoplasmata 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 members of in these order, however, the trivial name mycoplasma is also used (Levinsohn, 1992; Walker,1999). The members of the genus Mycoplsma (class Mollicutes) are the smallest organisms capable of self replication. Mycoplsmas DNA is poor in Guanine (G) and Cytosine (C), 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 and are nearest known mycoplasmas are parasites, which usually exhibits a rather strict host and tissue specify and many of them are clinical importance in human and veterinary medicine (Bolske, 1995; Walker, 1999): Mycoplasma comprises 200 species of class Mollicutes. Five distinct groups of mollicutes were identified by phylogenetic analysis of the 16S rRNA sequences, one of which, the Spiro-plasma group, contains *M. capricolum. capripneumoniae* which has been subdivided within the *M. Mycoides cluster. M. Mycoides cluster* are pathogens of ruminants and comprises six closely related subspecious that are subdivided in to two sub groups based on genetic similarity. The Capricolum sub group includes *M. capricolum subsp. capricolum*, *M. capricolum subsp. capripeumoniae*,and *Mycoplasma subsp. bovine group of 7 (BG7)*. The mycoides subgroup consists of *M. mycoides* subsp. *mycoides small colony,*and *M. mycoides* subsp. *mycoides large colony* (Weisberg *et al.*,1989).

This cluster contains six important ruminants mycoplasmas including *M. mycoides* subsp. *mycoides small colony*, *M. mycoides* subsp. *mycoides large colony* and *M. mycoides*

subsp.*capri* which cause MAKePS share cluster immunological and biochemical properties (Nicholas, 2002).

The mycoplasmas are non sporulating Gram negative, non – motile bacteria, which do not possess a determined shape of the cell. There are no internal membrane structure and no cell wall except plasma membrane however; many strains possess surface structure equivalent to a capsule with the exception of *Acholeplasma. Mycoplasmas* depend on supply of intact cholesterol which they incorporate in to the membrane creating sufficient osmotic stability for survival under normal physiological conditions. The *Acholeplasma s*ynthesizes cholesterol as substitute for cholesterol but incorporate cholesterol if it is provided (Quinn *et al.,* 1994).

Mycoplasma polymorphism is the consequence of missing cell-wall. Mycoplasmas are not only devoid of cell wall but the genetic capability to produce one, which also renders them completely resistant to β-lactam and other cell wall activity drugs. Due to their small size are able to pass through the usual bacteriological filter (0.1-0.3nm). The cell shapes include spherical, pears heaped, spiral and filamentous form (Quinn *et al.,* 1994). Cell sometimes appears as chain beads as a result of a synchronized genomic division (Gezahegn, 2006).

2.1.2. Growth characteristics

The mollicutes grow slowly and generally require 3 to 6 day incubation before colonies are apparent. Growth is best at 37^0 in atmosphere of increased CO_2. Sterols are required by all genera except *Acholeplasma* and *Anaplasm*a. Most genera are facultative anaerobes except *Anaeroplasma* and *Asteroplasmas*, which are obligate anaerobes. Optimum PH for growth ranges from 6.0 *Ureaplasma* and 7.5 to other Mollicutes. Colony sizes Vary from 0.1 mm to 1.0 mm. When observed with dissecting microscope, many species exhibit "fried egg" morphology (Jones, 1992; Adehana *et al*., 2004). The umbonate appearance is as a result of the central portion of the colony embedding in to 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.*, 2004).

2.1.3. Host Specificity

Another characteristic in mycoplasma infection is the high level of host specificity. Usually a mycoplasma species will infect only a single or few closely related animals' species. It has been suggested that the degree of host specificity is directly related to the intimate nature of the parasite relationship and dependence of the infecting mycoplsma on the host cell component to fulfill its fastidious nutritional requirements for successful colonization host may also be related to ability of mycoplasma to successfully invade the cellular host defense mechanism, which differ in various cell types (Levinsohn, 1992).

2.2. Mycoplasma species in goats

2.2.1. *Mycoplasma mycoides* sub species *capri* (Mmc)

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

2.2.2. *Mycoplasma mycoides* sub species *mycoides* large colony (MmmLC)

Mycoplasma mycoides sub species *mycoides* large colony (MmmLC) has one of the widest geographical distributed ruminant mycoplasmas, being found on all continents (Bolske *et al.*, 1988). The gross lesions seen include lung lesions with edema and

thickening of the interlobular septa. There may be septicemia accompanied by the enlarged spleen and the lesions present in several organs. Microscopically the alveoli contained mononuclear cells and scattered granulocytes, which also fills the bronchioles (Bolske *et al.*, 1988).

2.2.3. *Mycoplasma capricolum* sub species *capricolum* (Mcc)

The natural infection can be characterized by MAKePS syndrome including Mastitis, Arthritis, Kerato conjunctivitis manifestations in various organs appear after an initial septicemia; a stage which can be fatal for kids and adult animals in poor condition (Perreau and Breed, 1979).The gross pathology is confined to the joints and lungs. The lungs showed interstitial pneumonia and purulent bronchopneumonia. Interlobular septa were broadened due to edema and cellular infiltration mainly by mononuclear cells. The alveoli contained increased number of alveolar macrophages and there was marked peri bronchial lymphoid hyperplasia (Bolske *et al.,* 1988).

2.3. CCPP

2.3.1. History

Contagious caprine pleuro pneumonia (CCPP) was first described in 1873 in Algeria by Thomas and known under local name of "boufrida" because, in majority of diseased goats, only one lung was affected (McMartin *et al.*, 1980). A major outbreak is 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. *capripneumonia* (Mccp). This organism closely related to three other Mycoplasma species: *Mmm*LC, *Mmc* and *Mcc*. Unlike true CCPP, which is confined to the thoracic cavity, the disease caused by them is accompanied by prominent lesions in organs and 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 the identification of the strain F-38 by MacOwan (1976) and the subsequent

demonstration of its causal relationship with F-38 strain (MacOwan and Minette, 1976), Mycoplasma strain F-38 biotype is the only mycoplasma that fulfils Koch's postulates for CCPP and is believed to be the sole cause of CCPP (MacOwan, 1984).

2.3.2. Etiology

The causative agent of Contagious caprine pleuropneumonia is *Mycoplasma capricolum* subsp. *capripneumonia* (MccP), which was previously known by the strain name of its type species, F38 (Leech *et al.*, 1993).

2.3.3. Epidemiology

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 adult (Thiacourt and Bolske, 1996; Wesonga *et al.*, 1998).

Contagious caprine pleuropneumonia is transmitted by direct contact through inhalation of infective aerosols. Of the two known causative agents, *Mycoplasma capricolum* subsp. *capripneumonia* is far more contagious. Outbreaks of the disease often occur after heavy rains after the stress of sudden climatic change. 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 Bolske, 1996; Wesonga *et al.*, 1998).

