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ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

EPIDEMIOLOGICAL SURVEY OF CONTAGIOUS CAPRINE PLEURO-PNEUMONIA IN SOUTH OMO AND GAMO GOFFA ZONES, SOUTHERN ETHIOPIA

BY
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A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University as a partial fulfillment of the requirements to the degree of MSc in Tropical Veterinary Epidemiology

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Epidemiological survey of Contagious Caprine Pleuropneumonia in South Omo and Gamo Goffa zones, southern nation nationalities and people regional state, Ethiopia

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ABBREVIATION

bp	base pair
CCPP	Contagious Caprine Pleuropneumonia
cELISA	competitive Enzyme Linked Immuno Sorbant Assey
CFT	Complement fixation tests
CPS	Capsular Polysaccharides
Mab	Monoclonal antibody
MAKePS	Mastitis, Arthritis, Keratitis, Pneumonia, Septicemia
MccP	*Mycoplasma capricolum subsp. capripneumoniae*
Mmc	*Mycoplasma mycoides subsp.capri*
MmmSc	*Mycoplasma mycoides* subsp. *mycoides* small colony
MoA	Ministry of Agriculture.
NVI	National veterinary institute
PCR	Polymerase chain reaction
PPR	Pestis des Petits Ruminants
PRA	Participatory rural appraisal
SNNPRS	Southern nation nationalities people regional state.
VCM	Verona calcium and magnesium buffered solution
PBST	Physiological buffer solution with Tween-20
NMSA	National Metrology Service Agency
CSA	Central Statistics Authority
PA	Peasant Association

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ABSTRACT

Cross-sectional study was conducted in South Omo and Gamo Goffa zones of Southern Nations Nationalities and People Regional State from September 2004 to February 2005 to determine seroprevalence and associated risk factors, isolate the causative agent, evaluate test agreement, and validate community perception. A multistage sampling was used in pastoral and mixed production systems. A total of 1033 goats' and 80 sheep's sera were collected for serological study. Additionally nasal swabs, lung tissue and thoracic fluid were collected from sick goats and all samples were submitted to National Veterinary Institute for isolation. Participatory disease search in pastoral area and questionnaire survey in mixed farming area were conducted. Proportional piling and matrix scoring were used to characterize major diseases of goats including their causes and signs. Based on the CFT results seroprevalence was vary among selected woreda. In Hammer and Benna tsemay woreda it was almost equal 15.5%, whereas in Boreda (32%) and Arbaminch (23%) was observed. The overall mean seroprevalence in study area was16.5%. There was no significant difference observed in seroprevalence among age groups, even though seroprevalence increases as the age increases. Pastoral and mixed farming area showed 15.5 and 18.4% seroprevalence, respectively. However, seroprevalence obtained using cELISA showed significant difference between production systems, age category and flock size category by univariate analysis ($p<0.05$). The overall mean seroprevalence was 57.3%. Based on cELISA the mean seroprevalence at flock level was 90.3 %(n=58) in mixed and 100% (n=15) in pastoral production systems. Compliment fixation test result, using *Mycoplasma capricolum* sub sp. *Capripneumoniae* and *Mycoplasma mycoides* sub sp. *mycoides* Small colony antigens, showed substantial agreement with Kappa value of 0.62 and 0.78, in goats and sheep, respectively. The test agreement between CFT and competitive Enzyme Linked Immuno Sorbant Assay was negative with Kappa value -0.014 in goats and -0.005 in sheep. Using multivariate analysis production systems were not statistically associated ($P>0.05$) with seroprevalence except flock size and age category greater than 4years ($P<0.05$). *Mycoplasma capricolum* sub sp. *capripneumoniae* was isolated from specimen submitted to the laboratory. Contagious Caprine Pleuropneumonia (*Sompo*) was ranked as the first disease of goats in pastoral area. Local perception of disease causes, disease signs, and seasonal calendar were described. Matrix

scoring between groups (n = 12) and individuals (n = 44) revealed that disease signs and disease causes showed weak, moderate and good agreement by Kendall's coefficient concordance (W=0.21-0.99). Questionnaire survey in mixed farming area revealed that Contagious Caprine Pleuropneumonia was ranked first and introduction of sick goat into the flock considered as main source of infection. In this study participatory disease search, questionnaire survey, bacteriological, serological and postmortem findings substantiated each other and it is possible to conclude that Contagious Caprine Pleuropneumonia is a major disease of goats in the area. Participatory disease search shows indigenous knowledge used to generate more information with minimum cost, local materials and within short period of time. High seroprevalence result in sheep indicates that this species is a potential carrier of the causative agent. Therefore, appropriate control measures have to be implemented to mitigate the problem in the area. At a time of control measure attention must be given for sheep as itcould be a source of infection. Detailed study need to be conducted as to the role of risk factors for prevailing of the disease. Diagnostic kits need to be evaluated further based on this study finding.

Key words

Contagious Caprine Pleuropneumonia (*Sompo*), *Mycoplasma capricolum* subsp. *capri pneumoniae* (*MccP*), Seroprevalence, Participatory disease search, Risk factors

1. INTRODUCTION

Goats (*Capra hircus*) are thought to have been the first animals to be domesticated for economic purposes. There is a huge variation of size, color, and hair type among breeds of goats. Goats are now estimated to be about 592 million goats (Peacock, 1996), are highly adaptable to a broad range of climatic and geographic conditions and are more widely distributed than any other mammalian livestock (Smith and Sharman, 1994). Goats are found from Scandinavia to South America (Peacock, 1996). Goats contribute on the economies of people in developing countries: they provide for their owners meat, skins, hairs, horns, bones and manures (Smith and Sherman, 1994). The valve of goats is an immense, because of their manageable nature under every production system, including feral, transhumant, nomadic, extensive, intensive and total confinement systems (Smith and Sharman, 1994; Peacock, 1996). It is possible to estimate goats' contribution to the developing countries because of huge proportion (96%) exists in these countries. The continent, Africa hosts about 174 million goats, which are around 34% of the global total goats' number. Apart from economic valves they are relatively cheap to buy, and increasing the number will reduce the probability of losing all animals at once incase of some disaster. Goats reproduce very fast in most cases regularly produce twins and sometimes triplets. They can easily be sold in terms of urgent need such as sickness, death or the payment of school fees (Peacock, 1996).

Their natural behavior of feeding in different vegetation, the ability to tolerate harsh environment, cheap to purchase, easy to handle make them preferable animals by many livestock producers all over the world, particularly by poor farmers (Peacock, 1996). Despite all these advantages, little attention is given for this animal to improve their productivity in the world in general, in developing countries in particular.

In Ethiopia, though, there are about 13,834, 703 goats (CSA, 2004); less attention is given to increase their output. The majority of the populations do exist in lowland under pastoral production system, where the veterinary infrastructure is scarce. As the result a number of goats are easily affected by rampant disease in the area. Among the major diseases which affect goats include external parasites, internal parasites; blue tongue, peste des petits ruminants (PPR), brucellosis, Contagious Caprine Pleuropneumonia (CCPP) and etc are the

most common once (Peacock, 1996). Contagious Caprine Pleuropneumonia is the most important epidemic disease of small ruminants' esp. goats. It is characterized by severe fibrinous pleuropneumonia with morbidity of 100% and mortality ranges from 60 to 100% in susceptible flock (Nicholas, 2002).

Thomas reported the first clinical description of CCPP in 1873 in Algeria but it was not known that the disease is due to contagious agent. Hutcheon in 1881 proved that the disease was caused by a contagious agent after the occurrence of major outbreaks due to introduction of goats from Turkey to South Africa. In spite of this early description, the agent was isolated and characterized as *Mycoplasma capricolum* subsp. *capripneumoniae*[F38] by MacOwan and Minette in 1976 in Kenya after a century of disease occurrence because of isolation difficulty due to fastidious nature of the organisms (Thiaucourt and Bolske, 1996, Nicholas, 2002; and Maré, 2004). Contagious Caprine pleuropneumonia is a very precise entity, where it limits, its pathogenicity in the lung, whereas the others mycoplasmas that affect goats are belong to the syndrome "MAKePS", which stands for Mastitis, Arthritis, Keratitis, Pneumonia and Septicemia (Thiaucourt and Bolske, 1996). The causal agent of classical CCPP classified as *Mycoplasma capricolum* subsp. *capripneumoniae* (*MccP*) (Leach *et al.*, 1993 as cited by Nicholas, 2002). This organism is closely related to three other mycoplasmas: *M. mycoides* subsp. *mycoides*, *M. mycoides* subsp. *capri* and *M. capricolum* subsp. *capricolum*. All three mycoplasmas may confuse the diagnosis of CCPP, because the diseases they produce can resemble the disease caused by *MccP*, and because they share several serological and biochemical characteristics with *MccP* (O.I.E., 2000).

The presence of CCPP in Ethiopia has been suspected since 1983. It was confirmed later in 1990 by isolation and identification of *MccP* (Thiaucourt *et al.*, 1992). Since then the disease has become endemic in different regions of the country. To day there is repeated outbreaks found in different parts of the country from South to North and East to west with different frequency occurrence. Recent data from MoA veterinary service monthly outbreaks report showed that major CCPP outbreaks were high in South Omo and Gamo Goffa zone for the last seven years. The disease is widely spreading to the neighboring woredas of these zones alarmingly. In addition to this, the limited vaccine production, antibiotics shortage, high cost of treatment and scarce veterinary infrastructure aggravated the situation in the remotest part of the country, where more than 75% of the total goat population of the country is reared. (Thiaucourt *et al.*, 1992).

Despite these entire problems in the country, particularly in South Omo and Gamo Goffa zone little work has been done about the epidemiology of the disease, risk factors associated with spread of the disease, and isolation and identification of the organisms. Based on this introductory concept the study was conducted with the following objectives:

- To study sero prevalence of the CCPP and associated risk factors
- To isolate and characterize the causative agent in the area.
- To evaluate test agreement between CFT and cELISA
- To validate CCPP occurrence through participatory disease investigation approaches

2. LITRUTURE REVIEW

2.1. Generalities

'Mycoplasmoses 'are the infectious diseases due to mycoplasmas, which are known to be the smallest Prokaryotes with autonomous replication. The general term '*Mycoplasma*' is referring to a group of microorganisms that differ from other bacteria by lacking a cell wall. Due to this morphology an organism is classified as Mollicutes (Nicolet, 1996).

The causative agent of CCPP is one of the *Mycoplasma* infectious diseases of small ruminants. The isolates from CCPP have been classified as *Mycoplasma capricolum* subsp *capripneumoniae* (Leach *et al.*, 1993 as cited by Nicholas, 2002). It is one of the six genomically or serologically interrelated pathogenic mycoplasmas, which are affecting cattle, sheep and goats, are known as "*Mycoplasma mycoides cluster*" (Belton *et al.*, 1994).

Unlike *MccP*, other subspecies of *Mycoplasma mycoides*, which are pathogenic to sheep and goats, do affect different organs of the animals; but *MccP* affects only the thoracic cavity. In outbreak, other causative agents do cause fever; reduce lactation and swelling of joints that remains for long periods (Cottew, 1979).

2.1.1. *Taxonomy and General Characteristics*

Members of the *Mycoplasma mycoides* clusters belong to the class Mollicutes. Order Mycoplasmatale, Family *Mycoplasmataceae* and Genus *Mycoplasma*. All members of the class are small, simple, self replicating and distinguishable from walled and L- phase bacteria by a number of unique properties. The Mollicutes lack a cell wall and the genetic machinery to synthesize the cell wall; their DNA has a low guanine plus cytosine content typically less than 30 mol %; they have small genomes and consequently small numbers of synthesized proteins (Nicholas and Bashiruddin, 1995).

As it is shown in table1, there are six mycoplasmas that make up the group known as *Mycoplasma mycoides* cluster. This group is known to be pathogens of cattle, sheep and goats causing Pleuropneumonia. In addition septicemia, mastitis and arthritis occur.

Table 1. Members of the Mycoplasma mycoides cluster (Cottew *et al*., 1987)

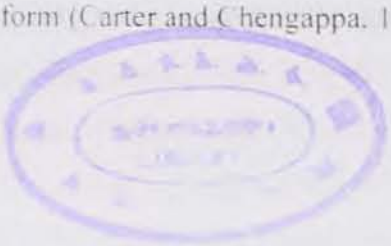
Name	Disease	Main / and others/ hosts
M. mycoides subsp. *mycoides* SC bovine biotype	Pleuropneumonia	Cattle/ Goats,Sheep,Buffalo/
M. mycoides subsp. *mycoides* LC	Peritonitis, septicemia poly arthritis	Goats, Sheep/Cattle/
M. mycoides supsp. *capri*	Pneumonia, arthritis	Goats
M. capricolum subsp. *capricolum*	Pneumonia, arthritis	Goats, Sheep/Cattle/
M. capricolum subsp. *capripneumoniae*	Pleuropneumonia(CCPP)	Goats
Bovine group 7	Arthritis, mastitis, calf pneumonia.	Cattle

Source: (Nicholas and Bashiruddin, 1995)

2.1.2. *Morphology and Staining Characteristics*

The *Mycoplasma* organisms have lost their gene for cell wall synthesis during degenerative evolution from the more complex eubacteria groups. The cells are confined by a lipoprotein plasma membrane and occur in a variety forms including spherical or pear shaped cell and

filamentous structures hence considered as pleomorphic organisms. Mycoplasmas in smears have the shape of cocco-bacilli, cocci, rings, spirals and filaments (Nicholas and Bashiruddin, 1995). They stain poorly with various methods because of their fragility and pleomorphism nature, although Giemsa is useful to stain the organism. They range in size from 0.2 to 0.8µm in diameter with variable length depending upon the form (Carter and Chengappa, 1991).



2.1.3. *Cultivation and Growth*

The Mollicutes are an extremely fastidious group of organisms. Nutritionally they are dependent on their host for a large variety of organic nutrients such as vitamins, nucleic acid precursors, amino acids, fatty acids and lipids. The large number of amino acids required by *M. mycoides clusters* indicates that the organisms have a limited synthetic capability. It will not grow in media lacking the preformed bases uracil, thymine, and guanine, which are needed for nucleic acid synthesis (Nicholas and Bashiruddin, 1995).

Cholesterol or related sterols are required for the growth of most mycoplasmas but others such as cholestanol and ergosferol can be substituted. Glycerol is required for the growth of most mycoplasmas for the synthesis of ά- glycerophosphate and thus glycerides. The polyamines spermine and spermidine are also used to stimulate the growth of mycoplasmas in defined medium (Carter and Changappa, 1991; and Nicholas and Bashiruddin, 1995). Cultivation for isolation of *MccP* was done at NVI using Hayflick's medium as described by Jones (1989) where they used basal media with heart infusion broth Difco 25gm(w/v), Neopeptone Difco 2.5gm, Bactocasitone Difco2.5gm, glucose 2gm and distilled H_20 700ml then autoclaved. *Mycoplasma* supplemental media: horse serum 200ml (source of cholesterol), yeast extract 25% 100ml (Precursor of nucleic acid), penicillin 200,000IU (.2ml) and thallium acetate (10%) 1.25ml at PH7.6-7.8. For biochemical test the respective chemical were added on the prepared broth (glucose50%, Arginine hydrochloride30%, triphenyl tetrazolium chloride 0.2%, dephosphatase horse serum 20ml added) for glucose fermentation, arginine hydrolysis, tetrazolium chloride reduction and phosphatase activity, respectively. Most Mollicutes, including *Mmm* clusters, are facultative anaerobic, growing well in both anaerobic and aerobic environments at a PH of 7.6- 7.8. They usually grow well in sealed liquid broth cultures, especially if the broth levels is a few inches deep to allow for an oxygen or air gradient (O.I.E., 2000; Nicholas and Bashiruddin, 1995).

2.2. Contagious Caprine Pleuropneumonia

Contagious Caprine Pleuropneumonia is a contagious disease that affects only goats. It is a classical disease of goats, which commonly confused with other serious pneumonias of goat and sheep. *Mycoplasma capricolum* subsp. *capripneumoniae[F38]* is now recognized as the cause of this highly contagious lethal disease. It is characterized by cough, dyspnea, lagging behind the flock, lying down, fever (40.5-41.5^{0}C) and in the terminal stages mouth breathing, tongue protrusion and frothy salivation with death in two or more days (Radostits *et al*., 1994; Thiaucourt and Bolske, 1996). The disease is confined to goats although there is isolation of the organisms from subclinically affected sheep. It is one of the devastating diseases of susceptible goat population with 100% morbidity and 60 to 100% mortality rate (Cottew, 1979; Thiaucourt and Bolske, 1996; Nicholas, 2002).

2.2.1. *Pathogenesis and Clinical Features*

In general the organism to cause the disease has to colonize the tissue or organ of the animals. Adhesion is a precondition for colonization and consequently, for infection. It has been suggested that by-products of mycoplasmal metabolism, such as hydrogen super oxide and superoxide radicals, may be toxic to the host cell cilia or to the cell membranes. The role of the capsules seen in some mycoplasmas, particularly the galactan of *M. mycoides* subsp *mycoides*, is not clear (Nicholas and Bashiruddin, 1995).

Recently performed studies indicate that the interaction of mycoplasmas with macrophages and monocytes induces the production of pro-inflammatory cytokines, such as the tumor necrosis factor α, interleukins and interferon γ. particular attention must be paid to the *Mycoplasma* membrane lipoproteins and certain lipids, which induce cytokine secretion by a mechanism distinct from that of bacterial lipopolysaccharides (Nicolet, 1996). There is very little information on the pathogenic mechanisms of *MccP*; a unique feature of CCPP is strict host and tissue specific, as the lesions are produced only in goat lungs. The expression of pathogenicity may depend on other factors that favor penetration and multiplication. Although *MccP* is present in high quantities in affected lungs, there is no dissemination to other organs. This may be due to a specific reaction of the lung tissue towards a mycoplasmas component, which leads to an exacerbated inflammatory response (Thiaucourt and Bolske, 1996). The

unique character of the disease is that it does not exhibit a bacteriemic phase and appear to be lung specific. It is suggested that either failure to isolate in serum or more likely, that soluble antigen (CPS) is only entering the circulation from the infected lung. PCR and direct culture were unable to detect *MccP* in the sera of infected animals, which strengths the hypothesis of capsular polysaharrides as an antigenic part of the organism. However, the role of CPS in disease pathogenesis and the mechanism by which it enters the circulation remain obscure (March *et al*., 2000).

Other mycoplasmas strains that affect goats are not localized in the lung and these strains due produce Mastitis, Arthritis, Keratitis, Pneumonia, and Septicemia (MAKePS) syndrome in goats (Thiaucourt and Bolske, 1996).

All goats can be affected, regardless of the breeds and age group. An acute case can be observed in regions where CCPP is introduced for the first time to susceptible populations. The incubation period generally lasts 10 days but may vary between two and 28 days. The first symptoms to appear are reluctance to walk, fever (41^{0}C), abortions, painful and accelerated respiration, accompanied by violent coughing. In terminal stages, the animals are unable to move, neck is stiff and extended, saliva continuously drips from their mouth and their nose is obstructed by a muco purulent discharge (Thiaucourt and Bolske, 1996). Nevertheless, according to Maré (2004) the clinical signs described differently because the involvement of other strain complicate the clinical picture of the disease by including other body system like reproductive and alimentary tracts.

In chronic cases animals show sporadic coughing, emaciation and diarrhea may be present. Animals recovered from this form of the disease almost completely and don't show symptoms or lesions after recovery. Though there is no sequestera animals may remain carriers (Seifert, 1996; Maré, 2004).

2.2.2. *Post Mortem Lesions and histopathological findings*

The lesions are generally confined to organs and tissues of thoracic cavity. Macroscopic lesions: one or both lungs could be affected. In acute cases the pleura (e) over the affected lung area(s) is usually covered with fibrinous material and there is excess straw-colored

thoracic fluid. Nodules of small diameter, which appears as congestion around the nodules can be observed in advanced cases. In chronic pleuritic causes visceral, parietal surface thickening and adhesions to the chest wall (Radostits, 1994; Seifert, 1996; Maré, 2004). It is emphasized that the lesions of CCPP do not resemble those of contagious bovine Pleuropneumonia (CBPP) in that "no thickening of the interlobular tissue" occurs, which is a classical lesion of CBPP. Contagious Caprine Pleuropneumonia diseased lung resembles granular liver, which is a description of the massive hepatization in CCPP affected lungs (Maré, 2004). Generalized lesions including encephalitis, meningitis, lymphadenitis, splenitis, genitourinary tract inflammations and intestinal lesions are not the feature of classical CCPP.

The histo- pathological lesions are mainly characterized by focal fibrinous pleuropneumonia with variable involvement of interstitial connective tissue. Common features in acute cases are capillary congestion with broncho-alveolar serofibrinous exudates containing macrophages and neutrophiles (Jones, 1989). According to the findings of Bolske *et al* (1989), during their experimental work on 38 infected goats. They have observed that five of them showed extensive lesions in the lungs. Microscopically, the alveoli contained mononuclear cells, oedema and scattered granulocytes, which also filed the bronchioles. The alveolar walls showed some thickening from hyperemia and proliferative changes of the interstitium, but little or no oedema. Dilatation of interlobular septa was not observed.

2.3. Epidemiology of contagious Caprine Pleuropneumonia

2.3.1. *Etiology*

Contagious Caprine Pleuropneumonia is caused by *Mycoplasma* strain called *M. capricolum* subsp. *capripneumoniae* (*MccP*) [F38] or vaccinal strain (Jones, 1989). For many years the causative agent of CCPP was considered to be *M.m capri* (type strain PG-3) because this was the agent most commonly isolated from goats with CCPP. However, in 1976 MacOwan and Minette have developed special media and isolated a fastidious new *MccP* [F-38] from a CCPP outbreak in Kenya and demonstrated that it was the cause of a highly contagious form of pneumonia resembling the original description of CCPP by Hutcheon (1881) as cited by Nicholas (2004). The taxonomic status of F38 has long been unclear and it was only in 1993

that it became a subspecies of *M. capricolum*, and classified as *MccP* (Leach *et al.*, 1993 as cited by Nicholas, 2002).

