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ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

SEROLOGICAL AND PARTICIPATORY EPIDEMIOLOGICAL SURVEY OF CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN AFAR PASTORAL AREAS, NORTH EAST ETHIOPIA

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A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University, in the partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Epidemiology

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXES

ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
ANRG	Afar National Regional Government
B-ELISA	Blocking Enzyme Linked Immunosorbent Assay
CAHWs	Community Animal Health Workers
CBPP	Contagious Bovine Pleuropneumonia
CCPP	Contagious Caprine Pleuropneumonia
cELISA	Competitive Enzyme Linked Immunosorbent Assay
CFT	Complement Fixation Test
CI	Confidence Intreval
CIRAD-EMVT	Centre de Coopération Internationale Agronomique Pou le Dévelopment
CSA	Central Statistics Authority
FAO	Food and Agriculture Organization
IFAT	Indirect Flourescent Antibody Test
IHAT	Inhibition Haemagglutination Test
LAT	Latex Agglutination Test
MAb	Monoclonal Antibody
MAKePS	Mastitis, Arthritis, Keratitis, Pneumonia and Septicaemia
MccP	*Mycoplasma capricolum subspecies capripneumoniae*
MccF38	*Mycoplasma capricolum subspecies capricolum* F38 strain
MF	Membrane Filtration
MmmLC	*Mycoplasma mycoides subspecies mycoides* Large Colony
MmmSC	*Mycoplasma mycoides subspecies mycoides* Small Colony
MoA	Ministry of Agriculture
NAHA	Nomadic Animal Health Auxilliaries
NGO	Non-governmental organization
NVI	National Veterinary Institute
OIE	Office International des Epizooties
OR	Odds Ratio
PA	Peasant Associations

PCR	Polymerase Chain Reaction
PDS	Participatory Disease Search
PHAT	Passive Haemagglutination Test
PPR	Peste des Petitis Ruminants
PRA	Participatory Rural Appraisal
RBC	Red Blood Cell
rRNA	ribosomal Ribonucleic Acid
SA	Settlement area
SNNP	Southern Nations and Nationalities People
SRBC	Sheep Red Blood Cell
VFG	Viande foie goat
X^2	Chi-square

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ABSTRACT

Contagious caprine pleuropneumonia (CCPP) is a respiratory disease of goats caused by *Mycoplasma capricolum subspecies capripneumoniae* (MccP). A Participatory Rural Appraisal (PRA) technique and two stage stratified cross sectional serological survey was conducted on CCPP from October 2005 to April 2006 in Afar pastoralist areas to determine the overall seroprevalence of the disease and assess the impact of community animal health workers (CAHWs) on the disease occurrence.

The study population was stratified into two based on differences in veterinary service providers in the areas. A total of 1,183 sera samples (1,158 goats and 25 sheep) were tested using complement fixation test (CFT) and competitive enzyme linked immunosorbent assay (cELISA) tests, and 40 informant groups (on average 12 persons per group) were interviewed. Results of the participatory disease assessment showed that among the livestock owned, goats are their second preferences next to camel because of their comparative advantages especially they are drought resistant and have short period of reproduction interval. The herders also identified that CCPP was one of most common diseases of goats next to pasteurellosis and other stress associated diseases, causing high morbidity and mortality. Out of 1,183 sera samples tested, 344 (29.08%) were seropositive for MccP antigen using CFT test and 19.19% in the cELISA.

In both CFT and cELISA tests, univariate and multivariate analysis of epidemiologically assumed risk factors showed that prevalence of CCPP is highly associated with the veterinary service provision through CAHWs. The seropositivity was higher (36.78%) in areas where CAHWs are absent than CAHWs intervention areas (21.42%). The seroprevalences observed between these two areas were highly significant ($p < 0.001$) using the CFT test. There was also a significant difference ($p < 0.001$) between non-CAHWs and CAHWs intervention areas using cELISA test with a mean seroprevalence of 23.90% and 14.50%, respectively.