In natural infections, susceptible goats acquire the micro organisms by inhalation of contaminated droplets from infected goats (MacOwan, 1984) while the clinical diseases has been reported in nearly 40 countries in Africa and Asia, *Mycoplasma capricolum* subsp. *capripneumonia* (MccP) has only been isolated in 13 countries (Table 1) because few have the facilities for isolating and growing mycoplasma (Nicholas, 2002). The only

African countries where *Mycoplasma capricolum* subsp. *capripneumonia* has been isolated are Chad (Lefevere *et al.,* 1987a), Eritrea, Ethiopia (Thiaucourt *et al.*, 1992), Kenya (MacOwan and Minette, 1976), Niger and Sudan (Harbi and El-Tahi, 1981), Tanzania (Kusiluka *et al.*, 2000), Tunisia (Perreau *et al.*, 1984), and Uganda (Bolske *et al.*, 1996).

The only reports of suspected CCPP in Europe date back to the 1920 when an outbreak occurred in Greece following the seizure of goats form Turkey. Goncalves (1982) reported a disease in goats in Portugal in 1980 which very closely resembled classical CCPP but from which *Mycoplasma mycoides* sub species *mycoides* large colony was isolated. In 1996, a suspected outbreak of pleuropneumonia clinically resembling CCPP was investigated in herd in England contains some imported goats and which had suffered severe respiratory disease resulting in many deaths. There have been no reports of the isolation of *Mycoplasma capricolum* subsp. *capripneumonia* on the American continent although other members of cluster have been described there (Nicholas, 2002).

Table 1: Prevalence of CCPP in different Parts of the World

	Confirmed by isolation of mycoplasma	
Africa	Chad, Eritrea, Ethiopia, Kenya, Niger, Sudan, Tunisia, Uganda, Tanzania	Algeria, Burkina Faso, Benin, Cameroon, Central Africa Republic, Djibout, Egypt, Libya, Mali, Nigeria, Somalis, 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 Clinical Signs

The classical disease as caused by *Mycoplasma capricolum* subsp. *capripneumonia* is purely respiratory illness. It is characterized by a fever of 106^0F (41^0c), coughing, and a distinct loss of vigor, Affected goats have labored breathing; later they may grunt or breath in obvious pain. Frothy nasal discharges and stirringly salivation are often see shortly before death. In the acute disease, which occurs in fully susceptible populations of goats, each occurs within 7-10 days of the onset of clinical signs (Thiacourt *et al.*, 1996; Mare, 1996). The disease causes interstitial, fibrinious pleuropneumonia, interlobular edema and hepatization of the lung causing high mortality rates of up to 80%. In fully susceptible flocks after outbreaks occur, morbidity reaches usually 100% and mortality of 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 carrier of the mycoplasmas (Gezahegn, 2006).

2.3.6. Pathogenesis

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 involved both, and an entire lobe may become solidified. The pulmonary pleurae become thickened, and there may be adhesions to the chest wall (Thiacourt *et al*, 1996).

The generalized lesions described include encephalitis, meningitis, lymphadenitis, spleenitis, 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 reflected various stages of fibrinous pneumonia. Extensive pleurisies are usually present, and various stages of hepatizaiton and marked dilation of interlobular septa is commonly seen. The cardiac and diaphragmatic lobes are the ones 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). CCPP-diseased lung

resembles "somewhat granular looking liver", which is described as massive hepatization seen in CCPP lungs. In sharp contrast, *M. mycoides subsp. capri* has been reported to cause lesions in a wide variety of organ systems and to produce lung lesions closely resembling those seen in CBPP.

Histological examination of the lung tissues may show acute serofibrinous to chronic fibrinonecrotic pleuropneumonia with infiltrates of serofibrinous fluid and inflammatory cells, mainly neutrophils, in the alveoli, bronchioles, interstitial septae and sub pleural connective tissue. Interlobular edema is more prominent but interlobular edema has also been reported. Peri bronchial and peri bronchiolar lymphoid hyperplasia with mononuclear cell infiltration is also present (MacOwan and Minette, 1976; Ojo and Obi, 1996; Wesonga *et al.,* 1998).

2.3.7. Diagnosis

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 hepatization and pleural adhesions warrants a field diagnosis of CCPP.

In the field, definite diagnosis cannot be established on clinical signs or on postmortem examinations alone. In classical acute CCPP, a high mortality and typical early thoracic lesions in goats are highly indicative of *Mycoplasma capricolum* subsp. *capripneumonia* infection, but all cases of caprine Mycoplasmosis need additional laboratory tests to establish a presumptive diagnosis.

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 an aseptically and if possible, placed in transport medium (heart infusion broth, 20% serum, 10% yeast extract, benzyl

penicillin at 250-1000 IU/ml), and kept cool and shipped on wet ice as soon as possible. If possible, placed in transport to laboratory is delayed more than a few days, samples may be frozen Blood can be collected for serum (Quinn *et al.,* 1994).

Serological tests

Several serological tests can be used in the laboratory for the detection of antibodies to the *Mycoplasma capricolum* subsp. *capripneumonia.* Antibody detection is not very useful for the detection of a new outbreak. 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 (Bolske *et al*., 1988).

IgM is the first to appear and has little specificity, which gives rise to pronounced cross reaction and remains in the blood for short duration while IgG is produced lately and lasts much longer than IgM and also more specific than IgM antibody isotopes (Staak *et al*., 2001). 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 *Mycoplasma capricolum* subsp. *capripneumonia* infection and it has been found to be specific, though less sensitive than indirect Hemaggluation (IHT) test (MacOwan, 1976; OIE, 2000). In comparison of blocking ELISA using monoclonal antibody and CFT using *Mycoplasma capricolum* subsp. *capripneumonia* and *Mycoplasma mycoides* sub species *mycoides* small colony antigen, blocking ELISA was found more sensitive to detect CCPP antibody (Sharew *et al*., 2005).

The direct and indirect fluorescent antibody test 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. Immunobinding on memberane filtration (MF dot) and dot blot on nitrocellulose paper are used to identify species of

Mycoplasma. They are rapid ready standardized and allow running many samples at time (Poumarat *et al.*, 1991; Poumarat, 1992).

Competitive 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 monoclonal antibody (Mab) that competes 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 show that sero conversion did not occur whatever test was used. Incase 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 *Mycoplasma capricolum* subsp. *capripneumonia* with exception of the PCR, Show cross-reactivity with bovine sero group of including a monoclonal antibody based ELISA (Thiacourt *et al.*, 1994).