2.3.2. *Source of Infection and Mode of Transmission*

The disease is readily transmitted by direct contact through inhalation of infective aerosols, through droplets released during coughing and very short periods of contact are sufficient, but the organism does not survive for long outside the animal body; therefore, carrier or newly infected animals are the main source of infection (Thiaucourt and Bolske, 1996; Maré, 2004). In extensive husbandry practices communal grazing areas and watering points are the major sites for disease transmission (Radostits *et al.*, 1994). Outbreaks of the disease often occur after heavy rains and after cold spells. This is probably because recovered carrier animals start shedding the mycoplasmas after the stress of sudden climatic change (Maré, 2004).

Some animals may become latent chronic carriers and thus play an important role in the transmission of the disease. Unlike in the case of CBPP, no sequestra have been described for CCPP. The exact location of the *Mycoplasma* in latent carriers is not known (Thiaucourt and Bolske, 1996).

2.3.3. *Factors Associated with Epidemiology of the Disease*

Host range

Under natural condition *MccP* infects only goats. It severely affects Caprine species accompanied with respiratory distress (Jones, 1989; Nicholas, 2002), sheep and cattle not reported as susceptible species for *MccP*. But study conducted in Kenya, indicates from farm which has experience of CCPP outbreak; *M. ovipneumoniae* and *MccP* were recovered from 31 goats and 22 sheep but none from calves. Antibody was demonstrated in sheep sera by SAST and CFT (Litamoi *et al.*, 1992). On the other hand, seroprevalence study in some wild herbivores and camels in Kenya indicates that *MccP* antibodies were found in buffalo (syncerus caffer) 32%, impala (Aepyceros melampus) 10% and camels (camelus dromedarius) 49% but not in bushbuck, eland, grant's gazelle, kongoni, oryx and thomson's gazelle (Paling *et al.*, 1978). The organism has been isolated and it has elicited antibodies in sheep according to the observation done by Dawit (1996), he observed that the detection of

MccP specific antibodies in 4(13%) of the 32 sheep herded with CCPP affected goats. These findings suggest that sheep could have an epidemiological role in maintaining causative agents. Recently it was reported from Qatar wildlife conservation farm that wild goats (Capra aegagrus), Nubian ibex (Capra ibex) were affected by CCPP and the case was confirmed using PCR (O.I.E., 2005).

Types of husbandry

The disease is more serious under intensive husbandry systems. Overcrowding and confinement favors contact and hence circulation of the agent. In extensive husbandry practices mixing of goats at watering, grazing areas, marketing sites and shelters play a great role in the spread of infections (Lefevre *et al.*, 1987).

Climate and seasons

The occurrence and severity of CCPP varies seasonally. This is mainly due to the effect of climatic conditions on the agent and the host. Raised environmental temperature and relative humidity predisposes to respiratory diseases by favoring the survival and replication of the pathogen and rendering the host vulnerable to infection by lowering its resistance (Thiaucourt and Bolske, 1996).

Immunological status

In regions where CCPP already occurs, the severity of the disease may depend on the different factors.

- Immune status of the host: the proportion of immune animals, as an animal that has survived a previous infection thought to be protected. This would reduce morbidity and mortality rate of new infection.
- Inter current infection. The occurrence of co-existing disease like viral infection (Orf, PPR), which may favor the development of CCPP, as immune status is compromised by concurrent infection.
- Stress: long distance movement, over crowded flock could predispose the host for infection (Thiaucourt and Bolske, 1996). They have suggested that agro-ecological situation and livestock rearing system (mixed and pastoral production) also affects the disease spread in the flock.

2.3.4. *Immunology*

The lack of a cell wall and endotoxins probably enables mycoplasmas to colonize the host without stimulating an aggressive immune response. The predilection of these organisms on mucosal surfaces must also limit the effectiveness of humoral immunity. The majority of naturally infected goats with lesions have a detectable antibody response but there appears to be no relationship between antibody titer as measured by the CFT and severity of lesions (Thiaucourt and Bolske, 1996)

2.3.5. *Geographical Distribution*

It is difficult to give an exact picture of the distribution of the disease. While considering *MccP* isolation record shows that affected countries are found in Africa and Asia. Numerous publications have documented the presence of *MccP* in Africa and Asian countries (O.I.E., 2000), but neither *MccP* nor *Mmc* has been isolated in North America (Maré, 2004).

The disease has been reported totally in nearly 40 countries in Africa and Asia. *M. capricolum* subsp. *capripneumoniae*, out of these it has only been isolated in 13 countries because few have the facilities for isolating and growing *Mycoplasma* organisms as it is shown in table 3.

Table 2. Distribution of CCPP in different Asian and African countries

Continents	*Confirmed case*	*Clinical disease reported or suspected*
Africa	Chad, Eritrea, Ethiopia, Kenya Niger, Sudan, Tunisia, Uganda.	Algeria, Burkina Faso, Cameroon, Central Africa republic, Djibouti, Egypt, Libya, Mali, Nigeria, Somalia.
Asia	Nepal, Oman, United Arab Emirates, Turkey, Yemen.	Afghanistan, Bangladesh, India, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Pakistan, Saudi Arabian, Syria.

Source: (Nicholas, 2002).

In England it was reported that there has been severe respiratory disease and many deaths occurred in 1998 due to imported goats. Contagious Caprine pleuropneumonia was ruled out by newly developed PCR tests (Nicholas, 2002).

2.4. Diagnosis

The diagnosis of CCPP has often been considered difficult. This is because of the confusion that can arise from other mycoplasmoses of small ruminants. Symptoms and lesions are similar and the isolation of *MccP* requires skilled technicians (Thiaucourt *et al*., 1996).
There are different techniques used for diagnosis of the disease. Out of these clinical signs supported by postmortem examination, isolation and identification, serological tests and PCR (polymerase chain reaction) techniques are common ones.

2.4.1. *Clinical signs and Post-Mortem Examination*

In case of an acute outbreak, the clinical signs and lesions observed may give rise to strong suspicions. Weakness, anorexia, cough, hyperpnoea, and nasal discharge accompanied by fever 40°C are often found. Exercise intolerance and eventually respiratory distress develop. Pathological lesions like thorax contain an excess of straw colored fluid, and there is acute fibrinous pleuropneumonia with over laying fibrinous pleurisy. Consolidation is some times confined to one lung (Kopcha, 1998). The affected lung is enlarged, firm and edematous, varying in color from gray to red, these colors forming a mosaic. Evolving lesions are characterized by round foci of hepatization with a gray pinpoint center of necrotisation and dark red hyperemic margins, which contrast markedly with the pink unaffected lung (Thiaucourt *et al*., 1996).

2.4.2. *Microbiological Examination*

The isolation of *MccP* may be a long and difficult process. Pleural fluid is the best sample and ten milliliters should be harvested aseptically from an animal that is in the acute phase of the disease. Dead animals should be avoided. Live animals need to be sacrificed for this purpose is the best method of sampling for isolation of the organisms. Samples from hepatized lungs

are also desirable. Square of 3cm sections should be taken from an area that borders normal lung tissue. Samples can be kept at +4°C if transport to the laboratory does not take place more than one or two days. They can be deep frozen at -20°C for long periods with out loss of *Mycoplasma* viability. In the absence of cold chain, Penicillin or Ampicillin should be added to the sample to limit contaminant growth (Thiaucourt *et al*., 1996).

There were several media assessed for their ability to grow strain *MccP* by different authors. Among these media Hayflick's, modified friis, viande foie goat (VFG), TPM, and GS are none of them proved to be satisfactory. A new medium, termed W1 is therefore used currently (Thiaucourt *et al*., 1996 and Jones and Wood, 1988).

All cultures are incubated at 37°C broth cultures aerobically and solid medium cultures in air with 5% CO_2. Negative broth cultivates are passed through one blind subculture at seven days and discarded if still negative at 21 days (Jones and Wood, 1988).

2.4.3. *Identification of Mycoplasma*

After the specimen has been cultivated and isolated from the culture, the organisms are further characterized using biochemical test. This procedure used to see chemical reaction of *Mycoplasma* species, which are glucose fermentative, occurs frequently in goats and sheep are shown in table 3.

Table 3. Biochemical reactions of *Mycoplasma* found in goats and sheep (Nicholas, 2002).

strain	glucose	Arginin Hydrolysis	Tetrazolium Aero/anaero		Film&spots Formation	Phosphatase Activity	Casein Digestion	Degitonin Sensitivity
MccP	+/-	-	-/+	w/+v	-	-	+	+
MmmLC	+	-	+	+	-	-	+	+
Mmc	+	-	+	+	-	-	+	+
Mcc	+	+	+	+	-	+	+	+
M.ovip	+	-	v	+	-	-	-	+
M.argi.	-	+	-	+	-	-	-	+
M.agal	-	-	+	+	+	+	-	+
M.putri	+	-	v	v	+	+	-	+
M.conj	-	-	-	+	-	-	-	+

V= variable, += positive, - = negative, w = weak.

2.4.4. *Serological Test for Antibody Detection*

There are different techniques in practice to detect the antibodies of infected animals. The most commonly used techniques are Complement fixation test, the passive haemagglutination test, agglutination of latex beads sensitized with capsular polysaccharide specific antigen and cELISA (Thiaucourt *et al*., 1996; Nicholas, 2002; Maré, 2004).

In CCPP the complement fixation test is the recommended test for detection of *MccP* infection and it has been found to be more specific, though less sensitive, than IHA test (O.I.E., 2000). However, it can produce positive titres for other mycoides cluster from apparently healthy animals and acute cases caused by *MccP* rarely show positive results before death. CFT is unable to detect early stage of infection until measurable immune response exists. It is insensitive prior to about 21 days post infection. In contrast, *MccP* CPS antigen has been detected in goats infected with *MccP* after 4 to 9 days following the onset of clinical signs using latex agglutination test (March *et al*., 2000).

Competition Enzyme- Linked Immuno Sorbent Assey (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), which is competing 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 in all animals infected whatever test was used. In the case of cELISA diagnostics test, the percentage of positive animals in affected flock varies between 30% and 60%. Therefore, the interpretation of serological results is often difficult, as these tests were not comprehensively validated. Precise information on sensitivity, specificity of antibody detection is lacking. Specificity is usually poor as these techniques detect antibodies to whole Mycoplasma antigens, many of which are shared by the different species of the mycoides cluster. Hence serological tests used to test at herd level than individual level (Thiaucourt *et al*., 1996).

2.4.5. *Polymerase chain reaction (PCR)*

This relatively new diagnostic method has radically improved the detection and identification of microorganisms, which do not grow easily in vitro. PCR is based on the amplification of specific DNA sequences with thermo stable enzymes and nucleotide primers that must be wisely chosen.

The PCR method, which has been described, for the detection of *MccP* involved two steps. In the first, which was based on amplification, a 548-bp segment of the 16S- rRNA genes from the members of the *M. mycoides* group were amplified. In this stage, all strains and isolates from the *M. mycoides* cluster have been positive in the PCR, whereas all other than 'mycoides cluster' species tested including several *Caprine Mycoplasmas* were negative. In the second step, the amplimers were cleaved with the restriction enzyme *PstI*. This resulted in a unique restriction pattern for *MccP*, differentiating it from the other members of the cluster. Three bands were generated for all *MccP* -like strains and two bands were observed with the other species. The presence of a *PstI* site in only one of the 16S-rRNA genes from strain *MccP* distinguishes it from the other members of the *M. mycoides* cluster (Bascanana *et al*., 1994 and Thiaucourt *et al*., 1996).

2.4.6. *Participatory Disease Investigation*

The most common epidemiological applications of participatory rural appraisal are to gain a rapid overview of the range a community's animal health problems. The method is achieved by directly asking livestock owners about animal health problems they have in their livestock. Researchers acted as facilitators and technical advisers, and research methods were often based on the interviewing, visualization and scoring methods of PRA (Catley *et al*., 2002a). To implement participatory epidemiology one has to follow practical procedures like identifying the area and the communities with which working and obtaining background information including literature on the communities of interest. Translators' selection, who do not have personal bias and have respect for local people. Epidemiological study consists of at least a translator and an epidemiologist. Key informants are necessary who have previous experience to be interviewed to begin the interview. These are mainly traditional leaders, religious leaders, community animal health workers and etc. The method of sampling in PRA

studies is based on the principal of key informants rather than randomization. The study actively seeks individuals who are likely to have specialized knowledge (mariner *et al*., 2001). The key informants can help to identify important clusters of livestock owners, entry points, community institutions and decision-making mechanisms.

The three main groups of participatory methods that have been mentioned by Mariner (2001) are informal interviewing, visualization and ranking or scoring methods. Triangulation refers to a process of crosschecking of reports or data provided by various independent respondents or methods. Triangulation is a simple and powerful method of quality control in the analysis of the results of participatory epidemiology. Laboratory confirmation, clinical signs and postmortem examination and direct observation are the important part of triangulation.

2.5. Control and Prevention

The disease in question is controlled using different methods at field level. Treatment, vaccination, movement control, and slaughtering infected animals are mentioned.
Treating diseased animals with broad-spectrum antibiotics (mainly tetracycline, tylosin, and tiamulin) are effective although early stage gives good result (Maré, 2004). Goats usually kept by small holders can be treated because of their small size, but it is difficult to eradicate based on treatment in a large flock size (Seifert, 1996).

In addition, the most active antibiotics belong to the tetracycline group and macrolide family (such as Spiramycin, Lincosamine and Erythromycin) as well as some new compounds belonging to the fluorquinolones may also be active (such as enrofloxacin) can be used. As for any other antibiotic treatment, the duration and the dosages prescribed by the manufacturer must be followed exactly. Duration of five days is deem necessary, and may be achieved by the injection of long acting formulations. All animals should be treated correctly, especialy in nomadic flock (Thiaucourt *et al*., 1996).

An inactivated vaccine with saponin, which is used as an adjuvant, protects goats for approximately a year has been produced in Kenya (Thiaucourt *et al*., 1996). In the former Soviet Union and in China, aluminum-hydroxide formalin vaccines have been applied (Seifert, 1996). However, recently *MccP* vaccine production in Ethiopia started at larger scale

producing relatively at large quantity to combat ever-increasing epidemics though the supply doesn't satisfy the national demand (Dr. Berhe personal communication).

Disease control can be achieved if there is strict control of animal movement and a prohibition of the importation of live animals from infected regions.
Slaughtering infected animals is recommended for countries, which are known to be free from infection. Swift and energetic action may then prevent dramatic losses and eradication can be achieved (Thiaucourt *et al*., 1996).

2.6. Contagious Caprine Pleuropneumonia Distribution in Ethiopia

In Ethiopia the presence of CCPP has been suspected since 1983. It was confirmed later in 1990 by isolation and identification of *MccP*. Since then the disease has become endemic in different parts of regions in the country (Thiaucourt *et al*., 1992).

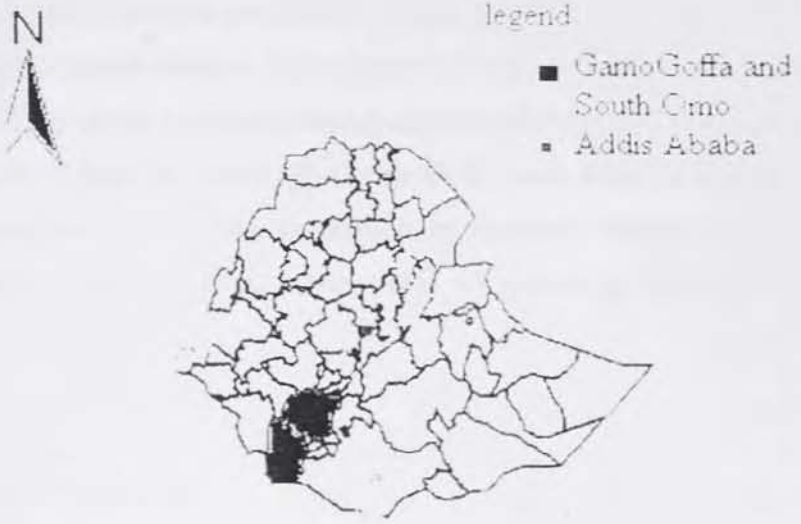
Data obtained from different workers indicate that seroprevalence varies from 6% to 51.5% in different part of the region as shown on the Annex 8. The wide variation may be attributed due to agro ecological zone, production system (mixed or pastoral), and overcrowded stock. The other factor for the difference could be the methods used during sampling procedure: diagnostic test used as well as number of examined animals also affects the seroprevalence of the disease.

In addition to research conducted in few areas of the country, there is an official monthly disease outbreak report to the federal MoA animal health department from different parts of the country. The compiled data from 1998 up to 2004 CCPP outbreak report in the country showed that 206 CCPP disease outbreaks were reported from different parts of the country, of which 88(42.7%) outbreak reports were from Southern Nation Nationalities People Regional state. In this region South Omo and Gamo Goffa zones share the highest proportion of outbreak number, showing its endemicity in the two zones. However, there were no sufficient epidemiological investigations systematically conducted to show its distribution and impact. In these areas vaccination against CCPP has been given intermittently with out the knowledge of infection status in the area.

3. MATERIAL AND METHODS

3.1. Description of the Study Area and demography

The study was conducted in SNNP regional state, South Omo and Gamo Goffa zones. Jinka and Arbaminch are the capital towns of South Omo and Gamo Goffa zones, and are located about 750 and 500 kms' far from Addis Ababa, respectively in 21" Southwestern directions. The study sites were selected purposely because CCPP outbreak reports from the region during the last seven years indicated highest frequency of the disease in these two zones. (i.e. 61 monthly outbreak reports from 88 regional outbreak reports) and reports were based on suspected cases only.



Map 1. Showing study sites of epidemiological survey of CCPP in South Omo and Gamo Goffa Zones, SNNPRS, Ethiopia.

Topographically, massifs, plains, steep slopes and gorges along the course of number of streams and rivers mark these areas. The biggest rivers are "Omo and Mago" that are found in South Omo zone. Lake Chamo and Abaya found in Gamo Goffa zones. The altitude ranges from 391 m.a.s.l. (Duss, in Hammer district) to 4200 m.a.s.l (Guge Mountain, Gamo Goffa). Mean monthly maximum temperature varies in these two zones 18°C-32.3°C and Mean monthly minimum temperature varies from11.2°C-19.2°C. Mean monthly rainfall distribution in Hammer Benna varies from 3.9- 276.5mm. In Gamo Goffa zone the rainfall has bimodal.

short rainy season occurs from January to April and long rainy season occurs from June to September. In South Omo rain fall distribution is erratic and usually appear bimodal from September to November and from March to May (CSA, 2004).

In the study area there are sixteen different ethnics groups. Those who are inhabited in Gamo Goffa zone practice mixed farming by producing crop and livestock, whereas those inhabitants in South Omo almost all produce livestock. Ari and Deme ethnics practice mixed farming though livestock production is predominant.

3.2. Livestock Production System in the study area

There are two production systems: pastoral and mixed farming system (sedentary). In the South Omo livestock production is transhumance type, where elders and children remain on their settlement villages and youngsters keeps their livestock. South Omo comprises of more than 43% of the regional states goat population (annex 9) and used to move long distance in search of feed and water. In Gamo Goffa zone almost all parts practice livestock and crop production system. The proportion of livestock number varies from cattle, sheep, goats, equines and poultry in each household. All species are local breeds.

3.3. Study Design

3.3.1. *Design*

The study type was cross-sectional investigation of CCPP in all age and sex groups of goats above 6 months of age used in the selected PA's of the two zones, namely South Omo and Gamo Goffa. Sera collection, questionnaire survey and participatory epidemiological investigation of disease search were conducted at the beginning of the study.

3.3.2. Study Animals

Goats that exist in South Omo and Gamo Goffa zones are typed as Woyto-Guji and originated from rift valley family goats. They are related to the Arsi-Bale goat. Key identifying features are brown, black or red color with a shiny, smooth coat and small head with a straight or concave facial profile. Coat colours are often marked with black or brown stripes along the back on the underside or on the front of the legs. Woyto-guji goat is a medium sized goat with a mainly straight (89%) to concave (11%) facial profile Farm-Africa (1996). Sheep are Ogaden black head in the pastoral area plus uncharacterized local breeds.

3.3.3. Study population

Goats that were found in South Omo and Gamo Goffa zones were used as target population and estimated as 1,384,479; out of which 1,157,201 goats were found in South Omo and 227,278 goats were found in Gamo Goffa zone. All age above 6 months and sex groups were included in the study population.

3.3.4. Sampling Strategy

To select the intended sample size from study area of two zones namely, South Omo and Gamo Goffa; It was classified into two strata based on livestock production system, i.e. the pastoral area and mixed farming system in South Omo and Gamo Goffa zone, respectively; because of difference in population size in the two zones, sample size were also allocated proportionally based on the existing goat population in two strata. Hence 66% of sample sizes were allocated to South Omo and the balance were allocated to Gamo Goffa zones. The sample size distribution at peasant association level in each stratum was allocated equally, because the population of goat in each PA's of Pastoral woredas were above 1000, therefore total sample size that were allocated for selected woredas of South Omo divided equally (about 88 sample size per PA's) and the population of goat per PA" in mixed farming area was less than 1000 then 40 sample size were allocated for each PA's. Multistage random sampling methods were applied to select study animals. The sampling frames at each stage were:

1. **Primary sampling units** were number of woredas that did not vaccinated their goats against CCPP for the last one year and woredas that have above 250 goat population per PAs used for random sampling. Out of the five woredas from South Omo two woredas, namely Hammer and Benna-Tsemay and out of six woredas of Gamo Goffa three were (Arbaminch zuria, Boreda and Humbo) selected. The sampling frame was a list of woredas that were found in two zones. Random selections of woredas were done at zonal level together with zonal veterinary officials.
2. **Secondary sampling units** were number of PA's randomly selected from each selected woredas. The sampling frame was a list of PA's found in each selected woredas. A total of 17 PA's were selected from five selected woredas. Eight PA's from South Omo and nine PA's from Gamo Goffa zones.
3. **Tertiary sampling units.** One village was selected from a list of villages from selected PA's. Individual animals were selected systematically as follows. Animals below six months of age were excluded from sampling. Animals were selected systematically at the interval of 5 animals starting from number one, until the required number of sample size in that village attained. During sampling history of goats whether they were the member of the flock or not were asked and a newly recruited goats replaced by existing flocks to avoid the risk of including vaccinated animals. Forty numbers of sheep were purposively sampled from each stratum.