The agreement between the two tests was slight (Kappa = 0.036) with 64.24% concordance. Both tests could be used complimentarily.

From the study it could be concluded that CCPP exists in its sub clinical level in goats and its prevalence was lower in areas where there is additional animal health service through NGOs supported CAHWs. This study has shown that CCPP is widely distributed in Afar Region.

The indigenous knowledge of pastoralists is highly appreciated which act as a bridge and basis for an active disease search and investigation and so it could be used complementarily with the conventional one in the prevention and control of livestock diseases.

Key words: CCPP, participatory epidemiology, CAHWs, seroprevalence, goats, Afar

1. INTRODUCTION

In areas where there are adverse climatic conditions like dry regions of the country, small ruminants, particularly goats next to camels have an outstanding ability to resist such conditions and survive with a better reproductive and productive performance.

According to the world goat population estimation, there are about 740,431,000 goats in the world, with 222,275,000 (30.2 %) of the goat population being inhabited in Africa. In Ethiopia, the goat population is estimated to be about 17 million, which makes the country second in Africa and fifth in the world (FAO, 2002).

Despite its significant economic contribution to the life of the rural community, the attention given to keep the well-being and increase the productivity of the goat industry is very little. Diseases, poor nutrition, traditional management, low genetic potential and inadequate research to improve the performance of animals are among some of the factors responsible for the low productivity and income. In Ethiopia, like any other tropical country, diseases particularly, peste des petits ruminants (PPR), and contagious caprine pleuropneumonia (CCPP) are major health constraints to goat production causing major economic losses due to high morbidity and mortality.

With respect to the wide geographic distribution of CCPP, historically, the first clinical description was reported in Algeria in 1873 more than a century ago (Nicholas, 2002). Soon after, a major outbreak occurred in South Africa in 1881 following the introduction of goats from Turkey, Duncan Hutcheon, the colonial veterinary surgeon, concluded that contagious caprine pleuropneumonia was caused by a contagious agent. In spite of this early description, the causative agent was only isolated and characterized a century later in 1976 (Thiaucourt and Bölske, 1996).

The presence of contagious caprine pleuropneumonia in Ethiopia has been suspected since 1983. It was confirmed later in 1990 by isolation and identification of the MccP or F38 (Thiaucourt *et al.*, 1992). Since then the disease was reported to be endemic in different regions of the country.

Because of its distribution and occurrence mainly in the developing countries, Africa and some Asian countries, the socio-economic impact attributed to CCPP is very huge, which aggravates the food security problem.

Although researches have been made during the last decade to characterize the disease, CCPP, isolation of the agent, taxonomy and diagnostic problems, the magnitude of the problem in each country are not known and sufficient to allow recommendations to be drawn up for formulating control strategies (OIE, 2004b). Apart from few research works on isolation, vaccine efficacy trials and prevalence studies, when compared to its significant importance, the efforts made towards to combat the problem are non-existing. Therefore, this study is conducted in its part to contribute to the better understanding of the disease with the specific objectives of:

1. To study prevalence of CCPP in selected areas of Afar pastoralist areas.
2. To assess the perceptions of the community towards important livestock diseases and CCPP in particular.
3. To determine the potential risk factors associated with the disease, which could contribute to the epidemiological occurrence of the disease.
4. To assess contribution of Community Animal Health Workers (CAHWs) in decreasing the morbidity and mortality rate of CCPP.

2. CONTAGIOUS CAPRINE PLEUROPNEUMONIA

2.1 Definition

Contagious caprine pleuropneumonia (CCPP) is an extremely contagious disease of goats caused by *Mycoplasma capricolum subspecies capripneumoniae* (MccP), characterized by acute pneumonia, high fever, respiratory distress, coughing, nasal discharge and high mortality rate and severe fibrinous pleuropneumonia (Aiello and Mays, 1998; Walker, 1999; Heldtander *et al*., 2001; Nicholas, 2002).