Isolation and Biochemical Characterization

Colony Morphology and growth characteristics may be of some help for the differentiation of mycoplasmas. Biochemical characterization and growth inhibition with known antisera are the main tools to identify the species of mycoplasmas although, more modern techniques like PCR and restriction enzyme analysis could reveal the confirmative diagnosis (Bolske *et al.,* 1996). Biochemical tests cannot identify an isolate unequivocally, which at present can only be done by serological or genetic means. Interspecies variation in some biochemical reaction 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 digitonin, phosphatase activity, protein digestion, arginin, 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; Bolske *et al.*, 1995; Nicholas, 2002).

Glucose fermentation, arginin hydrolysis, and film and spot fermentation tests are performed routinely in isolation and cultivation procedures. The member of the M. mycoides cluster strains of *Mycoplasma capricolum* subsp. *capripneumonia* appear not to metabolize sugars depend up on organic acids 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 arginin 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).

Table 3: Biochemical reactions of the Mycoplamas of goats and sheep

Strain	Glucose Fermentati	Arginine hydrolysi	Terazolium Pero/ana	Phosphate Se activity	Casein Digestion	Digestion Sensitivity
MccP	+/-	-	-/+W+V	-	+	+
MmmLC	+	-	+(+)	-	+	+
Mmc	+	-	+(+)	-	+	+
Mcc	+	+	+(+)	+	+	+
M. ovipneumuniae	+	-	W(+)	-	-	+
M. arginini	-	+	-(+)	-	-	+
M. agalaetiae	-	-	+(+)	+	-	+
M. Putrefaceiens	+	-	V	+	-	+
M. conjunctivae	+	-	-(+)	-	-	+

Source (Nicholas, 2002) V= variable, + = Postivive- = Negative, W= weak, aero= aerobic, ana= anaerobic.

Nucleic acid recognition methods

Diagnosis systems based on polymerase chain reaction (PCR) have been developed for the rapid detection, identification and differentiation of *Mycoplasma capricolum* subsp. *capripneumonia*. Polymerase chain reaction is used to amplify conserved segment of the 16S rRNA gene of the mycoides cluster. Ribosomal RNA offers the likely hood a finer identification of the strain circulating in the region. This tool can make use of full contribution to understanding the epidemiology of the CCPP (Lorenzon *et al.*, 2002). The PCR product is analyzed by restriction enzyme cleavage for the identification of the *Mycoplasma capricolum* subsp. *capripneumonia* amplification. The test is used directly on clinical materials such as lung tissue and pleural fluid. A DNA probe that differentiates *Mycoplasma capricolum* subsp. *capripneumonia* remains the confirmatory test (Taylor *et al.*, 1992; Bashiruddin *et al.*, 1994; Bolske *et al.*, 1996b; Stakenburg *et al.*, 2005).

2.3.8. Treatment

Successful treatment varies with affected site and time course of the disease. Commonly employed antibiotic includes tetracycline, spiramycin, erythromycin and tiamulins fumarate. In early treatment the prognosis is good (Walker, 1999). The prognosis for recovery with prompt treatment is approximately 8.7% and then animals recovered from clinical diseases may remain carrier (Onvorian, 1974; El-Hassen *et al.*, 1984).

After treatment of tetracycline and streptopencillin to infected goats for three consecutive days bring recovery within minimal pathological lesion. Treatment is ineffective after long periods of infection (Bereket, 1995).

2.3.9. Vaccination

Vaccine inactivated with saponin that protects goats against CCPP for approximately a year has been developed in Kenya. A single immunization with optimum formulation of 0.15mg of mycoplasma in saponin gives a protective immune response in goats that using sonicated *Mycoplasma capricolum* subsp. *capripneumonia* antigens in Freud'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).

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted in Afar pastoral areas, specifically in Gulina Wereda between October 2007-April 2008.

This study was conducted in Afar National Regional State which is situated in the North-eastern part of Ethiopia at $8^0$40' to $14^0$47N' and $39^0$51' to $41^0$23'E. The whole state lies in the Rift Valley. Topographically, the state is bordered by Eritrea in the North and North-east, Djibouti in the West, Tigray Region in the North-west, Amhara Region in the West, Oromiya Region in the South and Somalia Region in the South-east (Figure1). Administratively, this state, which covers a total area of 85,410km^2 is divided into 5 zones which are further subdivided into 29 weredas. The state capital, 'Semera', is located about 600km North-east of Addis Ababa in the main Addis-Djibouti road. The total human population of the region is estimated at 1.3 million. Over 90% of this population is composed of livestock-dependent pastoralist while the rest 10% are agro-pastoralists (Gezahgn, 2006).

The study area, Gulina, has an altitude that ranges from 1500 to1800 m.a.s.l. Its mean daily temperatures vary between 24 to 37^0C. The region receives three rainy seasons. The main rain season, "Karma", occurs between July and August followed by sporadic rainy showers from November to December called "Dedaa", and the short rainy season "Sugum", between March and April. The rainfall distribution also varies with an erratic bimodal pattern of 200-650 mm. In general the rainfall coverage is inconsistent and has wide variation in the amount of distribution within the state (Gezahgn, 2006).