3.3.5. Sample size

The determination of sample size for serum collection was based on 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, 95% multiplier = 1.96

At 95% confidence interval level with 5% absolute precision and 35.5% expected prevalence was used to determine sample size. A total sample size from the formula was 354, but since the sampling strategy that was used was multistage random sampling, to avoid uncertainty the sample size was multiplied 3 times and 1033 goats' sera were collected. The expected

prevalence was obtained from research work done at Arbaminch and Konso woredas, 36 and 35%, respectively. These two woredas found near and within study area. Average prevalence of the two woredas was used for sample size determination assuming that the finding can represent the prevalence of study area. Eighty sheep were purposively selected for serum sample collection from mixed farming and pastoral system. Geographical locations were obtained using "*Garmin-76*" GPS apparatus.

Table 4. Geographical locations of serum sample collected and numbers of sample size in each site.

Production systems	Woredas	PA	Altitude m.a.s.l	Sample size	
				Goats	sheep
Pastoral	Hammer	Dembayte	990	88	10
"	"	Kolakeja	967	88	10
"	"	Karoduss	391	88	10
"	"	Zeldaketa	987	88	10
"	Benna tsemay	Alduba	1334	89	
"	"	Sille	1250	88	
"	"	Shabba	1449	88	
"	"	Challi	1343	62	
Mixed	A/minch	Shellemille	1127	39	
"	"	Kolashelle	1182	46	
"	"	Gentamiche	1924	28	
"	Boreda	Z/manuka	1589	35	
"	"	Xenxele	1502	41	
"	"	Dubena	1717	45	
"	Humbo	Sippa	1643	40	
"	"	Kolshobo	1395	41	
"	"	Gaffetta	1224	41	
"	Mirab Abaya	Doshee	1300		40
Total				1033	80

3.3.6. *Questionnaire Survey*

Survey was conducted in the selected woredas of Gamo Goffa zones from the villages where sera samples collected. A total of 58 individuals were interviewed, out of which on the average six individuals responded from each village. The questionnaire included general description, which included name of the owner, location, goats' holdings, farming system, husbandry practice, movement of livestock and the purpose of raising goats.

The other portion covered related to veterinary services which included major goat diseases, measures taken to control diseases, source of drugs or vaccine and its efficacy, cost of drugs market value of study animals at health and sick status and finally, traditional practice what they have, were assessed. Structured open and closed ended questionnaires format were developed and administered to assess husbandry practices and their knowledge about the disease after pre-test has been done. The formats were coded for data analysis.

3.3.7. *Participatory Disease Investigation*

To validate the knowledge of the community about the disease, group and individual interviews were used by participatory epidemiological (PE) disease search methods. The methods were adapted from Mariner (2003). Participatory epidemiology research was conducted in Hammer and Benna tsemay woredas in more than 10 different villages at the beginning of November 2004 up to the end of December 2004. Eight sites that were selected for sera sample collection and another additional site were used. Key informants were used in each site in order to identify cluster of communities. Sampling of the informants is based on the information of key informants.

3.3.7.1. *Secondary data and interviews*

Secondary information and interviews were conducted with veterinary staff at zonal and woredas level regarding major goat disease, vaccination coverage and other relevant information-using questionnaire developed for veterinary staff.

3.3.7.2. Proportional piling of major goats diseases

Pre-testing done before real work started and used to determine major goat disease in the area. Proportional piling was used to rank major goats' disease in the study area. Different informant groups listed a numbers of diseases. The first five diseases that were frequently mentioned were used for matrix scoring and seasonal calendar. Using a pile of 100 beans more important diseases were ranked (i.e. the greater the pile of beans assigned to it the more important disease it is). Based on informants piling major goat diseases were *Sompo* (CCPP), *Quatsi* (Mange mite), *Shoko* (tick infestation), *Pirtsa* (Diarrheal syndrome) and "Other" diseases in this group different diseases included which were not mentioned frequently. The method was repeated with 10 informant groups. Median of the score and maximum and minimum response were calculated for each disease.

3.7.3 Matrix scoring

Matrix scoring was used to understand local perceptions of the main clinical signs and causes of CCPP. The matrix scoring technique was adapted from the method described by (Catley *et al.*, 2002a). Secondary information from veterinary staff indicated that pastoralists in two *woredas* used the disease name "*Sompo*" to describe CCPP, as it is locally known. Therefore, this disease name became the main topics of investigation in matrix scoring. However, in order to avoid exaggeration of responses to *Sompo*, and check that informants understood the matrix scoring method, list of major diseases were identified and included in the matrix and used to crosscheck diseases each other. To identify locally perceived clinical signs and causes of CCPP in goats, pair-wise comparison of the five diseases (*Sompo*, *Quatsi*, *Shoko*, *Pirtsa* and the disease called others) was conducted according to the method described by Mariner (2003). The pair-wise comparison was conducted with a group of 10 key informants. It was possible to produce 14 indicators. Researcher has categorized these indicators into 5 disease causes and 9 clinical signs.

The control diseases used were *Quatsi* (mangemite) and *Shoko* for tick infestation agreement created among informant by demonstrating the case and tick. This was used to confirm whether informants understood the matrix scoring procedure.

Five diseases mentioned above were represented using drawing on the piece of hard paper and placed along the top 'x-axis' of a matrix drawn on the ground. Each of the five diseases was

then scored against a list of clinical signs and causes of disease. The clinical signs and causes were represented using simple locally available objects that were placed along the 'y-axis' of the matrix. The clinical signs and causes were labeled using everyday objects. The arrangement of the matrix and each illustration was explained to the informants. For each disease sign or causes informants were asked to score the five diseases by dividing a pile of 30 beans. The more important a particular sign or cause, the greater the pile of beans assigned to it. After the scoring procedure was completed, informants were asked to check their scoring and confirm that as a group, they agreed that the scores were correct. The scores were then recorded and additional questions were asked to crosscheck and probe the responses. Especially to elicit additional information from those, which have highest scores.

Matrix scoring was repeated with 12 informants' group and 44 individuals at various sites of two woredas. Group sizes varied from 4 to 15 individuals. The level of agreement between informants groups and individual responses were assessed using Kendall's coefficient of concordance (W)(SPSS standard version inc.1989-2002). Maximum, minimum and median were used to see the difference between informants.

3.3.7.4. *Seasonal calendars*

Seasonal calendars were used to describe the seasonal occurrence of the diseases, used in the matrix scoring and weather condition rainfall distribution was also represented. The methodology for constructing the seasonal calendars was similar to the matrix scoring. Local names for seasons and months were used and each season was represented using an object placed along the top 'x-axis' of the diagram. Simple line illustrations of diseases and ticks were placed along the 'y-axis' of the diagram. Seasonal calendar method was done with group of informants from each village.

3.3.7.5 *Post-mortem and clinical sign Examinations* (Triangulations)

During Participatory investigation of CCPP, informants were asked in order to bring sick goats from their flock. In most sites the informants came with case history. Most of the cases were treated with oxytetracycline and recovered. The owners themselves used to administer oxytetracycline directly to the thoracic cavity. Recovery from the disease is common

according to the respondents. Temperature and respiratory rate were checked. Those goats that have in normal range of clinical signs were not autopsied. Five cases were found at different sites. Temperature, respiratory rate, pulse rate, mucus membrane of eyes, body condition were recorded, blood sample was taken from vein puncture. Case history from the owners was taken before postmortem examinations. All Postmortem examinations were conducted together with informants and they have indicated lesions that were used for the diagnosis of CCPP. Thoracic fluid, lung tissue from interface area of consolidated and unconsolidated region for agent isolation and lung lesion with 10% buffered formalin for histo-pathological examinations were taken.

3.3.8. Sample Collection

3.3.8.1. *Serum sample collection*

Five to ten ml of blood for serum extraction was collected by jugular venepuncture using sterile vacutainer tubes and needles. Then the tube was left for 6 hrs in slant position to allow clotting. Centrifuge was used to separate sera from coagulated blood mass. The supernatant (serum) was decanted into another tube. All samples were labeled and kept at +4°C in portable gas operated refrigerator and kept at -20°C at near by vet clinic until the samples submitted to the National Veterinary Instituted (NVI) for test. Data including the owners' name, PA's, Villages, age, sex and test tube number was recorded at the time of blood sampling.

3.3.8.2. *Sample for microbiological use and histopathological examination*

Pleural fluid was collected from suspected cases of CCPP diseased goat that was sacrified for postmortem examination. Ten ml pleural fluid was taken by aspiration. Then the tube was labeled and stored at +4°C for transportation. Kept at -20°C in a local veterinary clinic, until it was submitted to the NVI.

Nasal swabs were taken from the nasal cavity of clinically sick goats. At first the nasal openings were washed thoroughly with soap and disinfected using iodine tincture. Then sterile sticks rapped with cotton at one tip (which was kept under screw cupped test tube)

were inserted into the nasal cavity, and rubbed against the walls of the mucosal surface. The swabs were kept in sterile tubes. Then tubes were labeled and stored at −4°C in a portable refrigerator to be transported. Pieces of hepatized lung were excised from the interface between consolidated and apparently normal areas about $3cm^3$ sections was used and kept in sterile screw cupped glass tube and stored at +4°C and then deep freezed.

Sample for histopathological studies was taken from hepatized lungs. The sample was preserved in a glass tube with 10% buffered formalin until processed.

3.4. Sample Processing

The serological test and microbiological (cultivation and growth) investigation were done at NVI and histopathological examinations were performed Faculty of Veterinary Medicine (FVM), Addis Ababa University.

3.4.1. Cultivation of sample

Modified Hayflicks media was used to cultivate *Mycoplasma*. The preparation of the media was as described by Jones and wood (1988). The media comprises: basal components which contains, brain-heart infusion 37gm/l (w/v), Difco Bacto Neopeptone 2.5gm, Difco Bacto Casitone 2.5gm, D(+)- glucose anhydrous 2gm, and 1%(w/v) agar for solid media, where as for broth culture without agar. Supplement components include horse serum 200ml, yeast extract (25%) 100ml, Penicillin 200,000IU and DNA 0.2%, Glucose (50%), Thallium acetate 50unit and pH7.6.

Cultivation from nasal swabs sample: Four ml portion of modified Hayflicks' medium was dispensed into sterile screw cupped tubes. Each nasal swabs sample was soaked in peptone water and agitated thoroughly; then filtered by 0.45µm membrane filter. The filtered nasal swab sample was inoculated into broth then incubated at 37°C.

Thoracic fluid cultures: The frozen pleurisy fluids were thawed and 0.5 ml poured into 4ml liquid medium. The mixture was agitated and four serial passages were done: 0.1ml of the sample was simultaneously dispensed on solid media. Broth cultures were incubated at 37°C, while agar plates were kept in an incubator with 5% CO_2 and humidified atmosphere (to maintain the humidity water was placed inside the incubator).

Tissue Suspensions: The tissue samples were chopped with sterile scissors. Then it was vigorously smashed in 1ml sterile saline solution, using a mortar and pestle. Five hundred micro liter of the suspension was diluted into broth and an equal amount dispensed on solid medium, and broth culture was incubated at 37°C whereas solid medium incubated with 5% CO_2. For all broth culture there was control broth without inoculums.

Follow -up of broth and solid media: Broth cultures were examined daily for color change or turbidity, to compare the growth of *Mycoplasma* in cultivated medium and without inoculums. Cultures suspected of contaminations, by their gross turbidity within two days, were passed through a 0.45µm membrane filter before sub culturing. The broth culture was blind passage in 7-10 days regardless of turbidity of the suspension. The rate of growth of *MccP* organism was compared only with that of *M. ovipneumoniae*. All other members of the mycoides "clusters" grow within 24 and 48 hours by producing bigger colonies (1 to 3mm in diameter), but the diameter of *Mccp* colonies may be only 0.1mm after 4 to 5 days incubation and can only be seen by close observation with a binocular microscope (Thiaucourt *et al*., 1996). *Mycoplasma ovipneumoniae* were suspected when the colonies lack the classic "fried egg" appearance and do not stick to the agar surface.

Solid media were examined every 1 to 3 days using a stereomicroscope at 5 to 50x magnification. Sub culturing from solid media was made by transferring excised agar block that bears colony to new broth and agar medium by pushing block method. Repeated transfer of a single colony that has specific morphological structure was made for cloning.

Identification of Mycoplasma: This technique was applied after Mycoplasma cloned through consecutive sub culturing to characterize the isolate obtained. Media were prepared for each biochemical test in the broth culture form. Glucose fermentation, Arginine hydrolysis, Reduction of tetrazolium phosphatase test and protein hydrolysis test (protein liquefaction) biochemical test were done. After preparation of broth for each biochemical test, two milliliter of biochemical identification broth was dispensed in five test tubes containing different biochemicals for each test samples. Then after a thorough agitation of the test broth culture 0.1ml taken by pipetting and inoculated into the four test tubes; for tetrazolium reduction test second broth covered by paraffin to create anaerobic atmosphere. Each sample inoculums was checked daily for color changes of the media.

Interpretations of the biochemical reactions results of *Mycoplasma*.

S/n	test	first color	Positive	Negative
1-	Glucose fermentation	pink red	yellow	original color
2.	Arginine hydrolysis	red	deep red	"
3.	Phosphatse activity	yellow	* Red	"
4.	Tetrazolium reduction			
	aerobic	yellow	pink on the surface	"
	anaerobic	"	pink on the bottom	"
5.	protein liquefaction	coagulated	liquefaction	coagulation

* After NaOH added

3.5. Antigens Detection (Annex3)

3.5.1. Immunobinding on Nitrocellulose paper (Dot- blot)

Five milliliter of thoracic fluid was poured on the tip of nitrocellulose (NC) paper. Then NC was incubated for 10min. at 37°C and washed with tris buffer saline solution (TBS) for 3min. It was immersed into blocking buffer 200µl per well and kept for 30min with slow agitation. The paper was then transferred to hyperimmune sera 200µl aliquots per well and again kept for another 30min. with slow agitation. Then it was washed using TBS three times. Transfer in to developing solution for 2 min. then rinsed with distilled water to stop reactions. Appearance of red dot on the tip of the NC paper was considered as positive reaction and was ranked according to the color intensity strong (+++), average (++), weak (+). Negative (-).

3.6. Complement Fixation Test (CFT)

The O.I.E. standard test procedure is followed for the test (O.I.E. 2000). Even though this test is recommended by O.I.E., its sensitivity and specificity not indicated clearly, as the result of this condition, two different antigens were used to see their test agreement. The *MccP* and *Mycoplasma mycoides* subsp *mycoides* Small colony (bovine biotypes) antigens have been prepared and used, as these two strains are within the 'mycoides cluster' and cross- react (Bolske *et al*., 1994and Thiaucourt *et al*., 1996)(Annex 4).

Screening test was conducted instead of dilution test. Test samples were inactivated at 60^{0}C for 30 minutes to decomplement test sera. Heamolytic system and complement evaluation were done before test proper started. The test proper started by adding 25µl veronal buffer solution (VCM) per well on U-shaped microplates. Each 1:2 diluted test serum was dispensed in double wells. Then working dilution of antigen was added in one of the two wells of each test sera leaving one well to cross check anticomplementarity reactions. Working dilution of 25µl complement was distributed on top of reagents added, in each wells. After careful homogenization of mixtures, the plates were put in the refrigerator at 4^{0}C overnight. Next morning the plates were placed on the bench for at least 10minutes to warm up before 25µl of hemolytic system was dispensed per well. After completions of reagent addition, plates were sealed with sealing tape, homogenized before plates were incubated in 37^{0}C for 30 minutes under slow agitation and moisture condition. Before reading the results, the plates were left in the refrigerator at 4^{0}C, for some times in order to allow non-lysed cells to settle. Positive reactions were indicated by the absence of heamolysis or presence of sediment of SRBC, and negative reactions by the heamolysis of SRBC. Similar procedures have been used for both antigens (*MccP* and *MmmSC*) except that working dilution of antigens were 1:20 and 1:40 for *MccP* and *MmmSC*, respectively.

Table 5. Layout of the microplates for CFT test

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1*	2	2*	3	3*	4	4*	5	5*	6	6*
B	7	7	8	8	9	9	10	10	11	11	12	12
C	13	13	14	14	15	15	16	16	17	17	18	18
D	19	19	20	20	21	21	22	22	23	23	24	24
E	25	25	26	26	27	27	28	28	29	29	30	30
F	31	31	32	32	33	33	34	34	35	35	36	36
G	37	37	38	38	39	39	40	40	41	41	42	42
H	43	43*	44	44*	45	45*	46	46*	47	47*	48	48*

* Rows used to cross check anti complementarity reactions.

3.7. Competitive ELISA (cELISA).

The materials used for cELISA kit were obtained from CIRAD-EMVT, France. The guideline also provided by manufacturer but sensitivity and specificity of the kit were not indicated. On

Nunc polystyrene micro plate (U-type), antigen was coated in all wells of the micro plates overnight at +4^{0}C. Then the coated plates were washed with Phosphate buffer saline with Tween20 (PBST) two times. Before test proper started, pre-plates were prepared and in this pre-plate micro plate 100µl per well blocking buffer diluents dispensed. 11µl of the three control samples were dispensed on designed places (strong positive, weak positive and control negative) as it is shown in lay out below then test sera samples of 11µl added in the remaining wells. Working dilutions of Mab dispensed in all pre-plate test serum and control wells except on conjugate control, instead dilution buffer dispensed in the wells of conjugates. Using multichannel pipette mixtures of test serum/Mab were transferred from the pre-plate to the coated plate. The plates incubated for one hour at 37^{0}C under gentle agitation. After washing with PBS antispecies conjugate/substrate were added at recommended working dilution and incubated for 30min at 37^{0}C under gentle agitation. Washed and dried followed by revelation solution dispensed in all wells stay for 10min inside 37^{0}C incubation. When the time elapses stopping solution added per wells and homogenized gently. Then read at 450nm (Annex 4).

Table 6. Layout of the micro plates for cELISA

	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									20
C	CP++	CP++	21									30
D	CP+	CP+	31									40
E	CP+	CP+	41									50
F	Mab	Mab	51									60
G	Mab	Mab	61									70
H	Cn	Cn	71									80

Cc= conjugate; CP++= strong positive; CP+= weak positive; Cn = control negative

Mab= monoclonal antibody

3.8. Histopathological examination

Tissue samples collected from the field were processed and examined following histopathological procedures, according to Leeson and Leeson (1981). The hematoxylin-eosin stain procedures were applied. Fixation (dehydration clearing and impregnation)

embedding, sectioning, staining and mounting were performed according to established procedures.

Examination and interpretation of tissue sections: hematoxylin and eosin stains were used and nuclear structures of cells stained dark purple or blue, and practically all cytoplasmic structures and intercellular substances are stained pink. Then stained tissue sections was examined for characteristic lesions of CCPP infection, which was acute serofibrinous to chronic fibrino-necrotic Pleuropneumonia with infiltrates of serofibrinous fluid and inflammatory cells, mainly neutrophils, in the alveoli, bronchioles, interstitial septae and subpleural connective tissue (Nicholas, 2002)

3.9. Data Analyses

Participatory disease investigation and questionnaire data collected from field were entered in Microsoft excel spreadsheet. The data were edited as required after summarization and sero-prevalence (%) and 95% confidence interval analysis were performed. Test agreement Kappa used to see serological test agreement and Kendall's coefficient of concordance (W) analyzed by SPSS software (SPSS inc. 1989- 2002 USA) version 11.5.0 to observe agreement between participatory group and individual discussion. Risk factors against the outcome were analyzed using Pearson chi-square, univariate and multiple logistic regressions by STATA-7 (STATA software, STATA Corporation, Texas, 77845 USA). R-soft ware was used to select the best fitted model.

W (Kendall's coefficient of concordance) ranges from 0 to1. The higher the value of W is the higher the agreement amongst the informants. According to critical values for Kendall's coefficient concordance (W) provided by Seigel and Castellan (1994) agreement was termed as weak, moderate and good if W= values were less than 0.26, between 0.26 and 0.38($p<0.05$) and greater than 0.38($p<0.01$ to < 0.001), respectively. Kappa ranges between 1 (complete agreement) to 0(agreement is equal to that expected by chance). If it is negative value, indicates agreement less than is expected by chance. Arbitrary, benchmarks for evaluating observed Kappa value are: >0.81 (almost perfect agreement), 0.61-0.80 (substantial agreement), 0.41- 0.6 (moderate agreement), 0.21-0.4 (fair agreement), 0-0.2 (slight agreement) and 0 (poor agreement) (Thrusfield, 1995).

4. RESULTS

4.1. Participatory disease investigation

4.1.1. Socio-Economic Importance of Goats in Pastoral and Mixed farming area

Participatory investigation and questionnaire survey indicated that goats are important animals among livestock species. Out of 58 respondents from mixed farming area 47 (94%) of the respondents agreed that goats are drought resistance and do have market preference for immediate cash need and sometimes for meat consumptions; whereas in pastoral area a number of uses were listed through the technique called participatory rural appraisal methods.

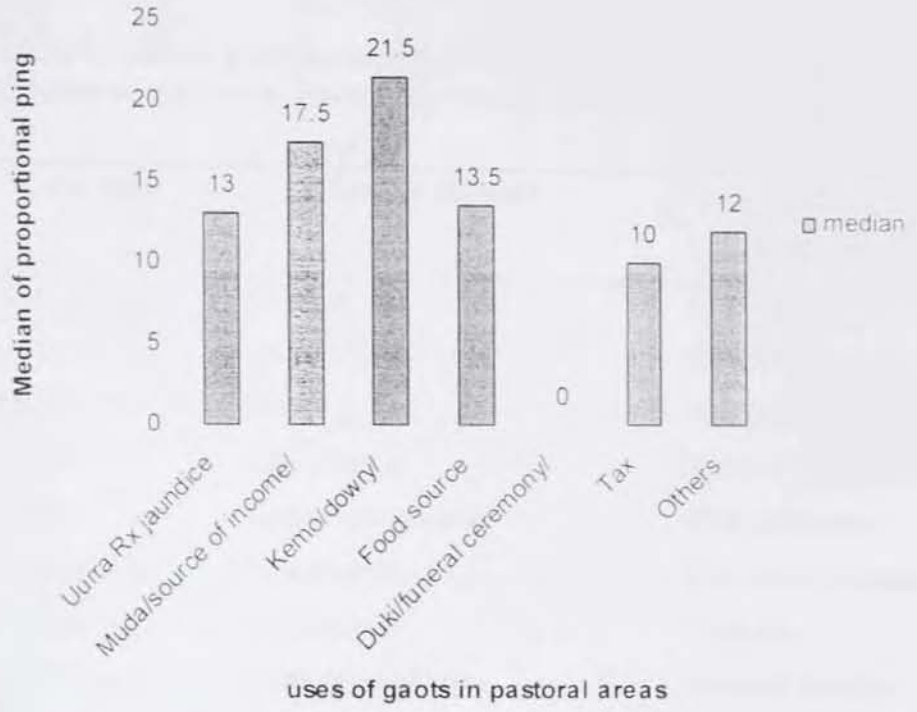


Figure1. The socio-economic importance of goats based on pastoral community responses.