2.2 Aetiology

2.2.1 The agent

There were many strong controversies in describing the causative agent of CCPP among researchers for a number of years, for many factors which could explain the long period between the description of the disease and that of the causative agent. First, MccP (Mcc38) is very difficult to grow *in vitro*, which explains why it was isolated only in 1976, whereas the causative agent of CBPP, a similar Mycoplasma disease of cattle, had been isolated in 1898. Moreover, MccP (F38) belongs to the group called mycoides 'cluster', a group of six *Mycoplasma species* or subspecies which share many antigenic and genotypic characteristics (Thiaucourt and Bölske, 1996).

Research into the control of CCPP was initially hampered by confusion over the exact cause of the disease. Two mycoplasmas, *M. mycoides subsp. mycoides LC* and *M. mycoides subsp. capri*, were for some time implicated in the etiology of the disease because they caused a pleuropneumonia in small ruminants that resembled CCPP. It was not until 1976 that a highly fastidious mycoplasma, designated F38 but later named *M. capricolum subsp. capripneumoniae*, was isolated for the first time *in vitro* by MacOwan and Minette (1976). Once these workers had developed a suitable medium for the mycoplasma and carried out experimental infections, its role as the primary cause of classical CCPP was confirmed (Nicholas, 2002).

However, in spite of these confirmations, respiratory diseases, caused by *M. m. capri* and *M. m. mycoides LC*, are still referred to erroneously as CCPP particularly in the Middle East and India (Thiaucourt and Bölske, 1996).

2.2.2 Taxonomy of Mycoplasmas

The mollecutes are members of the Order Mycoplasmatales and class Mollicutes. Mollicutes, also by their trivial name called mycoplasma, are characterized by pleomorphic shape, being devoid of cell wall, bounded by single trilaminar membrane composed of proteins, glycoproteins, glycolipids, phospholipids and sterols (Walker, 1999). They are poorly stained with the Gram-Stain, but producing better results with Giemsa and other Romanowsky stains (Quinn *et al.,* 1994).

Six genera of Mollicutes are recognized, of which, only Mycoplasma and Ureaplasma have veterinary importance. These Mollicutes are differentiated based on digitonin sensitivity, as a result mycoplasma and Ureaplasma are sensitive to it, but the others are not. Biochemically, Mycoplasma and Ureaplasma are urease negative and positive, respectively (Walker, 1999).

The taxonomic status of MccP (F38) has long been unclear and it was only in 1993 that it became a subspecies of *Mycoplasma capricolum*, and classified as *M. capricolum subspecies capripneumoniae*. The agent, *M. c. capripneumoniae,* belongs to the genus Mycoplasma and is one of the 200 species of the class Mollicutes. Five distinct groups of Mollicutes were identified by phylogenetic analysis of the 16S rRNA sequences, one of which, the spiroplasma group, contains *M. c. capripneumoniae* which has been subdivided within the Mycoplasma mycoides cluster (Table 1). This cluster contains six important ruminant Mycoplasma including *M. m. mycoides SC*, the cause of contagious bovine pleuropneumonia, *M. m. mycoides LC* and *M. m. capri* which share immunological and biochemical properties. Their close relationship can lead to problems for diagnosis (Thiaucourt *et al*., 1996; Walker, 1999).

CCPP is a very precise entity, whereas the others belong to what could be described as the 'MAKePS syndrome', for mastitis, arthritis, keratitis, pneumonia, and septicaemia.

The lack of precise definition of this syndrome reflects the diversity that exists among the mycoplasma strains that can cause such a syndrome and, sheep do not seem to be very susceptible to pulmonary mycoplasmoses, which is why little attention has been devoted to these animals (Thiaucourt and Bölske, 1996).