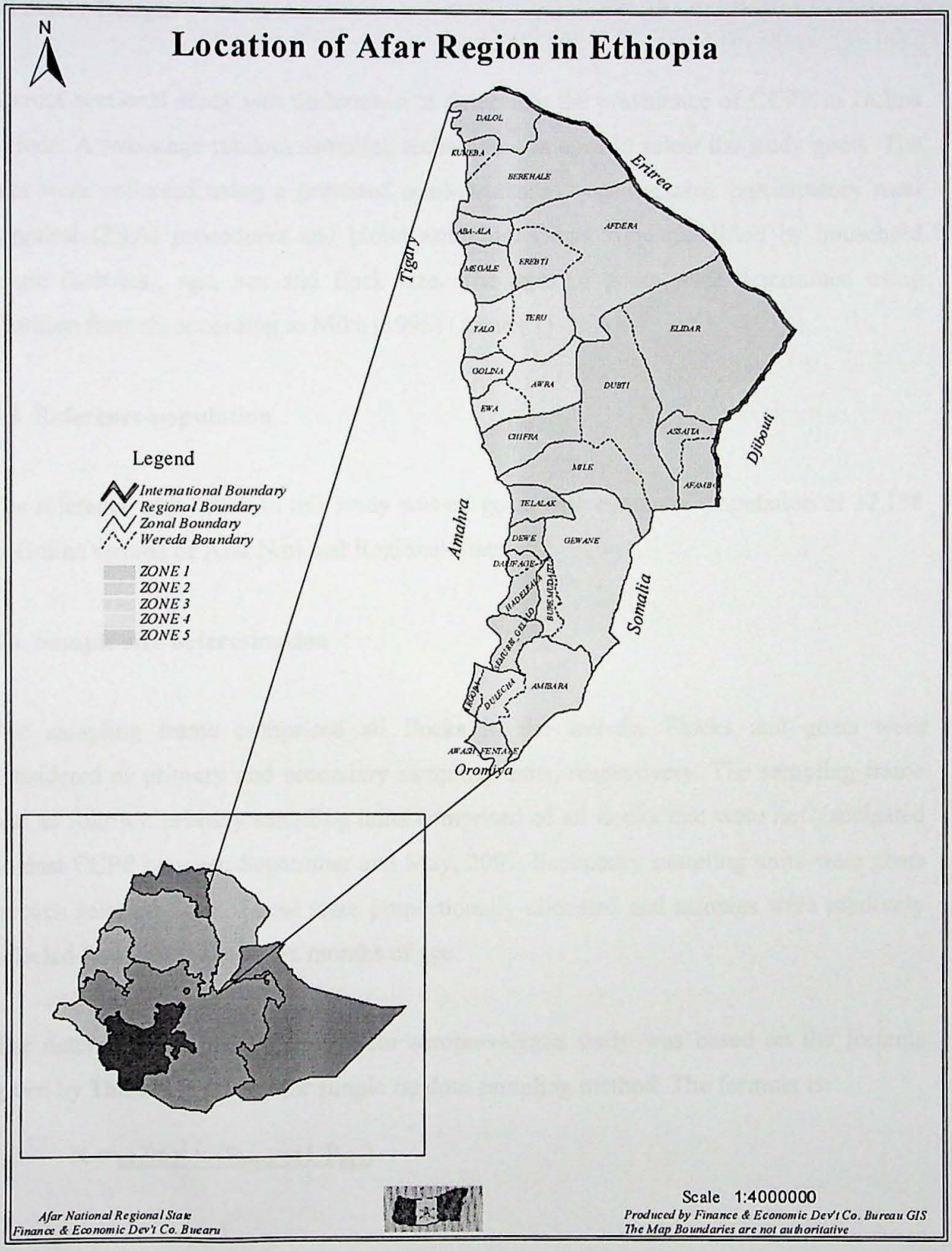

Figure 1: The Map depicts the Afar Region (Gezahgn, 2006)

3.2 Study Design

A cross-sectional study was undertaken to determine the prevalence of CCPP in Gulina wereda. A two-stage random sampling technique was used to select the study goats. The data were collected using a pretested semi-structured questionnaire, participatory rural appraisal (PRA) procedures and blood sampling. Goats were identified by household origin (kebeles), age, sex and flock size. The ages of goats were determined using dentition formula according to Mike (1996) (Annex 1).

3.3. Reference population

The reference population in this study was all goats with estimated population of 37,158 in Gulina wereda of Afar National Regional State.

3.4. Sample size determination

The sampling frame comprised all flocks in the wereda. Flocks and goats were considered as primary and secondary sampling units, respectively. The sampling frame was as follows: primary sampling units comprised of all flocks that were not vaccinated against CCPP between September and May, 2007. Secondary sampling units were goats in each selected flock. These were proportionally allocated and samples were randomly selected from goats above six months of age.

The determination of sample size for seroprevalence study was based on the formula given by Thursfield (1995) for simple random sampling method. The formula is:

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

Where: n = sample size, P_{exp} = expected prevalence and d = absolute precision

A study of CCPP in Afar pastoral areas using CFT showed an over prevalence of 15.46% (Gezahgn, 2006). Thus, 16% was used as the expected prevalence. Using the 95% confidence level and an expected prevalence of 16% and 5% absolute precision a sample size of 206 goats was obtained. To minimize chance of random errors and to increase the precision, this number was multiplied by 2 resulting in a sample size of 412. A further 34 goats were conveniently added. The final sample size was 446.

3.5. Questionnaire survey

A questionnaire survey was conducted in every settlement areas during sampling of study goats. The questionnaire was a pre-tested semi-structured type and generally covered information on the name of the owner, location, livestock owned, knowledge about the CCPP, movement of livestock and the purpose of keeping livestock (Annex 2).

3.6. Participatory CCPP Appraisal

Livestock owners were interviewed in groups and individual interviews. Informants assisted in selecting elders who could give information about the CCPP disease. Groups of six persons were interviewed. To validate the knowledge of the community about the disease, 12 groups were interviewed. Proportional piling, pair-wise comparisons, seasonal calendars for diseases and movements, simple and matrix-scoring techniques about indicators were used in all selected settlement areas. To perform participatory disease search, the community or clan leaders and community members were identified and orientations were given about the objective of the study intended.

Among communities, at least one language translator was used. Groups of informants were selected from the herders. The program was started by the local culture known as "Dagu", local information exchange. After general discussions and information exchange with the local communities, they were questioned on issues related to their livestock in general and particularly the animal health problems. All problems were listed. Specific disease survey was conducted. For detailed and further survey, particular attention was

given to the "communal disease of interest". The participatory methods used were based on guidelines and manuals by Mariner (2001) and Catley *et al.* (2002). The questionnaire used is indicated in Annex 2.

3.6.1. Clinical Observations of CCPP

The disease was mainly characterized by respiratory illness. Affected goats had labored breathing (may grunt and overt pain). Goats forced to run or rush in the field in order to see whether there was respiratory illness or not. Most of the affected goats fell down during the exercise.

3.6.2. Pair-wise Ranking

Pair-wise ranking of the list of the major diseases was "represented" by locally available objects. The informants were checked for understanding the meaning of named objects. After this, two diseases were chosen at time and compared. Questions like "which of these two diseases was most important was asked." "The reason why that disease was more important" was asked to obtain indicators. The way how on did these two diseases differ was asked. Then listing of the indicators and repeations of such questions were continued until each disease was compared with every other disease. Informants groups counts of that specific disease were taken and then ranking disease done.

3.6.3. Matrix Scoring

Matrix scoring was performed. This involved questions and discussions. By physically pointing to the particular scores, all the indicators associated with a particular disease were summarized. Open, leading and probing questions were used to explore the knowledge of the informant groups.

3.6.4. Proportional Piling

The disease names were represented by locally available objects for example stones on the ground. The informants were checked for understanding the meaning of named objects. After collecting 10 stones, the informants were allowed to distribute such stones for one indicator against each disease. All stones were used during distribution. After checking the scoring; we told them if they wish to change the scoring. The proportions of stones allocated were recorded on the note book. Then the procedure was repeated for the next indicators until we finished all the indictors listed."