The median shows that kemo (dowry) with the minimum and maximum of (3-36). *Mudda* (source of income) with the minimum and maximum of (14-36). source of food with the minimum and maximum of (0-34). *Uurra* (treatment for jaundice) with the minimum and maximum of (4- 40). funeral ceremony with the minimum and maximum of (0-16). tax with the minimum and maximum of (0-16) and others with the minimum and maximum of (7-20)took the highest rank accordingly as the major use of goats in the pastoral area as it is

shown on the (Fig1). However, despite their values, goats do have major health constraints aggravated by insufficient veterinary infrastructures and man power in the area.
Main diseases that were mentioned by pastoralists and mixed farming areas are listed below. Contagious Caprine Pleuropneumonia, Mange mites, and tick infestation share the highest percentage as compared to the other diseases.

Among the list of diseases mentioned (Table 7) during the participatory disease search, chance was given for different informant groups in order to prioritize according to the severity of the disease that affects their goats. The first four disease repeatedly mentioned by the communities and "others" category were used for further investigation. Diseases that were mentioned are *Sompo* (CCPP), *Quatsi* (mange mite), *Shoko* (Tick infestation), *Pirtsa* (diarrheal syndrome) and others.

Table 7. Lists of goat diseases mentioned by the communities and their veterinary equivalent in Hammer and Benna Tsemay woredas of South Omo zone Sept. to Dec.2004.

Local name	Species affected	Veterinary equivalents
Sompo	Goat/cattle	CCPP/CBPP
Quappa	Goat sheep, Cattle	Pasteurellosis
Quatsi	Goats/ cattle	Mange mite
Pirtsa	Goats/calves	Diarrheal Syndrome
Shoko	Goats/ sheep/cattle	Tick infestation
Shokomekese	Goats/ cattle	Tick-borne infection
Shokolo	Goats/cattle	Foot rot
Berdhet	Cattle/goat/sheep	Internal parasite
Uurramakese	Goat/cattle	Jaundice
Etmma	Human/ cattle/ goat/ sheep	Anthrax
Bosho	Goat/sheep	Orf/FMD

During group discussion major goat diseases mentioned were similar among groups except that disease called *Etmma* (anthrax) was emphasized by few groups as an important goats disease. Due to disagreement between groups about *Etmma*, it has been included in the disease group called 'others' for further research activity. According to proportional piling

showed that the highest median with their minimum and maximum value observed was 29(16-47), 27(7-41), 14.5(8-24), 9.5(4-14) and 11(3-21) for disease *Sompo*, *Quatsi*, *Shoko*, *Pirtsa* and disease called 'others', respectively. The minimum and maximum values of Etmma were (0-25).

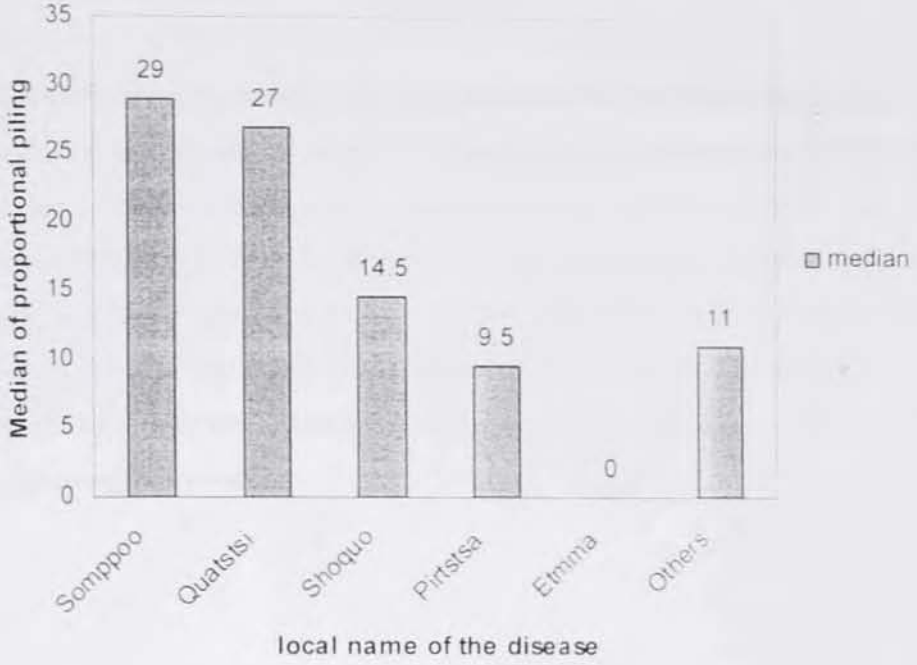


Figure 2. Major diseases of goats using proportional piling obtained from group discussion (n=12 groups) in Hammer and Benna tsemay *woredas*, South Omo zone.

4.1.2. Local perceptions of disease signs (group discussion)

Results obtained from individual and group informants' discussion about disease signs, disease causes and disease seasonal calendars were summarized in Figures 3-7. Agreement between informants was categorized as 'weak', 'moderate' and 'good'. According to critical values for Kendall's coefficient concordance (W) provided by Seigel and Castellan (1994).

The results of matrix scoring for disease signs showed good level of agreement (W=0.39-0.99) between 12 informant groups for the disease signs (Fig-3). The scores allocated to the control diseases indicated that informants understood the scoring method. For instance, *Quatsi* (mange mite) received high scores for itching and *Shoko* (tick) received very low scores for itching even though it is external parasite. The disease called *Pirtsa* was associated with

diarrhea & sudden death. Further probing questioning about the signs of *Pirtsa* showed that informants were able to differentiate sudden death due to *Etmma*. In the case of *Etmma* human being become sick if the carcass was consumed. Informant stated that spleen also becomes enlarged and bleeding from natural orifices exists.

4.1.3 Local perceptions of disease causes (group discussion)

The results of matrix scoring for disease causes showed moderate to good levels of agreement (W=0.37-0.68) among 12 informant groups (Fig.4). The score allocated to the control diseases indicated that informants understood the scoring method. For example, *Sompo* (CCPP), and *Quatsi* (Mange mite) received high scores for 'introduction of sick goats' as cause of disease. Marketing site also received high scores as a cause of CCPP and Mange mites. Further probing questioning revealed that these two diseases are transmitted by direct contact from sick animals. The informants said that grazing and watering site also contribute as the cause of disease occurrence.

Diseases

Signs	*Sompo* CCPP	*Quatsi* Mangemite	*Shoko* Ticks	*Pirtsa* Diarrhealsyndrome	Others
Weight loss W=0.39***	6.5(0-17)	11.5(9-16)	3.5(0-10)	1.5(0-2)	6(1-15)
Diarrhoea W= 0.78***	0(0-11)	0(0-6)	0(0)	18.5(9-30)	10(0-16)
Coughing W=0.84***	28(17-30)	0(0)	0(0-7)	0(0)	0(0-13)
Reduced appetite W=0.70***	17(9-30)	0(0-14)	0(0-3)	1.5(0-8)	7.5(0-18)
Nasal discharge W=0.51***	20(0-30)	0(0-10)	0(0-7)	0(0-9)	7.5(0-17)
Sudden death W=0.57***	15.5(7-24)	0(0)	0(0-7)	7.5(0-14)	6.5(0-19)
Abortion W=0.64***	15.5(7-24)	0(0-14)	0(0-2)	0(0-5)	7(0-30)
High mortality W= 0.65***	16(10-30)	7.5(0-14)	0(0-6)	0(0-10)	2.5(0-16)
Itching W=0.99***	0(0)	20(15-28)	10(2-15)	0(0)	0(0)

Figure 3. Summarized matrix scoring of disease signs based on group discussion. N=12 groups (4-15 informant members participated in each group) W= Kendall's coefficient of concordance (***P<0.001). The black dots represent the median scores. The Minimum and maximum scores are shown in parenthesis. Hammer-Benna words are shown in *italics*.

Disease

Causes	*Sompo* CCPP	*Quatsi* Mangemite	*Shoko* Ticks	*Pirtsa* Diarrhoeal syndrome	Others
Introduction of Sick goat W= 0.68***	16(11-25)	8.5(0-11)	0(0-8)	0(0-9)	2.5(0-11)
Wet season W=0.36**	11.5(0-15)	7(0-12)	5(0-11)	3.5(0-9)	2.5(0-11)
Overcrowding W=0.63***	11(8-16)	7(0-13)	5(0-9)	3(0-7)	3(0-10)
Grazingand watering Point. W=0.62***	14(9-30)	8(0-14)	4.5(0-10)	0(0-6)	1.5(0-9)
Marketing site W= 0.68***	16.5(10-27)	6.5(0-13)	2(0-6)	0(0-6)	0.5(0-9)

Figure 4. Summarized matrix scoring of disease causes based on group discussion.

N=12 informant groups in each group ranges of 4-15 members involved. W=Kendall's coefficient of concordance (**P<0.01; ***P<0.001). The black dots represent the median scores that were used during the matrix scoring. The minimum and maximum scores are shown in parenthesis. Hammer- Benna words are shown in *italics*.

4.1.4. Seasonal factors

Seasonal calendar for goat diseases are shown in Fig-5. These results were obtained from the discussion with informant groups, and identifying seasonal calendar according to the local perceptions. Local name of the seasons and months were recorded. Seasonality mainly depends on rainfall intensity and flood retreats were informants' main factors for classification. They have responded that almost for all diseases scoring were highest during *Bergi* and *Gidibergi*. Scoring was null at *Mishi* season and very small score were observed at *Bonna* season. Probing questions were made to classify each season with events that occur associated with different seasons. Mostly related with livestock movement for grazing and sorghum harvest are also the indications for them to classify seasons. For instance, livestock

move towards their settlement area during rainy season because feed is available and livestock are moved away from their villages during *Bonna* (dry season). Mean rainfall of three years (2002-2004) data were 194.53, 37.75, 79.18 and 67.43 mm in season *Bergi*, *Mishi*, *Gidibergi* and *Bonna*, respectively. Three years mean monthly ambient temperature in the area was (23^{0}C), maximum temperature was recorded in Feb (31.6^{0}C) and minimum temperature occurred in August (11.5^{0}C) (NMSA, 2004). Whereas, median of rainfall observed by proportional piling using 100 beans from 12 group discussions.

	Bergi (rainy season			*Mishi* (After long rainy)			*Gidibergi* (Short Rainy)			*Bonna* (dry season		
	Duka	*Putta*	*Zeqaa*	*Agay*	*Alet*	*Gemeragay*	*Alet*	*Mingi*	*Dalbit*	*Kilkila*	*Bere*	*Sorsa*
	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.	Jan	Feb
Mean Rainfall	197.53mm			37.75mm			79.18mm			68.43mm		
Rainfall median	52			5			30			13		
Sompo W=0.64***	11.5(6-18)			0(0-9)			10(7-12)			5.5(0-14)		
Quatsi W=0.46***	11.5(0-19)			0(0-30)			8.5(0-17)			5.5(0-9)		
Shoko W=0.63***	12.5(7-30)			0(0-7)			9(0-15)			5.5(0-9)		
Pirtsa W=0.72***	12(8-30)			0(0-5)			12.5(0-19)			3.5(0-8)		
Others W=0.72***	12.5(4-30)			0(0-10)			8.5(0-93)			5(0-15)		

Figure 5. Summary of seasonal calendars for major goat diseases.

N= 12 informant groups; W= Kendall's coefficient concordance (*P<0.05; **P<0.01; ***P<0.001). The black dots represent median of the scoring. Local names are written in *italics*.

4.1.5. Individual responses for disease signs

To crosscheck the responses of group discussion, forty-four individuals participated separately in matrix scoring for disease causes and signs. Individual matrix scoring for disease signs showed weak, moderate to good level of agreement (W=0.21-0.89) among 44 individual informants. Weak agreement showed for disease sign as weight loss (W=0.21) and moderate agreement were seen for disease signs as nasal discharge, reduced appetite and abortion at W=0.28, 0.37 and 0.38, respectively. For routinely observable clinical signs, scoring was corresponding with group discussion. Control diseases like *Quatsi* (Mange mite) and *Shoko* (ticks) scoring for respective disease signs indicated that informants understood the scoring method. The disease called *Pirtsa* scored high for diarrhea signs and sudden death. It doesn't affect human if the carcass was consumed unlike the so-called *Etmma*, where it can kill human being if the carcass was consumed. The response obtained from individuals showed similarity with that of group discussions and in both cases level of agreement is highly significant as shown in Fig 6.

4.1.6. Individual responses for disease causes

The result of matrix scoring for disease causes was shown in Fig 7. There was moderate to good agreement among individuals' responses (W=0.26-0.82) except for causes of wet season (W=0.05) the agreement was below W=0.21. This result was different from the result obtained by group discussion, where in-group discussion wet season was considered as a cause of disease occurrence.

Signs	Disease				
	Sompo CCPP	*Quatsi Magemite*	*Shoko ticks*	*Pirtsa Diarrhalsyndreme*	Others
Weight loss W= 0.21*	10(0-20)	8(0-22)	6(0-17)	0(0-16)	5(0-10)
Diarrhoea W=0.54***	0(0-15)	0(0-12)	0(0-5)	15(0-30)	14(0-30)
Coughing W= 0.89***	30(0-30)	0(0-10)	0(0-7)	0(0-5)	0(0-13)
Reduced appetite W= 0.37**	12(0-30)	0(0-10)	0(0-12)	0(0-19)	11.5(0-21)
Nasal Discharge W= 0.28**	13.5(0-30)	2.5(0-30)	0(0-11)	0(0-9)	7(0-30)
Sudden death W=0.60***	10(0-22)	0(0-9)	0(0-8)	8(0-17)	8(0-16)
Abortion W= 0.38**	10(0-30)	0(0-10)	0(0-14)	0(0-9)	10.5(0-30)
High mortality W= 0.66***	20(10-30)	7(0-16)	0(0-14)	0(0-11)	0(0-13)
Itching W=0.84***	0(0)	30(15-30)	0(0-15)	0(0-9)	0(0-5)

Figure 6. Summarized matrix-scoring results of major goat diseases and their signs based on individual responses.

N=44; W=Kendall's Coefficient concordance is (*P<0.05; **P<0.01; ***P<0.001). The black dots are median of scoring. Minimum and maximum represented in parenthesis. Hammer-Benna words written in *italics*.

Disease

Causes	*Sompo CCPP*	*Quatsi Mangemite*	*Shoko Ticks*	*Pirtsa Diarrhalsyndreme*	Others
Introduction of goats W= 0.26**	9(5-18)	8(0-15)	5(0-12)	4.5(0-9)	5(0-13)
Wet season W= 0.05	6.5(0-18)	5(0-11)	7(0-12)	5.5(0-12)	7(0-12)
Overcrowding W= 0.54***	11(5-17)	8(5-18)	3.5(0-18)	4(0-6)	4(0-9)
Grazing and watering Point. W= 0.34**	10(0-20)	6.5(0-17)	5(0-8)	2.5(0-17)	4(0-30)
Marketing site W= 0.82***	16(9-26)	9.5(3-15)	0(0-8)	0(0-8)	0(0-9)

Figure 7. Summarized matrix scoring results of major goat disease causes based on individual responses.

N=44 individual informant, W= Kendall's coefficient Concordance (*p<0.05; **P<0.001; ***P<0.001). Median of the score is represented in black dots. Minimum and maximum scores are presented in parenthesis.

4.1.7. Post mortem and clinical sign examinations (Triangulations)

All the cases presented for post-mortem examination due to *Sompo* were above one-year old and all of them were female except one male. Information on case histories, clinical signs and postmortem lesions were recorded. Frequent coughing was reported and observed in all cases, however the duration of coughing among cases varied from a week to months. Nasal discharge was reported and it was observed in half of the cases. Diarrhea cases were also observed in severely sick goats. The history of respiratory distress and lagging behind from the flock were reported and also observed in some of the cases. There was sign of weakness and frequent recumbancy. All cases of goat had high tick infestation. *Amblyomma* spp and *Hyalomma* spp were predominant.

Out of the gross pathological lesions observed, the communities emphasized adhesion of lung with chest wall, straw colored thoracic fluid, fibrinous tissue coverage of lung & consolidated parts of lung were commonly observed.

Table 8. Case histories, Clinical signs and Gross pathological lesions examined during participatory disease search.

Case history, clinical and Post-mortem findings.	Case No 1	2	3	4	5
Case history					
Coughing	+	+	+	+	+
Weakness	+	+	-	+	+
Lagging behind the flock	+	+	+	+	+
Nasal discharge	+	+	-	-	+
Diarrhea	+	+	-	-	-
Clinical signs					
Coughing	+	+	+	+	+
Weight loss	+	+	+	+	+
High respiratory rate (per minutes)	56	52	60	44	48
Temperature (^{0}C)	40.2	39.9	41	39.6	39.8
Pulse rate (per/min)	112	108	112	104	100
Nasal discharge	+	+	+	-	-
Diarrhea	+	+	-	-	-
Post mortem examined					
Adhesion of lung with chest wall	+	+	-	+	+
Yellow nodules surrounded by congestion	+	+	-	-	-
White patches over the lung	+	+	-	-	-
Strew colored thoracic fluid	+	+	-	+	+
CFT test	+	+	-	-	-

Questionnaire survey results

Responses of open ended and closed structured questionnaire survey were recorded from selected woredas of Gamo Goffa zones in mixed farming area. A total of 58 farmers were individually asked about major goat disease in their locality. The responses obtained were recorded, the frequencies of response for each disease were ranked and CCPP/*Goffina*/, Mange mites, (*Gandi*) trypanosomiasis ranked 1st, 2nd and 3rd, respectively (Annex-5).

In addition to major diseases, informants have responded about risk factors associated with the occurrence of CCPP. Each respondent ranked first main causes of disease then followed by the next causes. The scores were compiled as frequency of responses and then the disease that has highest score was ranked first. Introduction of sick stock was ranked first and grazing site ranked as second cause of disease spread and soon. Control measures were mainly by treating the animals and followed by vaccination. Marketing of healthy animals in fear of disease as well as segregation of sick from healthy ones are also practiced.

Table 9. Major causes of disease outbreak responded by GamoGoffa communities using questionnaire survey.

Causes	frequency	Rank
Watering sites	24	4
Grazing sites	35	2
Wet season	31	3
Overcrowding	11	6
Sick stock introduction	45	1
Others (gift, purchase)	19	5

4.2. Bacteriological isolation

Out of the total 14 cultures, 7 (50%) broth cultures and the corresponding 7 (50%) plates had signs of *Mycoplasma* growths. Those plates that showed *Mycoplasma* growth were again cultured in broth and solid media. After a number of passage colonies with dense center or nipple shaped morphology were cloned. The isolated *Mycoplasma* growth was further characterized by biochemical and immunobinding on nitrocellulose paper dot blot test.

Mycoplasma culture results from nasal swabs, thoracic fluids, and lung tissue in liquid and solid media.

	Nasal Swabs		Thoracic fluids		Lung tissue		Total	
	Broth	Plate	Broth	Plate	Broth	plate	Broth	plate
Cultures	6	6	4	4	4	4	14	14
Mycop. Growth	3	3	2	2	2	2	7	7
Contamination	-	3	2	2	2	1	4	6
No growth	3	-	-	-	-	1	3	1

4.2.1. Biochemical test

Seven isolates were cloned from the samples cultured and the isolates were used for biochemical identification of the field strain. All the isolates fermented glucose and reduced tetrazolium in both aerobic and anaerobic atmosphere. All isolates showed negative phosphatase activity and hydrolysis of arginine. All of the isolates including the control, liquefaction were not produced the protein as shown in table 10.

4.2.2. Immunobinding nitrocellulose paper (Dot-blot)

Samples that were characterized by biochemical test were further subjected to dot blot test using *MccP* monoclonal antibody to confirm whether the isolates have *MccP* antigen or not (Poumarat, *et al.*, 1991; Poumarat, *et al.*, 1992). Three broth cultures from thoracic fluids origin and broth culture from lung tissue origin were found to be positive for dot blot at different intensity. Thoracic fluid origins were strong positives and others were moderate to weak positives. But broth cultures from nasal swab origin were not positive as shown in fig 8.