Table 1- Relationship within the Mycoides 'cluster'

Mycoides subgroup	Capricolum subgroup
M. mycoides subsp.mycoides biotype SC	*M. capricolum subsp.capricolum*
M. mycoides subsp.mycoides biotype LC	*M. capricolum subsp.capripneumonia*
M. mycoides subsp. capri	*M. sp. Group 7 of Leach*

Source: Thiaucourt and Bölske, 1996

2.3 Epidemiology

2.3.1 Geographic distribution

The disease is reported to occur in more than 30 countries in West and Eastern Africa, Pakistan, India and Eastern Europe (Nicholas, 2002; OIE, 2004a; OIE, 2004b; Sharew *et al.*, 2005). In these countries where the occurrence of the disease was frequently reported, using either confirmatory or clinical disease of CCPP, are summarized in table 2.

Table 2- Distribution of CCPP

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

Source: Nicholas, 2002

2.3.2 Molecular Epidemiology

Unlike other members of the *M. mycoides* cluster, *M. c. capripneumoniae* shows a surprising degree of heterogeneity particularly in the sequence of its two rRNA operons which both contain the genes for 5S, 16S and 23S rRNA. These polymorphisms, representing point mutations in either gene, can be used to subtype strains, some of which may have epidemiological and, possibly evolutionary significance. Sequencing the16S rRNA genes of African strains identified two distinct lines, I and II, both of which were represented in Central, North and East Africa; isolates from the Middle East were of the type II only, although they could be further sub-divided (Nicholas, 2002).

Sequencing the amplified products of a different gene(s), the H2 locus (Lorenzon *et al.*, 2002), divided strains into 4 major groups, in which lineage A occurred exclusively in East Africa, B mostly in North Africa and the Middle East, C in Central Africa, and D, represented only by a single strain, was restricted to the United Arab Emirates. Sub-typing with amplified fragment length polymorphism (AFLP) strongly supported the 16S rRNA sequence analysis by identifying two main lineages, but all studies have been hampered by lack of strains; clearly more strains are necessary to provide a better understanding of the epidemiology and evolution of CCPP (Nicholas, 2002).

On a more local level, 10 of 11 strains of *M. c. capripneumoniae* isolated from four different regions of Tanzania had very similar profiles with AFLP (Kusiluka *et al.*, 2001).

These profiles were also indistinguishable from two Kenyan and one Ugandan strains indicating the close association between small ruminants in these three neighboring countries (Nicholas, 2002).

2.3.3 Host - Agent – Environment interactions

Goats are the only species known to be affected by MccP.The primary source or reservoir of infection of mycoplasmas is the host that they infect. Introduction of an infected animal into a susceptible population accounts for dissemination of infection. Young animals are very susceptible to infection and generally develop more severe disease than adult animals (Aiello and Mays, 1998; Walker, 1999; OIE, 2004b).

The disease caused by Mccp is highly contagious and fatal to susceptible goats of all ages and both sexes, but does not affect sheep or cattle (OIE, 2004a).

Serological and bacteriological studies on sheep have indicated the presence of sub clinical infection by MccP. In two reports, sheep have been shown to harbor MccF38 in the nares and lungs (Litamoi *et al.*, 1990; Bölske *et al.*, 1995). However, the pathogenicity of the ovine isolate to caprine and the role of sheep in the epidemiology of the disease have not yet been determined (Mekonen, 1996).

The causative agent, because of its fragile nature, doesn't survive for long in the external environment and requires intimate contact between infected and susceptible animals for transmission. Indirect transmission does not seem to occur as mycoplasmas are not very resistant, and are inactivated and destroyed by heat (50–55^{0}C), disinfectants, detergents, acids, UV- light, and putrifactive changes (Quinn *et al.*, 1994; Walker, 1999). Extreme climatic changes, poor nutrition, transportation, concurrent infection and other stress factors contribute to the occurrence of the disease (Walker, 1999).

2.3.4 Source of infection and modes of transmission

Transmission occurs predominantly by spread from animal to animal through direct contact and is mediated through inhalation of respiratory secretions (Walker, 1999). In extensive husbandry practices, communal grazing areas and watering points are the major sites for disease transmission.