3.7. Blood sampling and sera collection

Five to ten milliliters of blood sample were collected from the jugular vein of each goat using sterile vaccutainer tubes and needles. The sample was kept in a slanting position for 6-8 hours. The serum was separated from it and transferred to a sterile tube and stored at -20 ^{0}c until required for analysis with CFT.

3.8. Complement Fixation Test (CFT)

The recommended method of CFT by OIE (OIE, 2000) was adapted. Test sera as well as reference positive and negative control sera were decomplemented in water bath at 60 ^{0}C for 30 minutes. Series of two fold dilutions of each test serum were pipetted at 25 µl into µl plate wells. The first two columns of plates were left for positive and negative sera, complement and sheep red blood cells (SRBC). The MccP F38 antigen at 25 µl (1:20) was added into each well, except in wells for serum anti-complementary activity. The details of performing CFT and antigen preparation are given in Annex 3.

The plate was agitated and incubated at 37 ^{0}C for 30 minutes. 25 µl (1:20) of titrated complement was added into each well of test sera at +4 ^{0}C. It was incubated at 37 ^{0}C under constant agitation on orbital shaker for 30 minutes. A 2% one-day-old SRBC was prepared by washing them three times with Veronal Buffer Solution in calcium and

magnesium (VCM) and centrifuged at 2500 rpm for 5 minutes. An equal volume of diluted Amboceptor's (SRBC antisera) at (1:1000) was added to the sensitized SRBC, which brought hemolytic system to 1% instead of 2%. Twenty-five µl of sensitized SRBC (indicator) were pippeted into each well and the plates were sealed to avoid evaporation and incubated at 37 ^{0}C for 30 minutes with constant agitation. The plates were examined for sedimentation and hemolysis. The plates were kept in a refrigerator at +4 ^{0}C overnight so as to allow non-lysed cells to settle. The interpretation was done as given in Annex 3.

Table 4: Lay-out of the micro titer plate used in 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

NS = negative serum control, **HS** = Hemolytic serum control, **PS** = Positive serum control, **Cc** = complement control

3.9. Data Management and Analysis

All data were entered and stored in separate data bases in MS-excel files. Data were screened for proper coding and errors. The latter were corrected prior to performing statistical analysis. All data were transferred to intercooled stata 7.0 (Stata corp. 1984-2001) statistical software. Seroprevalence of CCPP were calculated based on CFT positive results. Flock and animal level seroprevalence were calculated by dividing the number of CFT reactors by total number of tested flocks and animals, respectively.

Univariate logistic regression analysis was used to determine the associations between the potential risk factors and sero prevalence. The strength of association of each putative risk factor and CFT was determined by the magnitude of Odd Ratio (OR). Questionnaire data were analyzed by using descriptive statistics in the Microsoft excel version 2000. Descriptive statistics used were median, frequencies and standard deviations. Data from participatory survey were analysed using simple scoring, matrix scoring, pair-wise comparisons and proportional piling. The SPSS (2000) version 11.5.0 was used to compute Kendall's coefficients of concordance (W).

4. RESULTS

4.1. Questionnaire Survey

According to the assessment of respondent to comparative importance of livestock species: camels had the highest proportion (44.6%) followed by goats (30.6%), cattle (8.3%) and of sheep 16.7% (Table 5).

Table 5: Proportional distribution of the relative importance of different livestock species

Relative importance of livestock species	Counts (number)	Percentage (%)
Camels	32	44.4
Goats	22	30.6
Cattle	6	8.3
Sheep	12	16.7

N.B: Number of informants groups, was 12 with an average 6 persons per group.

Of the 38.9% respondents mentioned, the possible source of CCPP as "watering points" followed by "grazing points" (31.9%). The justification pointed by groups was that goats gathered from different parts for the search of feed and water (Table 6).

Table 6: Frequencies and associated proportions of the presumed major sources of CCPP by informant groups

Major source	Frequency	Proportion (%)
Watering	28	38.9
Grazing	23	31.9
New introduction	14	19.4
Market center	7	9.7

Based on recall of the past experience, whenever any outbreak of diseases (single case) occurred, 34 (47.2%) respondents mentioned, that the main control tactic they applied was isolation of the affected goat(s) while only 2.8% of the respondents indicated that they used vaccination as the least measure (Table 7). While 27.8% and 22.2% used destocking and treatment, respectively.

Table 7: Proportional distribution of control tactics used by informant groups to combat CCPP

Control tactics	Frequency	Proportion (%)
Destocking	20	27.8
Vaccination	2	2.8
Treatment	16	22.2
Isolation of the affected goat	34	47.2

The twelve groups of informants divided a year into three seasons. Long rainy seasons ("karma"), from mid-June to mid-September followed by rainy showers ("Dedda") from mid-December to March and short rainy season ("Sugum") from March to April (Table 8).

Table 8: Categorization and score proportions of the seasonal calendar by informant groups for CCPP and goat movement

Seasons and peak goat movement	Score	Proportion (%)
June-September	22	15.6
December-March	46	60.5
March-April	32	23.9

4.2. Participatory rural appraisal

Participatory rural appraisal (PRA) methods are useful in particular for veterinarians involved in community-based animal health services. However, they are not the only assessment methods. They are useful where professionals adopt respectful, sensitive, and open approaches to communities (Catley *et al.*, 2002). The PRA collects information, using a tool kit comprising of interviewing, ranking, mapping and health condition scoring. In a typical survey, the combination of these tools allows cross-checking or "triangulation" of results while the researchers are still in the field. Results are also cross-checked by working with both men and women, and using information with varying experiences, skills, age, social status and wealth. When investigating Health conditions such as livestock diseases, "local experts" can be identified who are respected by communities for possessing special knowledge (Catley *et al*, 2002). Several types of field

data form the core of PA study. These data include spatial, temporal, social and technical data.

The semi-structured interview (SSI) has some relation with veterinary medicines. The SSIs require one to have a check-list of some questions. For the visualization, mapping has been used in animal health surveys. For temporal data, the 'tools' that have been applied are timeline, trend patterns, and seasonal calendars. Ranking and scoring methods have been widely used whereby informants are required to compare item or problems in pairs and decide the most important. The data are presented in a matrix. Another visual scoring method is proportional piling of stones. The method involves the use of piling of stones (Catley *et al*, 2002).

4.2.1. Clinical examination

Before sera collection, goats have forced to exercise to check their respiratory status. Suspected cases (developed difficult labor breathing or grunting) fell down during exercise, and muzzles dried due to increased body temperature. In severe cases, loss of body weight (emaciated) and starring of hair were clinically observed.

4.2.2. Pair-wise comparisons and ranking

A pair-wise comparison method was used to determine the most prevalent diseases of goats. The result indicated CCPP was the second economically important disease that causes significant losses (Table 9).