Table 10. Biochemical test results obtained from isolates samples

Isolates from	Glucose Fermentation	Arginine hydrolysis	Phosphatase activity	Tetrazolium reduction Aero/anaero	Protein hydrolysis
Positive control	-	-	-	+/-	-
Lung suspension	-	-	-	+/+	-
Lung suspension	-	-	-	+/-	-
Thoracic fluid-1	-	-	-	-/+	-
Thoracic fluid-2	-	-	-	+/+	-
Nasal swabs -1	+	-	-	+/+	-
Nasal swabs- 2	+	-	-	+/+	-
Nasal swabs- 3	+	-	-	+/+	-

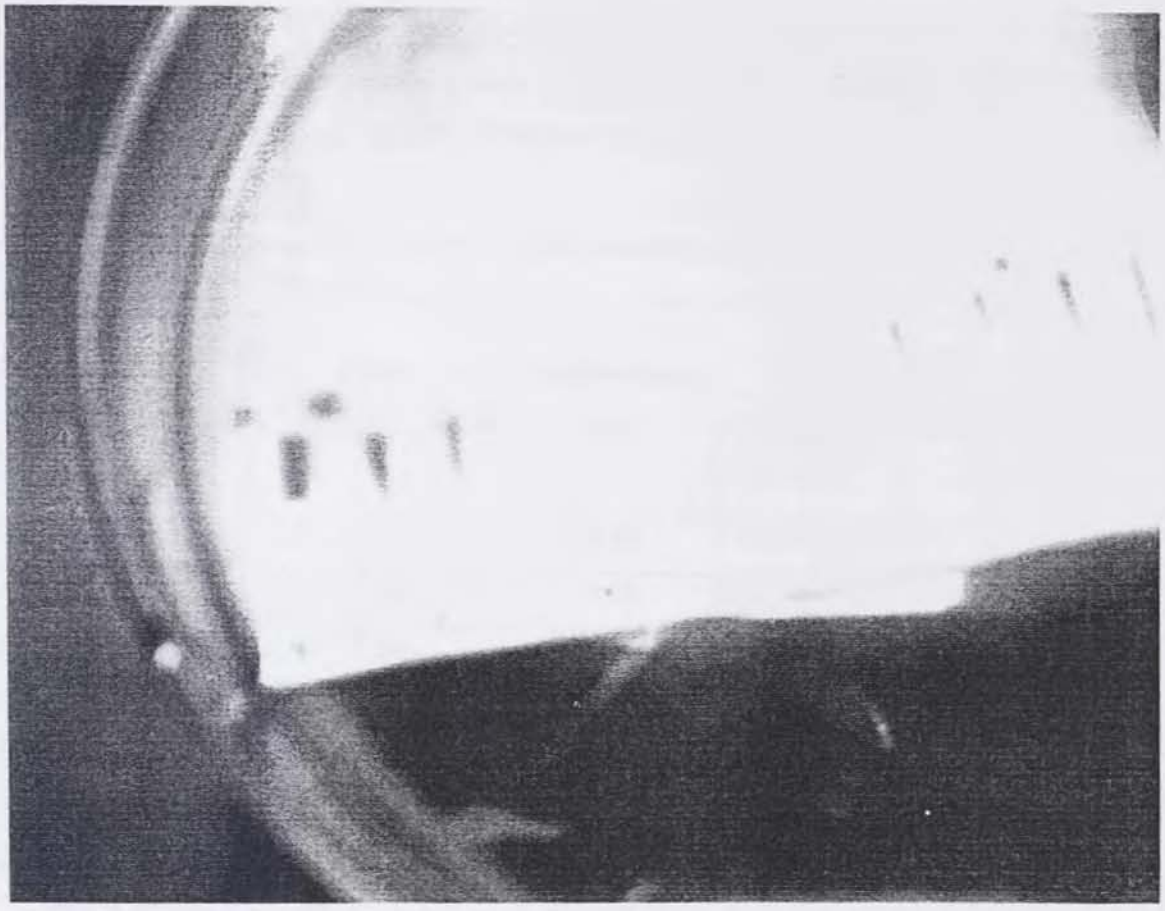


Figure 8. Picture shows immunobinding nitrocellulose paper (from the left to the right). Two thoracic fluid origins showing strong (+++) color staining intensity from the left side, next to these three tips of paper doesn't show any staining followed by moderate color staining observed next to empty staining(++). At the end of right side negative and positive control staining observed.

4.2.3. Histopathological examination

Tissue samples from lung were stained with hematoxylin eosin staining and observed under binocular microscope. The predominant lesion was congested alveolar septa. The walls of the alveolar septa were thickened and infiltrated with neutrophils and fibrin.

4.3. Serology

4.3.1 Sero-prevalence of CCPP infection in goats and sheep using CFT

A total of 1033 goat sera and 80 sheep sera were collected from selected *woredas*. All samples were tested for the presence of serum antibodies against CCPP infection using CFT with *MccP* and *MmmSc* antigens. The prevalence were 170 (16.63%) and 225 (21.78%) in goat sera, and 55 (68.75%) and 58 (72.5%) sero positive in sheep sera, for *MccP* and *MmmSc* antigen, respectively. Four hundred fifty goat and eighty sheep sera were also subjected to competitive ELISA. In this section, seroprevalence estimates presented with reference to CFT with *MccP* antigen result shown in table 11. The over all seroprevalence of CCPP infection in selected five *woredas* was 16.45% (95%CI=14.18-18.8%)

Table 11. Sero-prevalence of CCPP in goat sera sampled from five selected *woredas* of South Omo and Gamo Goffa zones.

woreda	Goats		Seroprevalence (%)	95% CI	OR	p-value
	Tested	Positives				
Hammer*	352	55	15.63	11.83 -19.41		
Benna-Tsemay*	327	50	15.29	11.34 -19.23	1.02	0.90
Arbaminch**	113	26	23.00	15.24 -30.75	1.6	0.06
Boreda**	121	39	32.23	27.98 -36.47	2.6	0.001
Humbo**	120	0	0	0	0	0

* Pastoral woredas ** sedentary woredas

As shown in table11 in spite of CCPP outbreak, CFT test was negative in Humbo *woreda*. There was no significant difference (P>0.05) in seroprevalence among Benna Tsemay, Hammer and Arbaminch zuria *woredas*. But there was significant difference (P< 0.001) in seroprevalence between Boreda and the other three *woreda* (Hammer, Benna-Tsemay and Arbaminch zuria).

Table 12. Seroprevalence of CCPP in South Omo and Gamo Goffa zones using CFT for the assumed factors

Variables	n	seropositive	seroprevalence (%)	95%CI	OR	P-Value
Production systems						
Pastoral	679	105	15.46	14.6 - 16.00		
Mixed farming	354	65	18.36	17.13 – 21.15	1.23	0.2
Sex						
Female	807	137	16.98	14.38-19.57		
Male	226	33	14.60	10.0-19.10	0.84	0.4
Age groups						
0.5 ≤2yrs	380	55	14.47	10.93 - 18.01		
2≤ 4yrs	414	70	16.0	12.52 - 19.58		
>4yrs	239	45	18.82	13.87- 23.78	1.5	0.06
Flock size category						
1- 10	195	66	33.8	0.00 - 40.5		
11- 20	11	9	81.8	59.9 -100		
> 21	12	10	83.3	62.2 -100	0.98	0.89
Total	218	85	38.99	32.52- 45.46		

Seroprevalence between pastoral and mixed farming production system showed 15.72% and 18.36%, accordingly. There was no statistical difference (P> 0.05) between two production systems in CFT test (table 12). Seroprevalence was relatively higher in females (16.98%) than males (14.6%), however, this difference was not significant (P>0.05).

Sero prevalence increased from 12.4% in younger to 18.82 % in age groups above 4 years old. However, there was no significant difference (P>0.05) between age groups in seroprevalence (P>0.05) as shown in Table 13. The age group was categorized into three groups: age group-1 includes (0.5 to ≤ 2 yrs), age group-2 (2 to ≤ 4 yrs) and age group -3 those animals above 4years old were compared. Three age groups showed no significant difference in seroprevalence (P>0.05).

Table 13. Seroprevalence of CCPP in South Omo and Gamo Goffa at different age groups using CFT results

Age/year/	sample size	sero positive	sero-prevalence (%)	95% CI
0.5	64	8	12.4	4.4 - 20.6
1	169	21	12.5	7.4 - 17.4
2	147	26	17.7	11.5 - 23.9
3	218	36	16.5	11.6 - 21.4
4	196	34	17.4	12.0 - 23.0
>4	239	45	18.8	13.9 - 23.8
Total	1033	170	16.69	14.2 - 18.2

The flock sizes in the study area were determined based on the average number of goats sampled per house hold and categorized into three groups: Group-1 flock size with average number of sampled goat between 1 and 10 per house hold, group-2 flock size between 11 and 20, and group-3 flock size greater than 21 average numbers of sampled goats. Flock was considered as seropositive when one positive animal is found. Seroprevalence based on flock size was shown in table14. Logistic regression analysis for flock size and seropositives with CFT test indicated that there is no significant difference (P>0.05) among flock categories.

Table 14. Flock level seroprevalence of CCPP in South Omo and Gamo Gaffa zones based on Compliment Fixation Test using *MccP* antigen result.

Woreda	flock size Tested	N° animals positives	N° Flock positives	Flock seroprevalence (%)	95% CI
Hammer	15	55	11	73.33	51.0 - 95.7
Benna Tsemay	29	50	18	62.06	44.4 - 79.7
Arbaminch	57	26	25	43.85	31.0 - 56.7
Boreda	54	39	31	57.41	44.2 - 70.6
Humbo	63	0	0	0	0
Total	218	170	85	38.99	32.5- 45.5

Seroprevalence of CCPP at flock level is given in Table 14. It was higher in Hammer (73.3%) and Benna Tsemay (62.01%) than Arbaminch (43.85%) and Boreda (57.41%) woredas. On individual animal basis, the prevalence was higher in Arbaminch (23%) and Boreda (32%).

4.3.2 Seroprevalence of CCPP using cELISA

The serological kit was provided by CIRAD-EMVT and the test was done according to the guideline supplied by the manufacturer. There were four categories of test results based on the percentage inhibition (PI); Negative, Doubtful, Weak and Strong positive corresponding to percent inhibition of <20%, 20-25%, 25-55% and above 55%, respectively. Among 450 samples tested, 320 samples from pastoral and 130 samples from mixed farming area were used.

For the purpose of analysis doubtful results were categorized as negative results and weak and strong positive percentage inhibition categorized as positive results, and sero-prevalence were calculated for the assumed risk factors.

Figure 9 shows the status of CCPP seropositivity in relation to flock size. The seroprevalence was higher in all flock size ranging from 93.33% to 100%. Univariate and multivariate analysis for flock size and seropositivity indicated that there was a significant difference ($P<0.001$) among flock categories. There was actually remarkable goats' population difference between these two areas. Number of flock used for sampling in mixed farming area was 174 and all of them were below 10 flock size as compared to 44 in pastoral area where the majority of flock sizes were above 11 goat's number.

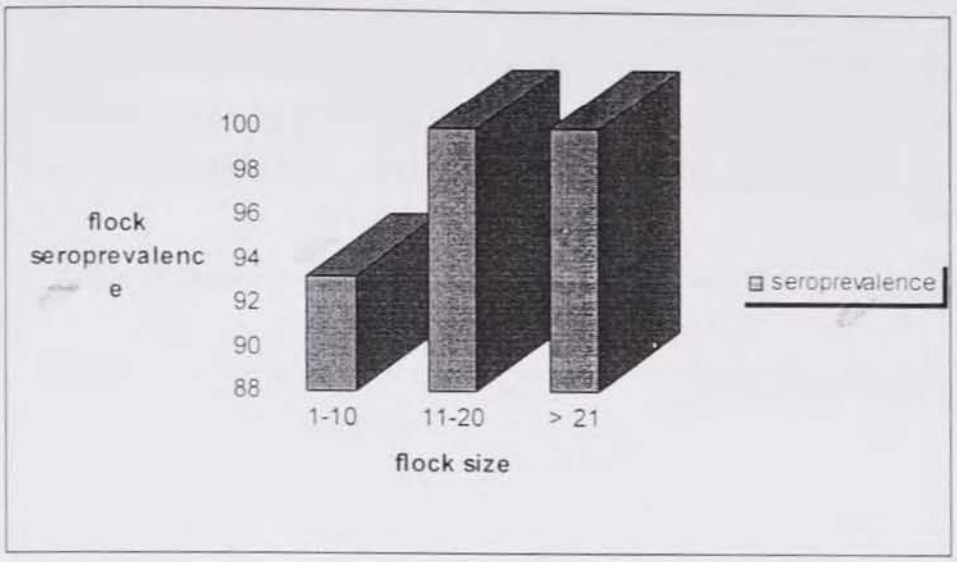


Figure 9. Seroprevalence of CCPP in relation to flock size cELISA.

The mean flock seroprevalence in the study area with at least one seropositive goat per flock was 93.15%. The seropositivity among the woredas was high ranging from 88.8% to 100% as shown in table 15. At the level of flock seroprevalence the average seroprevalence in mixed (Arbaminch and Boreda woreda) area was 90.3% and in pastoral (Hammer and Bennatsemay woreda) area was 100%.

Table 15. Flock level seroprevalence of CCPP in South Omo and Gamo goffa zones based on competitive Enzyme Linked Immunosorbent Assay.

Woreda	N° flock Tested	N° positive animals	N° positive flock	Flock level seroprevalence(%)	95% CI
Hammer	5	57/160	5	100	100
B /Tsemay	10	91/160	10	100	100
Arbaminch	49	75/89	45	91.8	83.8 - 99. 8
Boreda	9	31/41	8	88.8	78.4 - 99.4
Total	73	258 /450	68	93.15	89.3 - 99.7

As shown in Table16 seroprevalence of CCPP infection using cELISA in female and male was 60.75% and 45.54%, accordingly. This difference in seroprevalence was significant

(P<0.05). However, the ratio of female to male goats tested was 3:1. Significant difference was also observed between pastoral (46.25%) and mixed (84.6%) production system. The difference was significant (p<0.001). In the univariate analysis goats were 6.4 times more likely to be seropositive in mixed farming system than pastoral production system.

Table 16. Seroprevalence of CCPP in goats using cELISA test result for putative risk factors.

Variables	No of sample	No of positives	seroprevalence	95% CI	OR ratio	P-value
Production systems						
Pastoral	320	148	46.25	40.78-51.71		
Mixed farming	130	110	84.6	78.39-90.80	6.39	0.000
Flock size						
1-10	60	56	93.33	87.02-99.64		
11-20	5	5	100	100		
≥21	8	8	100	100	0.34	0.000
Age group						
0.5≤ 2yrs	176	96	54.54	47.18- 61.89		
2 ≤ 4yrs	204	114	55.88	48.93-62.69		
> 4yrs	70	48	68.57	57.69-79.44	1.82	0.045
Sex						
female	349	212	60.75	55.89- 66.11		
male	101	46	45.54	36.28-55.72	0.54	0.007

Seroprevalence using cELISA test did not show clear trend of seropositivity as the age increased. However, seroprevalence ranges from 36% to 68.5%. The highest percentage was observed in adult group above 4 years. The lowest percentage was in younger groups as shown in Table 17. The majority of goats were in the ranges of 2-4 years. The age was categorized in three groups in Table 16. The seroprevalence difference was significant (p<0.05) between age categories ranging from 54.54% in age categories 0.5≤ 2 years to 68.57% in those above 4 years.

Table 17. Seroprevalence of CCPP in goats in selected woredas of South Omo and Gamo Goffa zones at different age groups using cELISA test.

Age (year)	n	No of positive	sero-prevalence(%)	95%CI
0.5	32	16	50	32.7- 67.3
1	76	36	36	25.2 - 46.8
2	68	44	64.7	53.3 -76.1
3	108	67	62.0	52.8 -71.2
4	96	47	49.0	39.0 -59.0
>4	70	48	68.6	57.7 -79.4
Total	450	258	57.3	52.8 - 61.9

Table 18. Seroprevalence of CCPP in South Omo and Gamo Goffa zones using CFT test for the assumed independent variables on sera sample matching with cELISA.

Variables	No of tested	No of Positives	Seroprevalence (%)	95% CI	OR	P-Value
Production systems						
Pastoral	320	62	19.37	15.04 - 23.71		
Mixed framing	130	30	23.08	15.8 - 30.32	1.24	0.378
Flock size						
1 - 10	60	27	45	32.41 – 57.58		
11- 20	5	5	100	100		
≥ 21	8	6	75	45.00 - 100	0.80	0.083
Age category						
0.5 - ≤ 2 yrs	176	33	18.75	12.98 - 24.51		
2 - ≤ 4yrs	204	42	20.58	15.04 – 26. 12		
> 4 yrs	70	17	24.28	14.24 - 34.32	1.2	0.34

The seroprevalence of CCPP in pastoral area was 19.37% and 46.25%, and in mixed farming area was 23.08% and 84.6%, using CFT and cELISA, respectively. The difference in seroprevalence is significant (P< 0.001) based on cELISA test results as shown in Table 16 and Table 18.

There was no significant difference in all independent variables using CFT test result as shown in Table 18. However, as compared to the result of cELISA, all risk factors with seroprevalence were linearly related except at flock size category, greater than 21 flock sizes were lower in CFT test from the preceding flock size category.

4.3.3. Univariate and Multivariate analysis of risk factors

Univariate analysis was conducted for putative risk factors having epidemiological role in seroprevalence of CCPP. These factors were either intrinsic (age) or extrinsic (production system and flock size) the results are given in the Table 19. Each the assumed risk factors were analysised separately. Those significant risk factors by univariate further analysised by multiple logistic regressions.

Table19. Univariate logistic regression estimates for risk factors of CCPP infection in South Omo and GamoGoffa zones based on cELISA.

Risk Factors	OR	P-value	95% CI
Flock size 11-20 Vs 1-10	0.29	0.001	0.14 - 0.59
Flock size > 21 Vs 1-10	0.11	0.000	0.06 - 0.19
Age category 2 ≤ 4yrs Vs 0.5 ≤2 yrs	1.11	0.790	0.70 - 1.58
Age category > 4yrs Vs 0.5 ≤ 2 yrs	1. 81	0. 045	1.01 – 3.26
Age category > 4yrs Vs 2 ≤ 4 yrs	0.58	0.111	0.3 - 1.15
Production system	6.39	0. 000	3.78 – 10.80
sex	0.54	0.007	0.34 – 0.84

Further analysis were conducted using multiple logistic regression model adjusted for assumed risk factors showed significant association with flock size($p<0.01$) and age category >4 years($p<0.05$). However, the difference between flock size 11- 20 Vs 1-10 and in other age category were not significant ($P>0.05$) as shown in Table 20.

Table 20. Multivariate logistic regression estimates for risk factors of infection in goats in the study area

Risk factor	OR	P-Value	95%CI
Age category 2 ≤ 4yrs Vs 0.5 ≤2 yrs	1.35	0.188	0.86 - 2.14
Age category > 4yrs Vs 0.5 ≤ 2 yrs	1.94	0.046	1.01 - 3.71
Flock size 11-20 Vs 1-10	0.16	0.092	0.019 - 1.36
Flock size > 21 Vs 1-10	0.05	0.007	0.007 - 0.47
Production system	0.53	0.540	0.063 - 4.24
sex	0.84	0.490	0.506 – 1.38

The best fitted model obtained was logit [p] = 2.26 + 0.34age group – 1.09 flock size. Interaction between age group and flock size was not significant (p>0.05) using R-soft ware. Table that shows best fitted model

Variables	Coefficient	Std.	Z	P-value
Intercept	2.262	0.409	5.52	$3.29e^{-08}$***
Age group	0.339	0.155	2.18	0.029*
Flock size	- 1.099	0.132	-8.37	$< 2e^{-16}$***

df= 447 Resid. Deviance = 524.66. Deviance for interaction model was 524.65 the difference between two deviance was only 0.01, so it was not significant at (P>0.05, df=1 χ^2=3.84).

4.3.4. Comparison of CFT using MmmSc and MccP antigens

Contagious Caprine Pleuropneumonia seropositivity comparisons were made between two CFT tests as shown in Table 21. Positive results by complement fixation test using *MccP* antigen were found in 170(16.45%) samples, while positive results in the CFT using *MmmSc* antigen were 225(21.78%).

Table 21. Concordance and Kappa values on sera samples from South Omo and Gamo Goffa zones based on two kinds of CFT tests.

zones	*woreda*	Sera tested	CFT with		Concordance (%)	Kappa Value	agreement
			MccP	*MmmSc*			
South Omo goats	Hammer	352	55	81	85.8	0.55	Moderate
	B/tsemay	327	50	71	88.7	0.63	Substantial
	total	679	105	152	87.2	0.59	Moderate
Gamo Goffa goats'	Arbaminch	113	26	38	87.6	0.69	Substantial
	Boreda	121	39	35	83.5	0.61	Substantial
	Total	234	65	73	88.3	0.65	Substantial
	Humbo	120	0	0	0	0	0
	Overall total	1033	170	225	88.3	0.62	Substantial
South O. Sheep	Hammer	40	27	28	92.5	0.82	Almost perfect
Gamo Gofa Sheep	Mirababaya	40	28	30	90.0	0.75	Substantial
	total	80	55	58	91.25	0.78	Substantial

Test agreement for CFT using *MccP* and *MmmSc* antigen based on the crude agreement (concordance) and the agreement beyond chance (Kappa Value) were performed on sera samples from different localities. The mean concordance and Kappa value for over all goats sera samples regardless of the origin, was found to be 88.3% and 0.62, respectively. Where as in sheep sera mean concordance and Kappa value was 91.5% and 0.78. The statistical agreement between the two tests was substantial agreement as shown in Table 21.

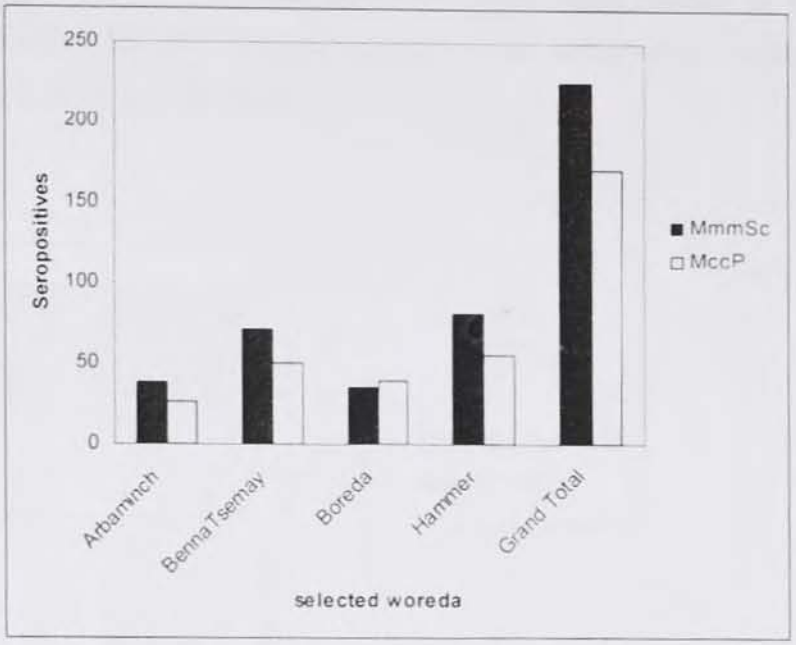


Figure 10. Contagious Caprine Pleuropneumonia seropositivity comparison between two CFT test using *MccP* and *MmmSc* antigen in study area.

4.3.5. Comparison between cELISA and CFT using MccP antigen

Table 22. Concordance and Kappa Values of sera samples using cELISA and CFT using *MccP* test from study area.