Table 9: A pair wise comparison of goat diseases

Goat disease	Mortality	Morbidity	Score	Rank
CCPP	457	432	889	2
PPR	542	371	913	1
Ecto-parasite	91	152	213	4
Pasteurellosis	310	484	794	3

4.2.3. Matrix scoring of diseases

The results of pair-wise comparisons were used for further characterization and selection of PA diseases for matrix scoring. Matrix scoring was used for the assessment of scoring of diseases among 12 informant groups. After identification and prioritization of major diseases, informants described such diseases against indicators (Table 10).

Table 10: Matrix scoring of disease indicator signs for diseases of goats

Indicators	Diseases			
	CCPP	**Pasteurellosis**	**Ecto parasite**	**PPR**
Nasal discharge (W= 0.931, = P<0.0001)	9 (6-12)	6.5 (5-8)	0 (0-1)	5 (5-6)
Chronic weight loss (W = 0.712 , P<0.0001)	8.5 (6-12)	4 (2-6)	3 (0-5)	5 (1-6)
Reduced milk yield (W = 0.776 , P<0.0001)	7.5 (5-9)	4 (2-7)	2 (1-4)	6 (4-8)
Diarrhea (W =0.865 , P<0.0001)	5 (4-8)	6.5 (4-8)	1 (0-3)	8 (5-11)
Erosive stomatitis (W = 0.909 , P< 0.0001)	3 (2-5)	4.5 (2-7)	0 (0-2)	11.5 (8-15)
Tearing and lacrimation (W = 0911 , P<0.0001)	3.5 (1-5)	6 (0-8)	0 (0-2)	10 (8-18)
Abortion (W = 0.921 , P<0.0001)	5 (2-7)	4 (2-7)	1 (0-3)	10 (8-13)
Highly contagious (W = 0.811 , P<0.0001)	8.5 (7-11)	2.5 (1-6)	0 (0-1)	8 (4-1O)
High mortality (W =0.884 , P<0.0001)	8 (7-10)	3 (2-5)	0 (0-1)	9 (7-11)
High morbidity (W =0.911 , P<0.0001)	7 (5-8)	4.5 (2-6)	1 (0-3)	7 (6-10)
Acute/Sudden death (W = 0.926 , P<0.0001)	8 (5-10)	3.5 (1-6)	0 (0-3)	8 (6-10)
Disease not treatable (W = 0.954 , P<0.0001)	8 (6-11)	3 (2-5)	0 (0-2)	8.5 (6-10)
Has no vaccine (W = 0.976 , P<0.0001)	6.5 (6-7)	8 (5-9)	0 (0-1)	6 (4-7)

Number of informant groups 12 (on average 6 persons per group), W = Kendall's coefficient of concordance (P< 0.0001) among informant groups. The median scores and with the respective minimum and maximum scores in parenthesis. Significant agreements (W=0.712 to 0.976, P< 0.0001) were observed among the 12 informant groups for all the disease indicators. These informant groups indicated that disease indicators against goat disease from high to low rank as follows: No Vaccination, Not Treatable, Nasal discharge, acute sudden death, High morbidity, Erosive stomatitis and High mortality, respectively.

4.2.4. Proportional piling

4.2.4.1. Composition of goat flocks

The results of proportional piling method used to assess the goat flock composition with sex and age are shown in Table 11. These results show that the majority (59.7 %) was females followed by males (40.7%). Informants pointed out that the proportions of goats less than 1 year old, between 1and 3 years and older than 3 years were 25.2%, 32% and 42.7%, respectively.

Table 5: The distribution of age-groups in male and female goats within flocks by proportional piling

Age categories and sex composition of goats	Number (n)	Proportion (%)
<1 Year old	289	25.2
Male	118	40.8
Female	171	59.7
1-3 Years old	367	32
Male	149	40.8
Female	218	59.7
>3 Years old	489	42.7
Male	200	40.8
Female	289	59.7

4.2.4.2. Morbidity and mortality of major goat diseases

Twenty piles of stones were given to each informant group in order to assess major goat diseases. Among the diseases that a causal mortality and morbidity and were ranked as follows: Pasteurellosis (33.6%) followed by CCPP (30 %). Significant number of goats died due to CCPP (31.7%) next to PPR (37.6%) (Table12).

Table 6: Major goat diseases based on Mortality and morbidity by proportional piling

Goat disease	Mortality	Proportion (%)	Morbidity	Proportion (%)
CCPP	457	31.7	432	30
PPR	542	37.6	371	25.8
Ecto parasite	91	6.3	152	10.6
Pasteurellosis	310	21.5	484	33.6

No. of informant groups were 12, with an average of 6 person per group.

4.2.4.3. Major challenges to goat production

Forty eight (65%) respondents indicated drought is as the major challenge to their livestock and that recurrent drought always accompanied by diseases (26.4%) due to the "shortage" of feed and water. Conflicts were among pastoralists and neighbor wereda were also a challenge (Table 13).

Table 7: Major challenges to livestock production

Major challenge	Frequency	Proportion (%)
Drought	48	65
Conflict	5	8.6
Disease	19	26.4

4.3. Serology

4.3.1. Overall seroprevalence of CCPP by CFT

The overall seroprevalence was 42.8 % (Table 15). The specific age-group seroprevalences were 42.4%, 36.8% and 49% in age group less than 1, 1-3 years and > 3 years older, respectively. Sex-specific seroprevalence observed was 40.9 % in males and 43.7% in females as shown in Table 15. Because of few males compared to females in each flock, the contribution of males to the total sero positive was only 32.9%.