Species	*woreda*	Sera tested	cELISA	CFT using *MccP Ag*	Concordance (%)	Kappa Value	Agreement
Goats	Hammer	160	57	27	61.25	0.043	Slight
	B/Tsemay	160	91	35	45.00	-0.021	Lessthan 0
	total	320	148	62	53.12	0.017	Slight
	Arbaminch	130	110	30	24.62	-0.014	Less than 0
	Total	450	258	92	44.88	-0.014	Less than 0
Sheep	Hammer	40	8	27	42.5	0.05	Slight
	Mirababaya	40	14	28	40.0	-0.071	Less than 0
	Total	80	22	55	41.25	-0.005	Less than 0

The matched 450 goat sera and 80 sheep sera, which were tested parallel by both CFT and cELISA, were observed for their agreement based on the seropositive results. The mean concordance and kappa results are shown in Table 22.

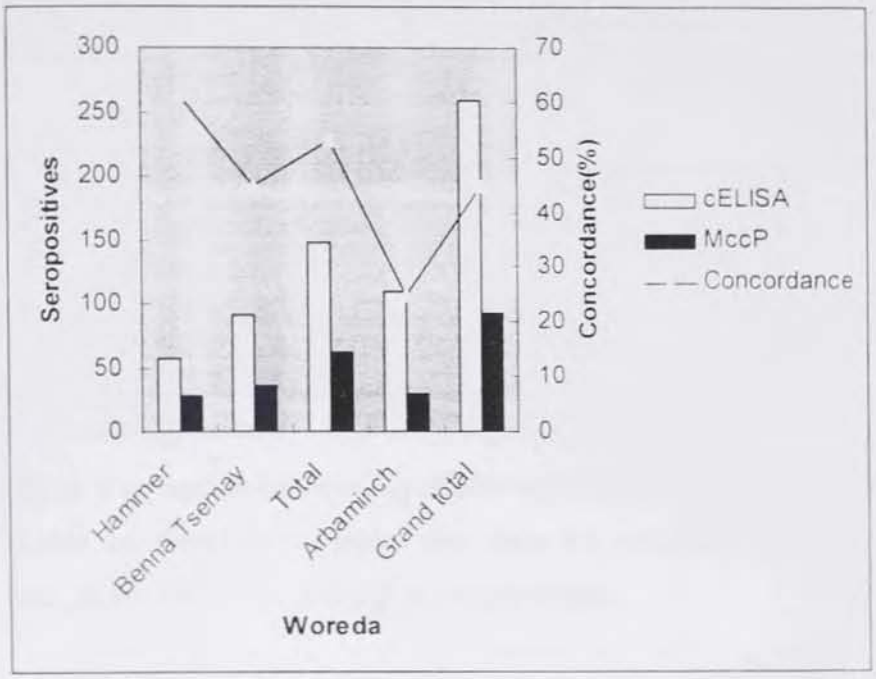


Figure 11. Comparison of CCPP sero positives between cELISA and CFT using *MccP* antigen based on 450 sera tested from goat samples.

As shown in Table 22 the test between cELISA and CFT test was not in agreement. The overall concordance and Kappa value were 44.88% and 41.25%, and -0.014 and -0.005, for goats and sheep, respectively. The agreement was less than expected beyond the chance.

4.3.6. Test agreement comparison between cELISA and CFT using MmmSc antigen

Parallel test were conducted on 450 goat and 80 sheep sera using cELISA and CFT by *MmmSc* antigen as shown in Table 23. The mean concordance and test agreement beyond the chance in goats and sheep were 43.5% and 35%, and -0.061 and -0.081 values, respectively.

Table 23. Concordance and Kappa values of 450 sera samples collected from pastoral and mixed farming area using cELISA and CFT with *MmmSC* antigen.

species	woreda	Sera tested	No of positives using		Concordance (%)	Kappa	agreement
			cELISA	CFT using *MmmSc* Ag			
Goats	Hammer	160	57	36	55.6	-0.054	Less than 0
	Benna tsemay	160	91	47	43.7	-0.065	Less than 0
	Total	320	148	83	49.6	-0.044	Less than 0
	Arbaminch	130	110	45	28.4	-0.180	Less than 0
	Over all total	**450**	**258**	**128**	**43.5**	**-0.062**	**Less than 0**
Sheep	Hammer	40	8	28	35.00	-0.048	Less than 0
	Mirababaya	40	14	30	35.00	-0.130	Less than 0
	Total	**80**	**22**	**58**	**35.00**	**-0.081**	**Less than 0**

There is no agreement between cELISA and CFT with *MmmSc Ag* in both species.

Table 24. Contingency tables that show the overall agreement between CFT and cELISA tests based on matched 450 goat seroprevalence.

		CFT using *MccP Ag*			CFT using *MmmSc Ag*		
		positive	Negative	Total	positive	Negative	Total
cELISA	Positive	**51**	207	258	**66**	192	258
	Negative	41	**151**	192	62	**130**	192
	Total	92	358	450	128	322	450

Crude concordance between cELISA and CFT *MccP* = (51+151)/ 450*100= 44.88%
Kappa = -0.014

Crude concordance between cELISA and CFT *MmmSc*= (66+130)/450*100= 43.55%
Kappa = -0.062

4.3.7. Seroprevalence of CCPP in sheep

Eighty sheep sera were sampled using purposive sampling method from pastoral production and mixed farming system. Forty sheep from Hammer *woreda* that were kept together with goats and equal number of sheep were sampled from Mirab abaya *woreda* in mixed farming system. Sheep sera were tested to observe epidemiological role of species: three parallel tests were conducted using CFT with *MccP*, CFT with *MmmSc* and cELISA (CIRAD-EMVT). The result of each test was shown in table below.

Table 25. Test results of sheep sera collected from different sites obtained by two CFT and cELISA

			CFT with MccP Ag		CFT with MmmSc Ag		cELISA	
woreda	*PA*	samplesize	Pos	%	Pos	%	Pos	%
Hammer	*Dembayte*	10	8	80	7	70	5	50
	Duss	10	6	60	7	70	0	0
	Zeldaketa	10	7	70	7	70	2	20
	Kolakeja	10	6	60	7	70	1	10
Mirbabaya	*Doshe*	40	28	70	30	75	14	35
Total		80	55	68.75	58	72.5	22	27.5

Seroprevalence using CFT using *MccP* and *MmmSc* antigen were high, 68.75% and 72.5%, respectively. Where as using competitive ELISA, 27.5% sero-positive animals observed as given in table 25.

Table 26. Seroprevalence of CCPP in sheep in pastoral and mixed farming system of South Omo and Gamo Goffa (Oct. 2004 to Mar 2005).

Variables	Sera tested	No of Positives	Seroprevalence (%)	95% CI
Production systems				
Pastoral	40	8	20	7.62 – 32.4
Mixed farming	40	14	35	20.22 – 49.8
Age category				
0.5 ≤ 2 yrs	41	11	26. 82	13.56 - 40.38
2 ≤ 4 yrs	34	9	26.4	11.60 – 41.23
> 4 yrs	4	2	50.0	1.00 - 99.0

As shown in table 26 seroprevalence of CCPP was higher in mixed farming (35%) than pastoral system (20%).It was also higher in sheep older than 4 years (50%) compared with animals younger than this age group. However there was no significant difference between each category of these factors ($p>0.05$).

5. DISCUSSION

5.1. Participatory disease Investigation

5.1.1. Local perceptions among groups and individuals

Participatory disease search included the use of proportional piling, matrix scoring and seasonal calendar to understand community perception about the major uses and diseases of goats in Hammer and Benna tsemay woredas of South Omo zone.

Proportional piling of beans about the importance of goats in pastoral area revealed that there is a wide range of socio-economic importance of goats. The significance of goats' values was attributed due to their highly prolific, drought resistance, manageability by children and women's and easily marketable for immediate need as well as resource for cultural taboos (Peacock, 1996). Especially for the cultural practice known as "*kemo*" which means "dowry", this can be performed in the presence of large number of goats, from 20 to 127 goats depending on the ability of individual person to offer the bride price. Goats are valuable assets for food and social security in the area. A finding of this study agrees with Farm Africa observation. Farm Africa (1996) reported that blood used as source of food, traditional medicine, and goatskins for traditional leather garments for women, social occasions like payment for bride price, burial or other cultural ceremonies.

The problems of CCPP and mange mites were also indicated by Farm Africa (1996). Monthly disease outbreak reports show CCPP is being reported routinely (MoA, 2004). The findings of participatory discussion substantiated that CCPP is considered as a major disease in the area. The repeatability of the methods used was, analyzed by 'W' Kendall's coefficient concordance. Response agreement among groups and individuals indicates that the standardized participatory method was repeatable. As compared to the work done by Catley *et al.* (2002a) and Catley *et al.* (2001) the methods were reproducible. It was possible to say that the methods were valuable for understanding local characterization of goat diseases. Especially after matrix scoring method was completed, probing questions were valuable for crosschecking scores and relate local name of the disease with the clinical signs and postmortem findings. Probing questions in "Duss" one of the sampling sites, revealed that sudden death accompanied by diarrhea was different from sudden death caused by

Etmma(Anthrax). The later was anthrax that causes sickness even death if the carcass consumed by human being. As it was shown from matrix scoring of diseases and their respective clinical signs, the communities are well aware of the diseases and their clinical signs. For instance, "*Sompo*" means lung and "*piskilsa*" means coughing. It is possible to see that community have recognized that coughing is associated with lung disease. The other control disease called "*Quatsi*" means "Mange mite" is to indicate that the disease causes itching, communities were able to select and show goats infected by mange mite in their flock.

These findings had weak, moderate to good agreement (W=0.21 to 0.99) in both group discussion (n=12) and individual participatory disease search (n=44) except in individual disease cause matrix scoring where wet season was W=0.05.The difference might be individual awareness varies enormously; therefore, disagreement could appear during responses whereas group discussion could bring ideas that can be agreed by groups, because of discussion which can help them to memorize the events or causes of the diseases.

This shows that indigenous knowledge about animal diseases in the area developed through generation in the pastoralist area. Further probing questions revealed that pneumonia due to pasteurellosis differ from CCPP by its clinical signs and postmortem lesions. According to the participants "*Quappa*" means pasteurellosis the name derived from the clinical sign of throat suffocation, which means the disease signs is mainly difficulty in breathing especially in the upper respiratory tract. They have stressed that in cases of *Quappa* there is no gross lesion like adhesion and yellow color thoracic fluid. *Sompo* kills a number of goats in flock within short period of time.

Community perceptions about disease name and their signs largely related with modern disease signs. Findings indicated that *Sompo* with clinical signs of coughing, weight loss, sudden death, and high mortality coincides with modern CCPP clinical signs (Nicholas, 2002; Bereket, 1995). Itching signs was strictly allocated for "*Quatsi*" (mangemite). Assessment of disease causes shown that the disease "*Sompo*" and "*Quatsi*" were strongly associated with the introduction of sick goat, marketing site, grazing and watering site and overcrowding (direct contact). Hammer and Benna community clearly indicated that these two diseases are their major problem and transmission is through contact between sick and healthy goats.

5.1.2. Seasonal factors

The occurrence of "*Sompo*" in different seasons varies according to the matrix scoring allocated by the community. It was indicated that most cases occur during long and short rainy season, *Bergi* (March to May) and *Gidibergi* (Sept. to November), respectively.

Seasonal calendars have diagnostic value, because results can be crosschecked against other findings like "matrix scoring" which was demonstrated by associations between fasciolosis with snails in the wet season (Catley *et al.*, 2001). This study has also shown that the occurrence of "*Sompo*" is associated with wet season. Matrix scoring described that wet season is one of risk factors for the occurrence of the disease. The study of seasonal calendars used to describe seasonal variations by their own definitions of seasons. All the participatory disease search process helps people to feel confident about their own language and knowledge, and strengths the relationship between researcher and livestock keeper, especially seasonal calendars were used to open discussion on topics such as the best time to control livestock disease and external parasites. In seasonal calendar analysis, informants were remarked that the given seasonal pattern information is based on normal weather conditions when drought exists such types of pattern won't exist (Lienhardt, 1961, Evans-Pritchard, 1940 cited by Catley, *et al.*, 2002b). Similar suggestions were observed from respondents in this fieldwork during seasonal calendar matrix scoring.

5.1.3. Postmortem and clinical sign examinations

Postmortem and clinical examination of the sick goats that were presented by the farmers revealed the classical gross lesions and signs of CCPP. Gross pathological lesions were restricted to thoracic cavity. There was an adhesion of lung with pleura, and sero-fibrinous fluid inside the cavity. Yellowish pea sized nodules on the surface of lung surrounded by congestion. The diameter of nodules was varied in different observation; a case of one lung was fully consolidated. Maré (2004) and Thiaucourt (1996) indicated that postmortem lesions and clinical signs could be used for field diagnosis of CCPP, characterized by respiratory distress, high mortality and postmortem lesions of fibrinous Pleuropneumonia with pronounced consolidation and pleural adhesions. Unilateral consolidation, accompanied with accumulation of strew-colored pleural fluid and pleuritis are common features.

5.2. Bacteriological Isolation

Mycoplasma capricolum subsp. *capripneumoniae* is one of the fastidious organisms to grow in vitro and very susceptible for external environment (Thiaucourt, 1996) sick goat and some thoracic fluid kept in cooler jug with ice used during sample collection, to submit specimen for diagnosis at NVI. The isolated colony had dense centered and nipples like morphology were observed and biochemical tests confirmed that isolates were glucose fermenting and arginine negative and phosphatase negative group of *Mycoplasma* (Nicholas and Bashiruddin, 1995). Slow growing characteristic of the *MccP* were observed by (MacOwan, 1976) and confirmed by Nicholas, (2002) and Thiaucourt, (1996) that *MccP* are fastidious and slow growing organisms. This slow growth rate compared with *M. ovipneumoniae*, but morphologically the colony of *M. ovipneumonie* does not show dense centered (center less) as well as not related antigenically.

Mycoplasma mycoides cluster grown within 48 hours (Thiaucourt, 1996; Nicholas, 2002; Nicholas and Bashiruddin, 1995). In this study sub culturing were made after membrane filtration to remove contaminants and fast growing *Mycoplasma* following the first culture. Several serial subcultures were made to clone slow growing *Mycoplasma*. To confirm the causative agent immunobinding nitrocellulose paper was used (Poumarat, *et al.*, 1991 and Poumarat, *et al.*, 1992)

5.3. Serology

5.3.1. Seroprevalence

The findings in this study area show that 16.45% and 57.33% sero-prevalent using CFT and cELISA, respectively. Low seroprevalence of CFT may be related to the ability of the test to detect antibody that appear first and span short duration in the blood. Complement fixation test clear limitations were observed by March *et al.* (2000) where 80 to 100% of acute phase animals experimentally infected with *MccP* was not detected. In addition, significant complement fixation titers in infected goats were not observed until 21 days post infection. Therefore, at an early stage in a field outbreak false negative results based upon serological

diagnosis are a real possibility (March *et al.*, 2000). This idea substantiates negative result obtained in Humbo woredas where antibody was not detected by CFT test, despite in the presence of CCPP disease outbreak.

However, cELISA was not conducted in this woreda because of shortage of reagents. Higher seroprevalence using cELISA in other parts of study sites might be due to the presence of long lasting antibody and repeated infection. This finding agrees with Thiaucourt (1996) who reported that antibody detected by cELISA persisted longer than detected by CFT. In his observation sero-conversion did not occur in all animals, in any test. In case of cELISA, the seroprevalence animals in affected flocks varied from 30% to 60%. Consequently, none of the tests gave an indication for individual test rather it is used for flock test. Isotypes of antibody production against antigen varies in their duration in the serum (Tizard, I.R.1996). IgM is the first to appear and has little specificity which gives rise to pronounced cross reactions 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 isotypes (Staak, *et al.*, 2001). Based on this report it indicates that seropositive case by CFT test shows that there is recent infection and circulation of the causative agent within the flock. Where as, the result associated with cELISA might be due repeated infection.

5.3.2 Comparison of serological tests

The concordance and kappa value were compared for two CFT tests using *MccP* and *MmmSc* antigens for detecting CCPP antibodies in both goats and sheep. The agreement found to be substantial agreement. The mean concordances were 88.3% and 91.25% in goats and sheep, respectively. The mean kappa values were 0.62 and 0.78 in goat and sheep, respectively.

The findings in this study agree with different workers. Belton *et al.* (1994) and Nicholas and Bashiruddin (1995) have stated that *Mycoplasma mycoides* 'cluster' are pathogenic mycoplasmas of cattle, sheep and goats, which are genomically or serologically interrelated. Great homogeneity of the sequences were obtained within two subspecies (*MccP* and *MmmSc*) observed regardless of their geographical origin (Thiaucourt, 2000). Isolates of *MmmSc* from goats have serologically cross-reacted with mycoides cluster and showed identical biochemical and growth characteristics (Nicholas and Bashiruddin, 1995). Serological investigation of CCPP using CFT with *MccP* and *MmmSC* antigen and blocking-

in CCPP reported area, respectively (Sharew *et al.*, 2005). In this study 16.45% and 5% seroprevalence in goats and 68.75% and 72.5% seroprevalence in sheep obtained, g *MccP* and *MmmSC* antigens in CFT tests, accordingly showed very close relation. efore, any one of the tests could be used for screening and particularly with *MmmSc* gen.

concordance and kappa values were compared for cELISA and CFT using *MccP* antigen detecting CCPP antibodies in both species. The agreement was negative. The mean ıcordance for goats and sheep was 44.88 and 41.55%, respectively. The mean kappa value s -0.014 and -0.005, in goats and sheep, accordingly. The difference might be due to ferences in detecting different immunoglobulin. This finding agrees with other observers ıt antibody detected by cELISA are long lasting than antibody detected by CFT hiaucourt, 1996). The complement fixation test detects antibody that appear first and span ort duration (March, *et al.*, 2000). So this might be the reason for different seroprevalence ıd negative agreement. Therefore, these tests could complement each other instead of one eing gold standard or confirmatory test for another.

.3.3 Risk factors

Seroprevalence of CCPP infection appeared to be higher in mixed farming system (23.05% and 84.6%) than pastoral area (19.4% and 46.25%) using matched number of samples tested by CFT and cELISA. There was significant difference ($P<0.01$) based on the result of cELISA. The high seroprevalence result obtained from this study may be attributed to the frequent livestock movement from high susceptible area to endemic area because of marketing access. Thiaucourt (1996) explained that higher rates of seroprevalence occur during epidemics where as in endemic situations rates are much lower. This might be due to introduction of high susceptible population since rapid replacement of stock occurs from non-infected area. It could be also over stock density might have contributed for the high seroprevalence. This finding agrees with that of Lefevre *et al.* (1987), which stated that extensive husbandry practices, mixing of goats at watering, grazing areas, market access and shelters play a great role in the spread of infections.

Contagious Caprine Pleuropneumonia is known as a major disease of goats in Africa and Asia. Morbidity is very high and mortality may reach 80% (Thiaucourt, 1996; Maré, 2004;

Nicholas, 2002). It was also suggested by other workers that subclinical infection occurs in sheep and cattle and reactors were also found in impala, buffalo and camels (Paling *et al.*, 1978). In present study due to limited biological and reagents wide ranges of host were not subjected for testing, but test result from 80 sheep have shown that 68.8% and 27.5% seropositive result using *MccP* antigen CFT and cELISA test, respectively. Previous studies on sheep in Ethiopia have reported seroprevalence of 5% in Arbaminch (Mekonnen, 1996), 13% in Yabello (Dawit, 1996) shows that sheep can be infected subclinically. In addition, Yigezu *et al.* (2004) isolated Mycoplasma from two nasal swab samples out of 20 collected from apparently healthy sheep inhabiting in CCPP outbreak site around Arbaminch. This finding agrees with the previous observations of Litamoi *et al.* (1992). Current study and the previous study give insights about the role of sheep that can harbor and remain a continuous source of infection. Keeping sheep with goats favors for the continuous circulation and maintenance of the causative agent from subclinically infected sheep. This is because livestock owners are not aware of subclinical infection of sheep with CCPP could occur. The communities have clearly suggested that sheep are not diseased by CCPP, even if both species are kept together.

The occurrence of *MccP* other than goats and the occurrence of *MmmSc* in goats and sheep (Nicholas and Bashiruddin, 1995) could indicate some epidemiological role especially from serological cross-reaction point of view, either of the two strains could be used for disease prevention.

The absence of significant association ($p>0.05$) between sex and seropositivityto CCPP in current investigation using multivariate analysis was in agreement with the report of Dawit (1996), Mekonnen (1996), Beyene (2003) and Zenebe (2004).

In both univariate and multivariate logistic regression significant association ($p<0.05$) between seroprevalence and age category > 4 years Vs age category 0.5 to ≤ 2years was observed. This might be associated as result of animals get older and stayed in the infected area for a long period; which can contribute to be infected with the causative agent. However, study conducted in Ethiopia by Dawit (1996), Mekonnen (1996), Beyene (2003) and Zenebe (2004) have observed no difference in age groups. Nicholas (2002) indicated that goats of all ages and sex groups are equally susceptible to the disease.

(2004) have observed no difference in age groups. Nicholas (2002) indicated that goats of all ages and sex groups are equally susceptible to the disease

Community response using participatory disease search and questionnaire survey indicated that introduction of sick goats, grazing and watering site, and wet season were mentioned as risk factors. Individual and group responses were in agreement statistically with Kendall's coefficient concordance. The idea raised by community agrees with other reports (Thiaucourt, 1996; Nicholas, 2002; Maré, 2004) because the disease is contagious and transmitted only by direct contact between sick and healthy goats. Climatic change could also contribute for disease out break occurrence.

The odds ratio (OR) value of the seropositivity showed that the goats in mixed farming area in Boreda and Arbaminch woredas were 6.4 times more likely to be seropositive than goats in pastoral area. Goats in age groups greater than 4 years were 1.8 times more likely to be seropositive than goats in age group between 0.5 to ≤ 2years using univariate analysis. However, odds ratio of production system was less than one and was not significant ($p>0.05$) during multivariate analysis, where as flock size category above 21 was significant ($p=0.007$) and age category above 4years was significant ($p=0.046$). In multivariate analysis odds ratio of age category above 4 years were 1.94 times more likely to be seropositive than goats in age group between 0.5 to ≤ 2years. There was no interaction between flock size and age group. The best fitted model obtained from this risk factor analysis was Logit [seropositive] = 2.26+0.33 age group – 1.09flock size.

It was observed the difference between flock and individual seroprevalence in pastoral and mixed farming system. The highest individual seroprevalence was seen in mixed farming area 26.8 and 84.26%, as compared to 16.8 and 35.6% in pastoral area, using CFT and cELISA, accordingly. On the other hand, flock seroprevalence in pastoral area was 90 and 100% as compared to 36 and 91.8% in mixed farming area, according to CFT and cELISA test results. It is difficult to suggest the finding. It might be attributed that large flock category dominant in pastoral area compared to small flock size category in mixed farming system. Therefore, the probability of getting one seropositive animal from large flock size is greater than small flock size. This could be the reason, despite relatively low individual seroprevalence in pastoral area flock prevalence is higher than mixed farming system.

6. CONCLUSION AND RECOMMENDATIONS

The participatory disease search, structured questionnaire survey, bacteriological isolation, serological and post mortem findings strongly indicate that CCPP infection is one of the major goat health problems in pastoral and mixed production systems in South Omo and Gamo Goffa zones of Southern Ethiopia.

Participatory epidemiological study shows indigenous knowledge used to generate more information with less cost and simple local materials within short period. Local description of the disease using matrix scoring and seasonal calendar shows that CCPP and mange mites were regarded as major causes of goats' loss in the area.

Individual seroprevalence of 20.4% and 57.33%, and flock prevalence of 52% and 93.2% using matched number of sera by CFT and cELISA test, respectively is suggestive of a wide occurrence and distribution of CCPP infection in both pastoral and mixed farming production system in these two zones. The isolation of causative agent proves that disease out break associated with respiratory clinical signs in the area is mainly due to CCPP.

In final analysis among the risk factors that were associated with seroprevalence, flock size and animal older than 4 years were found to be significantly associated ($p<0.05$) with the outcome (seroprevalence).

Comparison of serological tests between CFT with *MccP* and *MmmSC* antigens shows that there was substantial agreement with Kappa value of 0.62, whereas the agreement between CFT and cELISA was negative with kappa value of -0.014 in goats, though both are known to be used as diagnostic tests for CCPP.

In the present study sheep were highly seropositive (68.5 and 27.5%) by CFT and cELISA tests despite no clinical disease has been detected or indicated in the PDS, which indicates that this species could be a potential source of infection for the causative agent.

Based on the findings, the following recommendations are forwarded

- Participatory disease search was used as one of the tool to investigate major diseases in the area and the findings were in agreement with clinical, pathological and laboratory disease search methods, hence the technique could be applied for disease investigation in pastoral productions systems.
- Contagious Caprine Pleuropneumonia is found to be the most economically important disease in the study areas. Therefore, control measures including routine vaccination and movement control measures should be implemented.
- Strengthening of the veterinary infrastructure at grass root level is necessary in order to provide immediate service during outbreaks in the remotest part of the country, where market access for drug purchase and private veterinary service is scarce.
- Risk factors that were observed in this study and other unseen factors that could aggravate the occurrence of disease should be thoroughly studied.
- Further detailed experimental and field studies on the diagnostic ability and merits of CFT and cELISA and whether they have complement each other or used individually has to be conducted.
- Attention must be given for sheep as it could be source of infection for *MccP* and *MmmSc*. Thus CCPP control program should include control in sheep too.

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8. ANNEXES

Annex 1. Questionnaire for CCPP survey in mixed farming zone (Gamo Goffa)

1. Address: Region Zone District PA

---------- ---------- ---------- ----------

Village---------- GPS ----------Date----------

2. Name of Owner ----------. sex----------. Age---------- ---

3. No of goats Owned

3.1 Age and sex distribution of goats owned

<1year(1) 1-2 years (2) >2years(3)

F(0)___M(1) F(0)___M(1) F(0)___M(1)

4. Comparative use/ importance/ keeping animal species (1to3)

	Cattle	goat	sheep
Disease resistance	----------	----------	----------
Drought resistance	----------	----------	----------
Market preference	----------	----------	----------
Economic valve	----------	----------	----------
Milk use	----------	----------	----------

5.What is objective of raising goats?

Dairy (1) Cash income (2) Meat consumption (3) Others (4)

---------- ---------- ---------- ----------

6. What kind of production system do you practice?

Sedentary (1) Transhumance (2) Pastoralist (3) Others specify

---------- ---------- ---------- ----------

7. How do you manage during day? Separately or communally. If it is communal

7.1 Is it with the same community, other community or both?

---------- ----------

7.2 If the contact is with other community or both where do flocks contact.

At grazing---------- watering point ---------- Market --------- -----

7.3. Is there any contacts between species at grazing site --------- Mixed the whole year(1)

Some times in the year (2)

Separated the whole year (3)

Is watering point---------- Permanent (1)

Temporary (2)

8 Did you encounter goat health problems? Yes/No

9. What are the major goat diseases, according to their importance (1to5)?

1---------- 2---------- 3---------- 4----------and 5----------

10. Which disease out of you have mentioned is severely affect goat flock?

1---------- 2---------- 3---------- 4----------

11. Do these diseases involve Sheep? Yes/No

12. Is CCPP present in your herd/ neighboring herd / districts zones currently? Yes (1)/No (0)

13. Which age and sex groups were more affected?

< 1year ------- 1-2 years ------- > 2years------ All age and sex group

F(0)----- M(1)----- F(0)----M(1)--- F(0)----M(1)-----

14. What possible reasons for the disease outbreak?

Watering point --------- season----------

Grazing point ---------- large flock size----------

Sick stock introduction----------

Gift----------

Purchased----------

Others----------

15. What measures do you take to combat the disease during outbreaks?

1. Marketing of affected/Health/ ----------
2. Vaccination ----------
3. Treatment ----------
4. Segregation, ----------
5. Slaughter----------
6. Others----------

16. Did you vaccinate goats? Yes/No

17. Against which disease was vaccination given?

18. What type of drugs do you use? How many times do you use?

Oxytetracycline---------- ----------

Penicillin---------- ----------

Streptomycin---------- ----------

Others ---------- ----------

19. Who treated your goats?

Public service----------

Private service----------

Local trader ----------

Traditional----------

Others----------

20. Markets values of goats and sheep

Age	Healthy			
	Goats		Sheep	
	F	M	F	M
< 1year				
1-2 years				
> 2 years				

Age	Sick			
	Goats		Sheep	
	F	M	F	M
< 1year				
1-2 years				
> 2 years				

21. Was there any traditional treatment used? Yes/No

If yes what was the results

Annex 2. Record sheet Format

Zone---------- Woreda---------- PA's----------

Village---------- Spp---------- Flocksize----------

GPS---------- Date----------

S/n	Name of Owner	No of goats	Age	sex	Altitude	Prod. Systems	season	CFT With *MccP*	cELISA	CFT With *MmmSc*

Annex 3 Antigen detection procedures

1. Immunobinding on Nitrocellulose paper (Dot- blot) or indirect immunoperoxidase test.

Materials and methods

- Test samples of pleural fluids or tissue suspensions, broth culture stored in deep freeze at -20^{0}C.
- Nitrocellulose paper cut in small sizes (0.3cm x 1.5cm) that can easily fit in micro plate wells.
- Micro plates (greiner U); micropipettes and tips.
- Incubator, and agitator
- Tris Buffer saline (TBS), 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 at -20^{0}C (polyoxyethylene sorbitanolaurat).

- Hyperimmune sera diluted in blocking solution.

  Monoclonal antibodies (Mab 1: 5.000): Mouse hyper immune serum against *Mccp*.

  - Conjugates: peroxidase labeled goats antirabbit (1: 200) and anti mice (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.)
  - Reference Mycoplasma strains of *MccP*[F38].

Procedure of Dot-blot test

1 5µl of the thoracic fluids was poured on the tip of the nitrocellulose (NC) paper.
2 The papers was incubated for 10 minutes at 37°C and washed with TBS for 3 min.
3 Then immersed into blocking buffer 200µl per well and was kept for 30 min with slow agitation.
4 The NC was then transferred to hyper immune sera, 200µl aliquots per well and kept for another 30 min. with slow agitation.
5 Washed three times in TBS, 250µl per well (3minutes per washings).
6 200µl conjugate was added per well and the NC paper immersed in it for 30 min., with slow agitation.
7 Then repeated washings as procedure 5. Washed three times in TBS.
8 Transferred into developing solution, pipetted 150µl per well, for 2min.

Finally, rinsing in distilled water stopped the reaction. Appearance of red dot on the tip of the NC paper was considered as positive reaction and was ranked according to the color intensity, (+++) strong, (++) average, (+) weak, and (-) negative or no reaction.

Annex 4 Serological test procedures

1. Complement Fixation Test (CFT) (O.I.E., 2000).

Materials and methods

- Test sera of goats
- Microplattes U- from (greiner labortechnik), multichannel micropipettes and tips.

- Guinea pig complement 5u and complement diluent's (Diagnostics CIRAD EMVET, France).
- Veronal buffer solution, PH7.2

MccP(F38) antigen , 1:20 Dilution in Veronal buffer and MmmSC antigen 1:40 dilution in veronal buffer solution.

- Sheep red blood cell (SRBC): Sheep blood collected from jugular vein in Alsever's Solution 3:8 ratios (75ml blood in 125mlAlsevers).
- Titrated haemolytic serum: rabbit anti sheep RBC (Diagnostics Pasteur, France).
- Water bath, incubator with an agitator.

Antigen preparation

Mycoplasma capricolum subsp. capripneumonie antigen preparation.

MccP was isolated after it has been characterized by an immunoperoxidase test and protein electrophoresis, and preserved as a lyophilized pure culture (Thiaucourt *et al.*, 1992), was obtained from CIRAD-EMVT and used as the source of antigen. A portion of the freeze-dried material was inoculated into 3ml of modified Newing's tryptose broth growth medium and incubated at 37°C for a further 5 days. Sub culturing was done for a second time into 100ml of growth medium, with incubation at 37°C for another 5 days. The culture was then centrifuged at 7000g for 15 min at + 4°C and the sediment was washed three times using distilled water. The packed organisms were resuspended in 1ml-distilledwater, diluted 1:60 and ultrasonicated by ultrasonicator for 3min at lowpower in a container of iced water. The suspension was centrifuged at 1250g for 30min to remove any debris (O.I.E., 2004). The antigen was then stored at -20°C in aliquats of 10ml in screw capped glass bottles until it was used.

MmmSC antigen preparation

A freeze dried strain of *MmmSc*, obtained from CIRAD-EMVT, was inoculated into 10ml of *Mycoplasma* broth medium and incubated at 37°C for 10 days. The organisms were then harvested by centrifugation at 7000g for 15min at + 4°C. Subsequently, they were washed three times with distilled water and the packed organisms were resuspended in 100ml-distilled water. The suspension was autoclaved for 10-15min, coded and sonicated for 20min at low power in a container of iced water, to give an even suspension. The suspension was distilled in distilled water until its opacity at a

dilution of 1:10 matched that of Brown's tube N 2 (Campbell and Turner. 1953). To preserve the suspension, 0.5g phenol was added to each 100ml and it was stored at $+4^{0}$C for 6 weeks, with shaking for 5min at weekly intervals. The antigen was then titrated for use by checkerboard titration and tested for ant complementary activity. If this was present, the suspension was re-agitated, stored as before and re-tested after 6weeks. When there was no longer anti complementary, the antigen was used in the CFT as recommended by O.I.E. (2004).

Antigen titration

To determine range of suitable antigen concentrations (working dilutions of an antigen stock of certain concentration) Positive control serum was used. Checkerboard titration was used to combine range of antibody concentrations which produced clearly positive reactions margin was considered the working dilution that could create the optimum reaction with test sera. According to antigen checkerboard titration, *MccP* and *MmmSC* antigen working dilutions was 1:20 and 1:40, respectively.

Complement Evaluation

Serum harvested from guinea pig was used as complement. The working dilution of complement was evaluated daily before test proper underwent. The C' evaluation was done without addition of antibody and antigen, instead of these diluents was used to replace the volume. Then C' was serially diluted with diluent's in micro plates and equal amount of heamolytic system added according to recommended amount and time limits. Instead of 3 hemolytic units five hemolytic units were used as a working dilution.

Preparation of hemolytic system

2% SRBC preparation.

One day before preparation sheep blood was harvested by Alsever's solution at a rate of 75ml blood in 125ml Alsevers' solution. The SRBC was washed by centrifugation at 2500 rpm for 5min using test tube. The procedure repeated three times. Packed cell volume was measured before the last supernatant discarded. Then PCV was diluted by veronal buffer solution into 2% SRBC.

Amboceptor Titration:

To complete heamolytic system anti-sheep red blood cells was tittered. Amboceptor was diluted at 1:2 rate up to 1:256 after equal amount of diluent dispensed into each wells. Equal volume of 2% SRBC added to each wells and incubated at room temperature for 10min. then C' added at working dilution and incubated at 37^{0}C for30 minutes. The minimum heamolytic dilution (MHD) was read and recorded then one unit back from MHD was used for working dilution.

Test Proper

1. Test sera were decomplemented in hot water bath, 60^{0}C, for 30 minutes.
2. 25µl veronal buffer solution was dispensed in all wells of microplates.
3. Each test sera (1:2 diluted) dispensed in double wells one well was used for anti complementary reaction control.
4. The working dilutions of 25µl antigens (MccP and MmmSc) were added into one of the two wells of each test sera simultaneously in different micro plates, for both antigen tests, to check serum anti complement activity.
5. The working dilution of complement was added 25µl in all wells.
6. The content was carefully homogenized and kept overnight at +4^{0}C
7. Next morning the plates were placed on the bench for at least 10minutes to warm-up before 25µl of hemolytic system was dispensed per well.
8. The plates were sealed with sealing tape, homogenized gently then incubated in 37^{0}C for 30 minutes under moisture condition.
9. Before reading the results, the plates were left in the refrigerator at +4^{0}C, for some times in order to allow non- lysed cells to settle.
10. Positive reaction was seen by the absence of haemolysis, and presence of SRBC, and negative reactions by the hemolysis of SRBC.

2. Competitive ELISA (cELISA).

Materials

- Test sera of the sample
- Nunc- immunoplates (Maxisorp), multichannal micropipettes and tips.
- Freeze dried *MccP* antigen with diluents.
- Monoclonal antibodies *MccP*(CIRAD-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 ant-mouse IgG labeled with horseradish peroxidase.
- Substrate (1mm ABTS solution 40.05m citrate buffer PH5 $+H_2O_2$)
- Reference sera. strong positive. weak positive and negative serum (CIRAD-EMVT)
- Incubator with an agitator. sphectrometer.
- Stopping solution

Test proper Procedure

1 Nunc polystrene microplates were coated with *MccP* antigen lysed with sodium dodecyl sulphate(SDS) diluted at 1:100 with phosphate buffer saline(PBS) and incubated overnight $+4^0$c.

2. washed with washing solution 300µl /well two times

3. Pre-plate test sera. Mab. conjugates. control serum were prepared at recommended dilution rate and incubated at 37^0c.for one hour.

4. Transferred 100µl of the mixture of test serum/Mab from the pre-plate to the coated plate using a multichannel pipette. Controls sera. conjugate and Mab were dispensed in their respective wells.

5. The plates incubated for 1hour at 37^0c.Under gentle agitation.

6. Washed two times at 300µl/well washing solution.

7. Rabbit anti-mouse immunoglobulin conjugated to horse raddish peroxidase was diluted at 1:50 and dispensed 100µl/well then incubated for 30 minutes at 37^0c under gentle agitation.

8. Revelation solution of 100µl/well was dispensed in all wells and incubated at 37^0c for 10min

9. Stopping solution of 100µl added per wells. Shaked gently until the colored solution was homogenized. Incubated for 5-10 min to develop the color. Wiped carefully the underside of the plate.

10. Read at 450nm

The percentage of inhibition (P.I.) was given by the formula.

P.I.= 100[ODMab- Odtest]/ (ODMab- ODCc).

Where ODMab was the mean of the 0% control and 100% the OD conjugate was the mean of 100% control.

Interpretations

P.I. $\leq$20% considered as negative

P.I. 20- 24.99% considered as doubtful but for data analysis added with negative results.

P.I. 25-55% considered as weak positives (p+)

P.I. 55-85% considered as strong Positives (P++)

Annex 5. Major Diseases of goat mentioned in Gamu Goffa zone based on the questionnaire survey.

Local name	Veterinary equivalent	Frequency of response	Rank
Goffinna	pneumonia/CCPP/	36	1
Pusso/pussele/	Mange Mite	31	2
Chirunch	Ticks	12	4
Tirre	Internal parasite	10	6
Gendi	Trypanosomosis	23	3
Shotte	Diarrhea	10	6
Ketsa	Orf	4	7
Wozena	Heart water	2	9

Annex 6. Sero prevalence of CCPP in South Omo and Gamo Goffa zones at selected PA's and 95% confidence interval of seroprevalence.

PA	No of samples tested	No of positives	Seroprevalence(%)	95% CI
Abela Kolshobo	41	0	0	
Abela Sippa	40	0	0	
Abella Gaffeta	39	0	0	
Alduba	89	13	14.60	7.26- 21.93
Challi	62	8	12.90	7.64- 21.24
Dambayte	88	12	13.63	6.42- 20.77
Dubenabullo	45	14	31.11	17.57-44.62
Duss	88	16	18.18	10.12-26.23
Ganta Meche	28	7	25	8.96-41.03
Kolakeja	88	13	14.77	7.35-22.12
Quola shele	46	9	19.56	8.09-31.02
Shabba	88	6	6.81	1.54-12.05
Shelle Millae	39	10	25.64	11.9-39.30
Sille	88	23	26.14	16.95-35.31
Xenxele	41	11	26.83	13.27- 40.39
Zefene Menuka	35	14	40	23.77-56.0
Zeldeketa	88	14	15.91	8.26-23.55
Grand Total	1033	170	16.46	14.18-18.18

Annex 7. CCPP outbreak report 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
DIREDAWA	10	576	413	111

Annex 8. Seroprevalence of CCPP in different parts of the Ethiopia

Source	Study area	No of animals Examined	Prevalence %
Mekonnen.L. (1996)	Arbamich	254 goats 108 sheep	36 5
Gezahegn. M. (1993)	East Shoa	200 goats	51.5
Dawit, K. (1996)	Yabello	273 goats 28 sheep	24 13
Teshome. F.Y. (1997)	Afar.Arsi. North Omo. Somali Wollo (pooled)	375 goats	17.9
Beyene. N. (2003)	Dire Dawa	319	6
Bereket. Z. (1995)	Konso	122	35

Annex 9. Annual CCPP outbreak reports from 1998-2004 in Ethiopia

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 disease outbreak report

Annex 10. CCPP outbreak in SNNP regional state from year 1998-2004

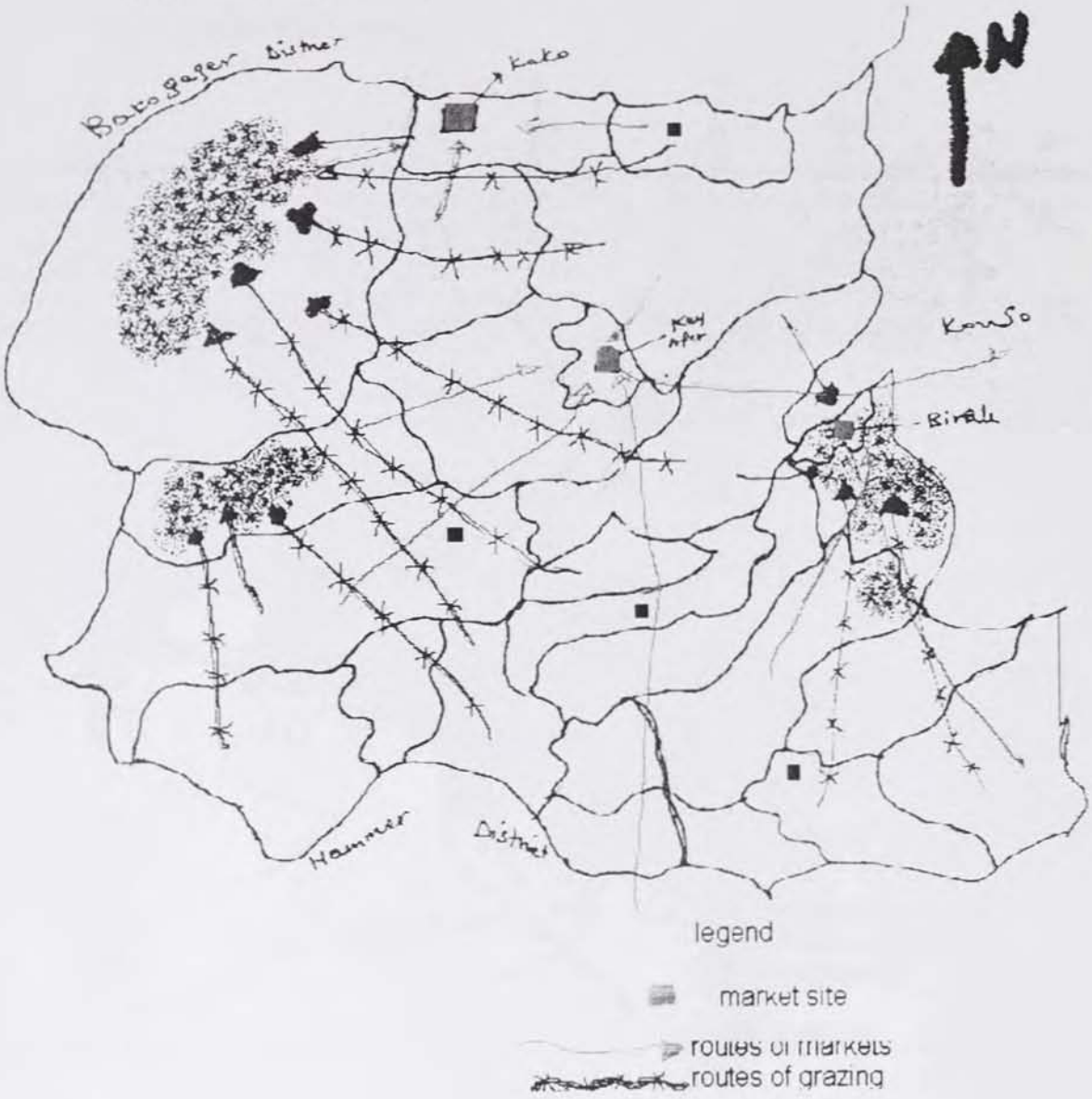
Zone/esp.woreda	No of outbreaks	cases	deaths	slaughtered
Gamo Goffa	36	1525	929	121
South Omo	25	1281	330	10
Konso esp.woreda	3	10.000	1422	711
Amaro esp.woreda	16	322	142	20
Derashe esp.woreda.	8	1215	137	114

Annex 11. Livestock Distribution in the study areas of Gamo Goffa and South Omo zones (CSA, 2003).