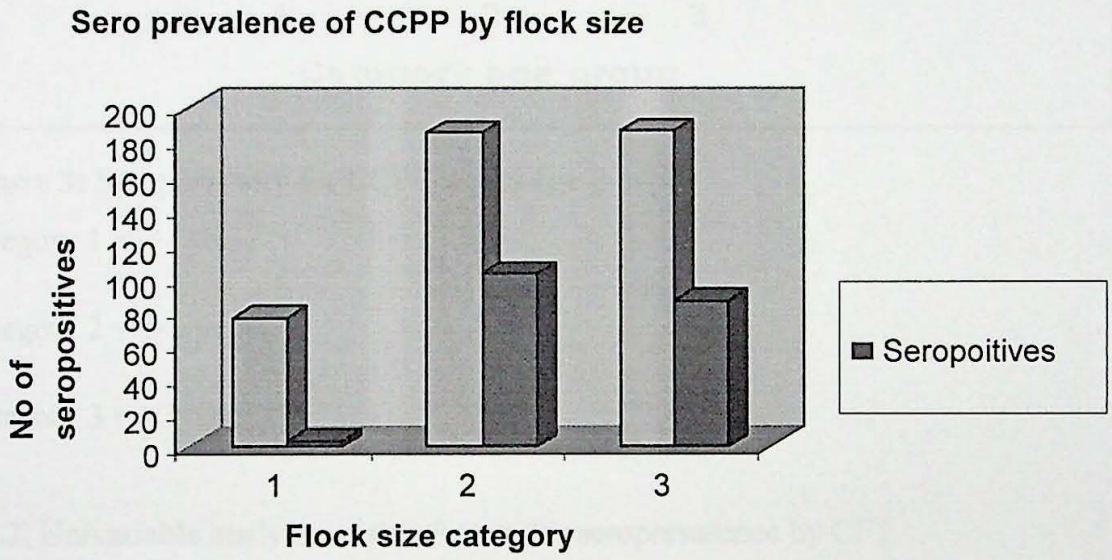

Figure 2: Sero prevalence of CCPP by flock size

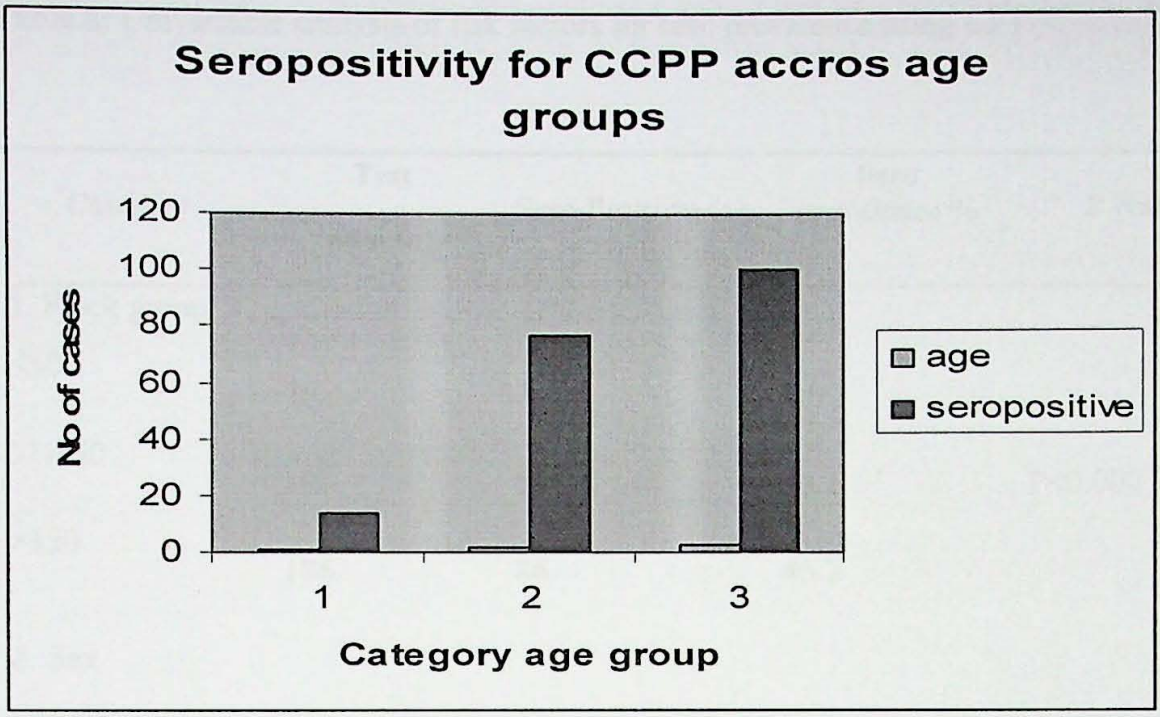

Figure 3: Seropositivity for CCPP across age groups

Category 1 = < 1year

Category 2 = 1-3 years

Category 3 = >3years

4.3.2. Univariable analysis of risk factors for seroprevalence by CFT

Univariable logistic regression was conducted for the assumed potential factors which could have epidemiological importance of the results are shown in table 14. The effects of age, sex and flock size on the seroprevalence of CCPP were analyzed.

Table 8: Univariable analysis of risk factors for sero prevalence using CFT

Category	Test sera (n)	Sero-Positives (n)	Sero prevalence %	P -value
1. Flock group				
<50	76	3	3.9	
51-150	185	102	55.1	P<0.000
>150	186	86	46.2	
2. Sex				
Female	293	128	43.7	
Male	154	63	40.9	p>0.05
3. Age group				
< 1 year	33	14	42.4	
1-3 years	209	77	36.8	P<0.000
> 3 years	2O5	100	49	
Total	446	191	42.8	

Flock size (P= 0.000) had significant effect on the occurrence of CCPP. The Age groups were important factors for the transmission of the CCPP (P= 0.000). However, sex has no effect on CCPP or the disease affects equally both male and females (P>0.05).

4.3.3. Multivariable logistic regression analysis of risk factors for CCPP seroprevalence by CFT

Those factor that having associations were used in analysis. These enabled to exclude confounding effects of the different intrinsic and extrinsic factors. Results of the logistic regression of risk factors associated with CCPP seropositive by CFT.

Table 9: Multivariable logistic regression analysis of risk factors for CCPP seroprevalence by CFT

Variable	Odd Ratio (OR)	P-Value	95% CI for OR	
Flock size			Lower bound	Upper bound
< 50 goats	3.4	0.065	0.976	0.238
51-150 goats	25.6	0.000	0.014	0.151
> 151 goats*	-	-	-	-
Age group				
< 1 Year	0.974	0.324	0.301	1.486
1-3 Year	8.4	0.004	0.348	0.815
> 3 Year*	-	-	-	-

* Reference group

Strong significant associations between flock size 51-150 (P=0.000) and CFT sero positivity for CCPP were observed, as one positive case is enough to infect the whole goat in flock size 51-150 due to overcrowding (lack of ventilation). In the mean while, goats of less than 1 year were found to be more safer (P=0.324) as they kept home.

The results shown no significant associations between flock size category of less than 50 goats (OR= 3.4, p=0.065). Also they show OR=25.6 for flock size of between 51-150 goats indicating very high significance (p=0.000). As for the age-group categories significant association was observed in age group 1-3 years (OR=8.4, p=0.004).

5. DISCUSSION

In this study the participatory disease search methods have shown that the afar pastoralists have the knowledge and skills to prioritize the prevalent diseases according to their severity and economic importance. Relatively camels had the highest proportion followed by goats. This proportional classification was based on the importance of the livestock species, (Sources of food and cash), socio-economically, and culturally (as prestige and wealth status) over the others. In addition to these, they are relatively less affected by the drought.