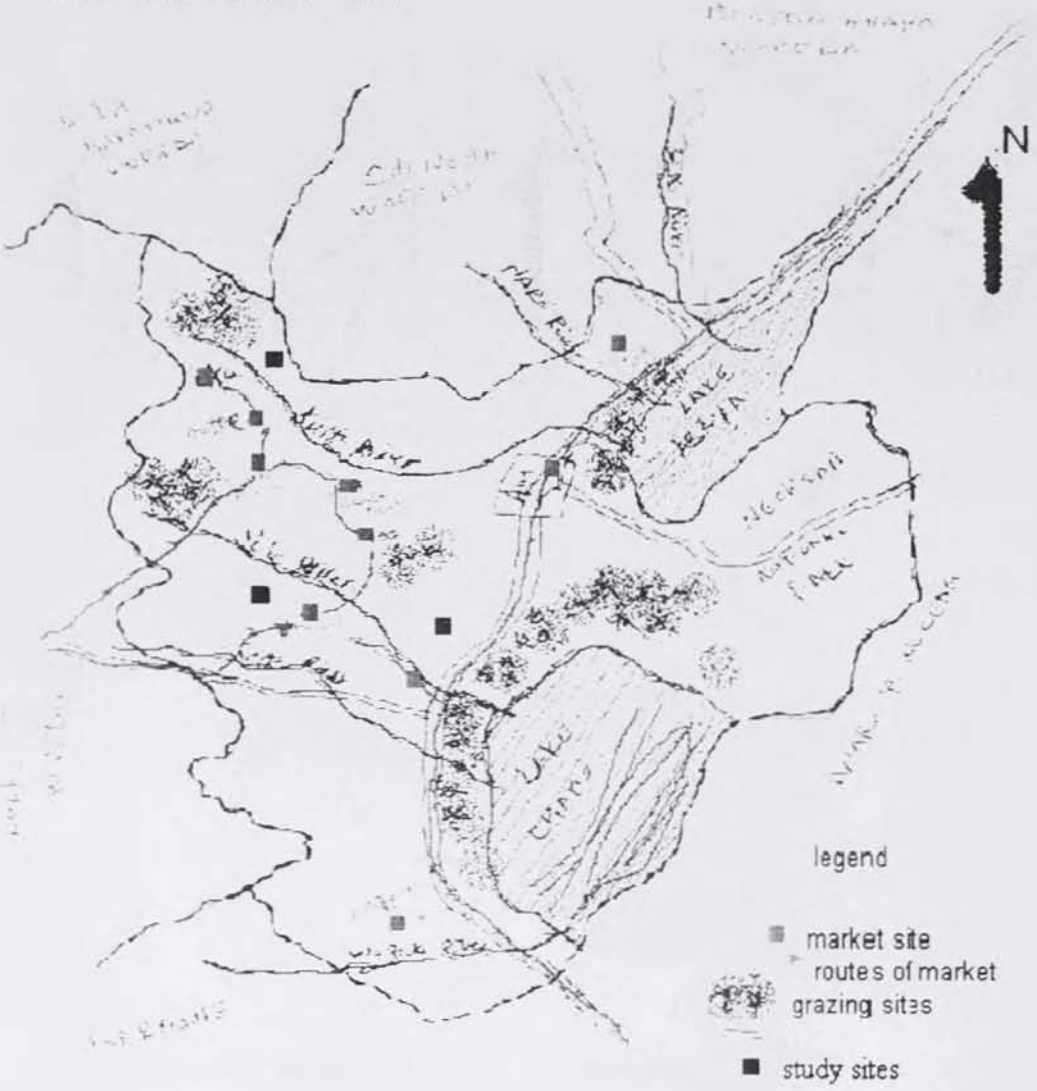
Zones	Species	Number	% Of the region
South Omo	Cattle	1,392,821	15.77%
	Sheep	1,013,331	31.96
	Goats	1,157, 201	43.65
	Horses	7,214	2.46
	Asses	48353	15.85
	Mules	3,091	4.41
	Camels	1300	100
	Poultry	13,925	4.04
Gamo Goffa	Cattle	850,290	9.6
	Sheep	381,533	12.0
	Goats	227,278	8.8
	Horses	23194	7.9
	Asses	6340	2.1
	Mules	9,397	13.4

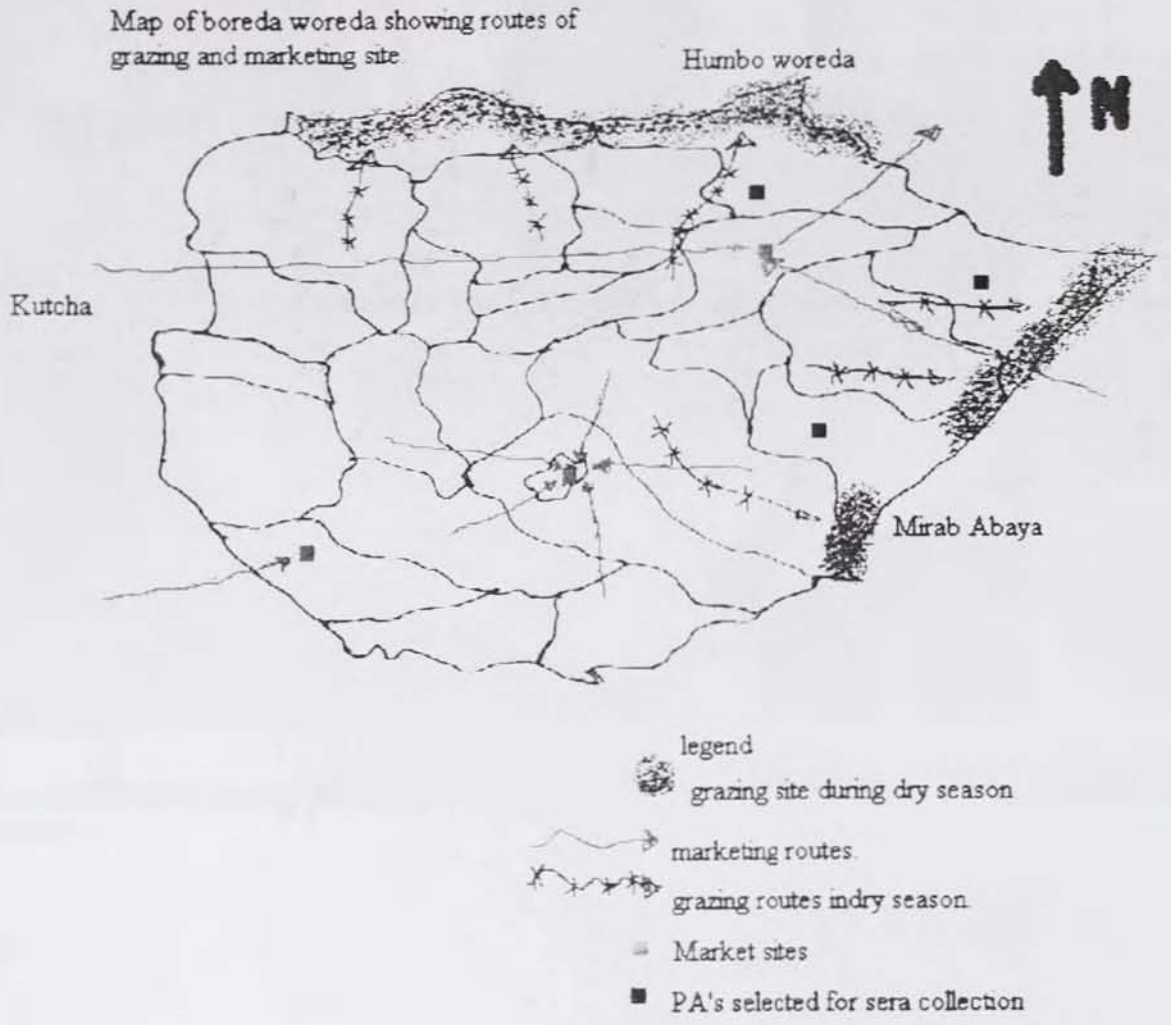
Map of Hammer woreda shows route of market and grazing.

Map of Benna-Tsemay woreda



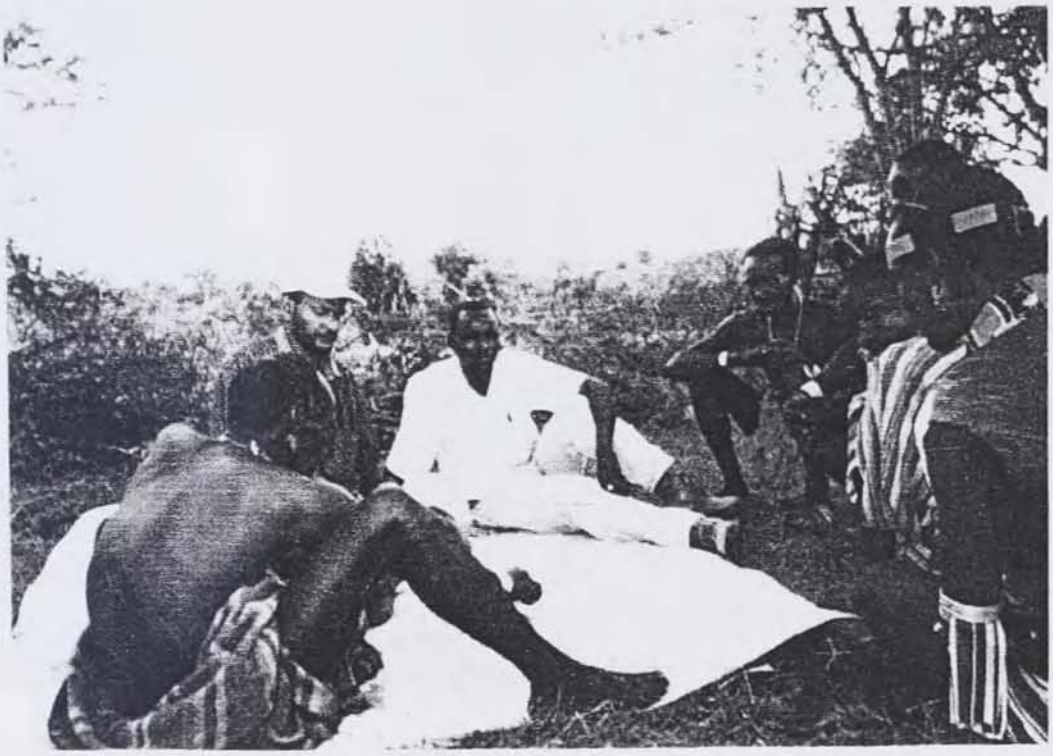
Map of Arbaminch zuria woreda



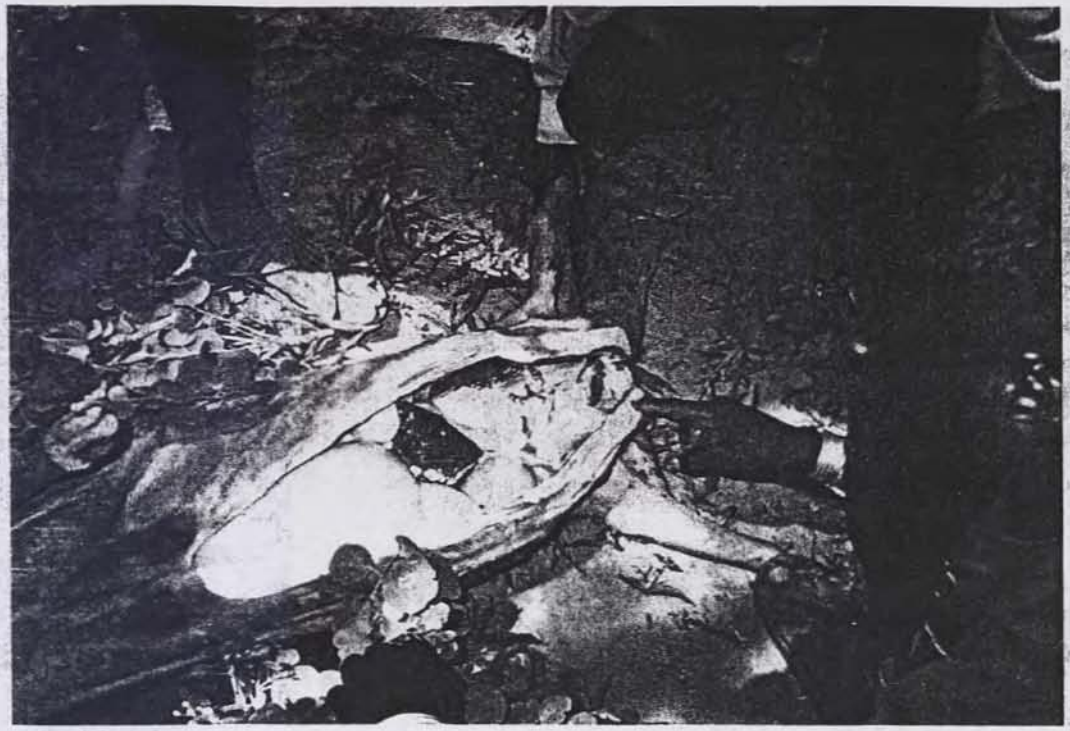


Map 2- 4. Shows infrastructure of grazing and marketing routes

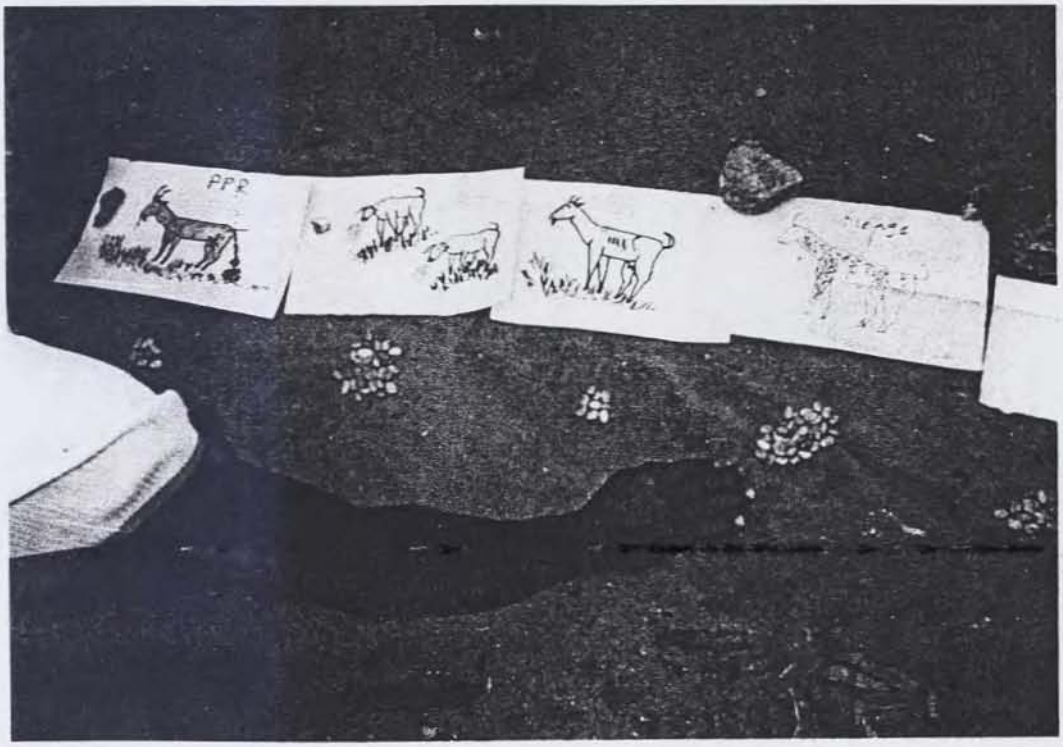
Picture 1. Group discussion while scoring clinical signs for each disease. translator is at the middle



Picture 2. Group matrix scoring



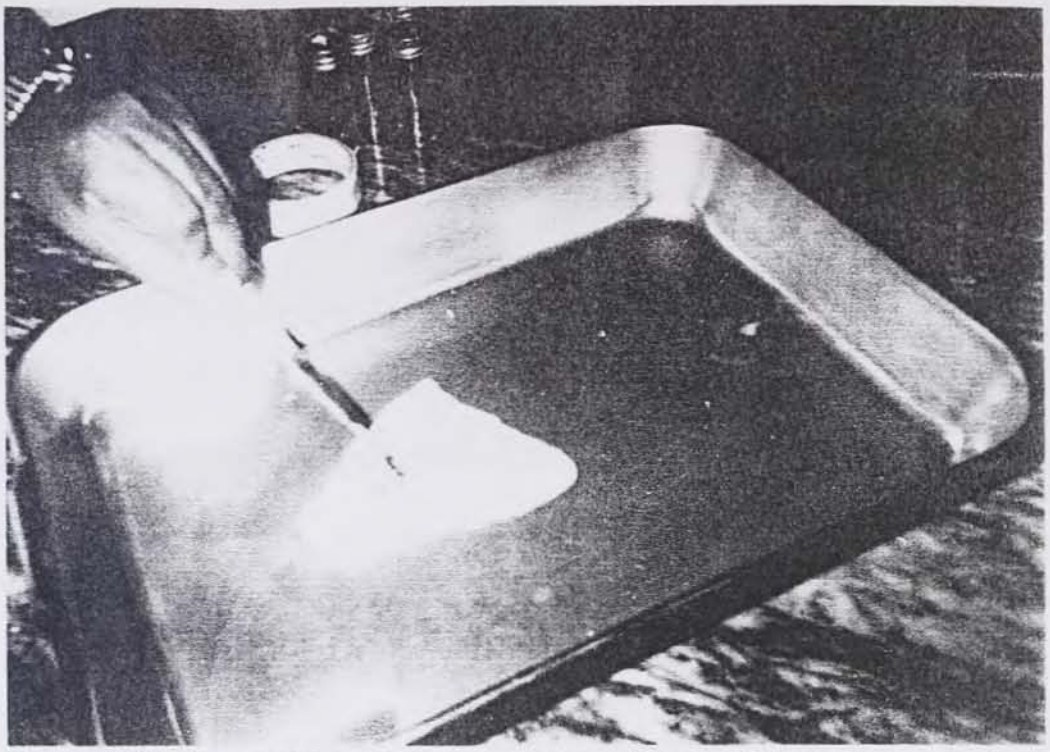
Picture 3. Pastorals were able to show gross pathological lesions of CCPP case: yellow-colored thoracic fluids, adhesion and fibrinous tissues were covered on the lung. Congested nodules were not pronounced.



Picture 4. Shows completed proportional piling of major goat's diseases.

Picture 5 Sick goats were submitted to NVI for isolation of the causative agent.

Picture 6 One lung shows consolidation and enlargement, the second lung partially congested



Picture 7. Small part of lung shows congestion and consolidation, there was adhesion of lung with pleura during postmortem examination

Picture 8. Goat shows diarrhea, emaciation, depression and nasal discharge

CURRICULUM VITAE

I .Personal Data

1.1 Solomon Mekuria Wudineh

1.2 Nationality- Ethiopian

1.3 .Date of birth Sept.1964G.C

1.4 Place of birth Hossana-Hadiya zone

1.5 Language ability. Amharic, Hadiya, Siltigna, Gurage and Engilish

1.6 Current address

1.5.1 Awassa /Ethiopia p.o.box 766 and P.o.box 80

Tel o6 –201077, 201317 offices

Tel 06-202480 residence

II. Educational Back ground

2.1. Addis Ababa University Faculty of vet Medicine Graduate in 1990

Obtained degree of veterinary medicine (DVM)

III-Work experience

3.1 Field veterinarian and woredas Animal health team leader from 1990 to 1993

3.2 Zonal veterinary field officer from 1993 to 1997

3.3 Zonal veterinary section team leader in 1998

3.4 Regional field vet expert 1998 to 2000

3.5 Regional veterinary team leader and PACE branch coordinator from 2001 to 2003

3.6 Technical advisory committee member for southern tsetse eradication project in 2003

3.7 Training of community animal health workers at their locality using PRA techniques 1999 to 2002

3.8 Active disease surveillance and monitoring of trans-boundary diseases 2001 to 2003

IV workshop Attained (international and local)

1. Participatory epidemiology, and lesson learned, Addis Ababa, Ethiopia, 2001
2. Regional and international meeting on CBPP disease control, Addis Ababa, Ethiopia, 2001.
3. Regional O.I.E. meeting at Ghion Hotel, Addis Ababa, Ethiopia, 2001.
4. Conflict resolution within Karamonjon clusters Umbale, Uganda, 2000
5. Conflict resolution within Karamonjon clusters Lodwar, Kenya, 1999
6. Training on trainers of trainee by international training on intermediate technology (ITIT), Nairobi, Kenya, 1998
7. Several workshops at national level, issues related to animal health activities
8. Regional training on strategic planning and management regional level.

V. Additional experience.

1. Computer literate.
2. Researches work on traditional medicine "*Bole* and its effect on GIT parasite of domestic animals". On proceeding of six cycle local grant research workshop held at Ethiopia science and technology commission. Dec. 15-17th 1999
3. Need assessment of the community in Salamago and Backogazer using PRA methods 2003.

Reference:

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Dr. Ademe Zerihun Faculty of veterinary medicine, Head for Microbiology and Public health Department.

Dr. Alemayehu Lemma FVM Head of registrar office.

SIGNED DECLARATION SHEET

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

Name SOLOMON MEKURIA WUDINEH

Signature: _____

Date of submission: _____

This thesis has been submitted for examination with our approval as University advisors

Advisors/ Supervisors

1. Dr. Berhe G/Egziabeher _____

2. Dr. Ademe Zerihun _____

EPidemiological Durvey Of Contagiou s Caprine Pleuro-Pneumonia In South Cmo& Gamo Goffa Zones Southern Ethiopia

Solomon Mekuria