The informant study groups indicated that among the different sources and transmission routes of CCPP infection were watering points and mixed animal grazing as the two major sources. In extensive husbandry, communal grazing and watering points are the major pathways for disease transmission (Siefert, 1996).

The same groups scored CCPP as the second problem of goat rearing among other health problems. They also identified the most important seasons when the CCPP outbreaks occured. During "Sugum", drought is very common and this predisposes goats to CCPP infection. This is in agreement with Gezahgn (2006) who reported similar findings in 3 zones of Afar regional state.

The current study recorded an overall CCPP sero prevalence of 42.8%. In contrast to studies by Gezahgn (1993) and Mebratu (1988) reported sero prevalence of 51.8% and 50% respectively. Their findings are very close to that of MacOwan and Minnett (1976) who obtained 53.1% sero prevalence by CFT. Sharew *et al.*, (2005) reported a sero prevalence of 77% using CFT from field samples in CCPP endemic areas in Ethiopia. The higher sero prevalence does not agree with that of Gezahgn (2006) who reported low sero prevalence of 16% in 3 different zones of Afar National Regional State. The sero prevalence recorded in this study most likely was due to cross-reactivity between members of *M. mycoides* complex which infects goats such as *Mmm*LC, *Mcc*, *Mmm*SC

(Bolske *et al.*, 1988), Thiacourt *et al.*, 1994; Kusilka *et al.*, 2000 and Sharew *et al.*, 2005. Further, use of CFT gives the result of recent infections since it only detects IgM, antibodies that are produced immediately post infection.

A variety of studies carried out in many countries have shown that all ages of goats are equally susceptible to CCPP (Nicholas, 2002; Lefevre *et al.*, 1987b; MacOwan and Minnette, 1976). Variations among age-groups in this study could have occured due to the inclusion of a few numbers of older goats relatively to young ones. Movements of goats especially adults away from homestead for search of water and pasture probably exposes them to infection. Although, all ages are equally susceptible to CCPP infection, the chances of being infected increases due mixed goats from different households during these long distance movements. Likewise, Solomon (2005) reported that large flocks were more affected with CCPP than smaller ones. This may due to the fact that Mccp infection regulations both close proximity to the sources of infection and large susceptible population (Lefevre *et al.*, 1987).

There are several advantages and disadvantages of PA methods compared to conventional methods (Catley and Mariner, 2002; Mugenda, 1999). The use of PA tools normally generate largely qualitative data that are detailed, dynamic defines local peoples' problems and solution where as the conventional methods only yield data that are chiefly quantitative and stable (data whose facts do not change). Thus the PA methods have been quantified as a bottom approaches compared to the top-down approach of the conventional approach.

The PA approaches are holistic in nature where by all aspects of the phenomenon questions are studied by the use of multiple methods in a process referred to as triangulation. The focus of conventional method is usually on selected predefined variable. The community has a greater access, control, understanding and analysis of information, when PA tools are employed, but in conventional ones, the community does not apparently own information. In addition, this makes the project unsustainable, because researchers in conventional methods are seen as 'outsiders' and, decision-making

is entirely from them. In contrast, the PA methods attempt to break the cultural barrier and the researchers seen as 'insiders' which in turn, lead to collection of useful information about the community, particularly with involvement of local people in decision making.

6. CONCLUSSION AND RECOMMENDATIONS

In participatory assessment results, CCPP, Pasteurellosis and ecto-parasites were indicated as the major goat diseases in study area. The detection of *M. capricolem* subsp. *Capripnumonia* antibodies by CFT from goats indicated that CCPP was widely distributed in the study wereda.

Based on the above study the following recommendations are forwarded:

- Further study should be conducted to assess epidemiological patterns of pathogenic *Mycoplasma* spp. involved in caprine pleuropnemonia.
- Participatory disease search (PDS) strongly recommended for use in animal disease diagnosis and investigation processes in pastoral areas.
- Since vaccination is cost-effective to control contagious caprine pleuropnemonia, annual vaccination programs should be regularly implemented.
- Legal control of livestock movements should be effectively implemented and well coordinated with in the Afar state and neighboring states.

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8. ANNEXES

Annex 1. Determination of 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 pairs 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. Questionnaire for CCPP Survey

PA name ……., Kebele…... Date of Sample collection ……

1. Owners name……, Sex……..... Age……, Ethnic…..
2. Address: Region ……., Zone……, Wereda……., PA……
3. What is the comparative importance of keeping livestock specious on the basis of Diseases and drought resistance, Marketing, Socio-Economic Value (Camel, Cattle, Sheep, Goat)?
4. What is flock composition of goats on age basis? <1 year, 1-3 year, >3years old.
5. What is the major goat disease according to their importance?
6. Is the disease present in your / neighbored currently?
7. Which sex is more affected by CCPP, male or female?
8. What are the possible sources of CCPP? Watering point, Grazing point, new introduction, and marketing.
9. What measures do you take to combat the disease? Destocking, Vaccination, Isolation, Slaughtering.
10. At what season of the year animal movement is common?

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Annex 3. 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* (F-38) 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 tests and sheets of plate lay out for records.

Procedures: The OIE standard test procedures of O.I.E. (2000) were adopted.

1. Test sera including positive and negative control sera control were decomplmented in hot water bath at 60^0c for 30 minutes.
2. Series of two fold dilutions of test sera pipetted 25 micro liter per well. The first two column were left for control of the Positive and Negative serum, Complement and SRBC that was hemolytic system.
3. Twenty five micro liter (1:20) antigen was added in to 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. Twenty five micro liter of titrated complement were added in to the wells of test sera. It was incubated under constant agitation on orbital shaker for 30 minutes.
5. Two percent of 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 sensitize the red corpuscles, which brings hemolytic system to 1% instead of 2%.
6. Twenty five micro liters SRBC (indicator) was pipetted in to each well and the plates were sealed to avoid evaporation then incubated at 37 ^{0}c for 30 minutes with constant agitation.
7. The plates were examined for sedimentation 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 reaction in sedimentation of SRBC and its hemolysis indicate negative reaction. The results were ranked according to the extent of SRBC sedimentation hemolysis (-), weak (+), moderate (++) and strong (+++).When the sedimentation in test serum and control serum for anti complimentary (without *Mccp* antigen) were equal the test serum was taken as negative.

9. SIGNED DECLARATION SHEET

I, the undersigned, declare that the thesis is my original work that has not been presented for a degree in any other university, and that all sources of materials used for the thesis have been dully acknowledged.

Name: Amare Getu

Signature _____

Date of submission June 2012

The thesis has been submitted for examination with my approval as university advisor

Advisor Signature

Dr. Tesfaye Sisay _____