Unlike contagious bovine pleuropneumonia, chronic carriers of CCPP are not as such important. Carrier animals may shed more organisms after times of stress and changes in climate. CCPP spreads to new areas by the movement of infected or carrier goats to susceptible goat population (Thiaucourt *et al.*, 1996).

2.4. Pathogenesis

There is very little information on the pathogenic mechanisms of MccP, although some hypothesis can be drawn from comparison with other mycoplasmosis and especially with

CBPP. A striking feature of CCPP is the strict host and tissue specificity of the causative agent, as lesions are produced only in goat lungs (Walker, 1999; Thiaucourt and Bölske, 1996).

Mycoplasmas usually have adhesions, but no such component has yet been described for MccF38 the expression of pathogenicity may depend on other factors that favor penetration and multiplication, which explain why it is sometimes difficult to reproduce the disease experimentally (Quinn *et al*., 1994; Thiaucourt and Bölske, 1996).

Although MccF38 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 mycoplasmal components which leads to an exacerbated inflammatory response (Thiaucourt and Bölske, 1996).

2.5 Clinical signs

Different clinical forms of the disease, namely, per acute, acute and chronic forms are noticed. In most cases, per acute and acute forms of the disease could occur when infected and carrier animals are introduced into fully susceptible population, whereas chronic cases occur in endemically affected areas.

In per acute form, animals die within 1–3 days with minimal respiratory signs, and the acute cases are characterized by high fever (41-42^{0}C), high morbidity and mortality rates in susceptible flocks affecting all ages and both sexes, and abortions in pregnant goats. The incubation period is often 6–10 days though it is sometimes as long as 3 – 4 weeks. After approximately 2-3 days of high fever, respiratory signs become apparent, accelerated and painful, and in some cases are accompanied by a grunt. Coughing is frequent, violent and productive (Aiello and Mays, 1998; OIE, 2004b).

In the terminal stages, animals are unable to move, they stand with their front legs wide apart, the neck is stiff and extended, and sometimes saliva continuously drips from the mouth (Nicholas, 2002). In chronic forms, goats are debilitated with chronic cough and nasal discharge.

2.6 Post-mortem findings

In chronic cases, swelling of bronchial lymph nodes with encapsulation, serofibrinous and pleuropneumonia of lungs are observed. Unlike CBPP, no sequestrate formations have been described for CCPP (Bölske *et al.*, 1995; Thiaucourt *et al.*, 1996). Post-mortem examination reveals unilateral or bilateral pneumonia, with massive lung hepatisation, consolidation and pleurisy, accompanied by accumulation of straw-colored pleural fluid (Aiello and Mays, 1998; Nicholas, 2002).

2.7 Histopathology

Histological examination of the lung tissues often revealed an acute serofibrinous to chronic fibrino-necrotic pleuropneumonia with infiltrates of serofibrinous fluid and inflammatory cells, mainly neutrophils, in the alveoli, bronchioles, interstitial septae and sub pleural connective tissue. Intralobular edema is more prominent but interlobular edema has also been reported. Peribronchial and per bronchiolar lymphoid hyperplasia with mononuclear cell infiltration is also present (Walker, 1999; Nicholas, 2002).

Ultra structural examination of the lungs of goats naturally affected with CCPP confirmed histopathological findings of congested septal capillaries with inflammatory cells invading thickened septal walls. The alveolar lumen contained serous fluid mixed with neutrophils and lymphocytes, some of which were necrotic (Walker, 1999). The most significant findings were a widespread hyperplasia of type II pneumocytes that had lost most of their characteristic lamellar ultra structure and large numbers of mycoplasma-like structures lying close to the microvilli and membranes of these cells (Nicholas, 2002).

2.8 Immunity

Little is known of the immunology of CCPP despite a number of reported experimental infections. More recently, (March *et al.*, 2000 and 2002) monitored the humoral response of goats infected with a multipassaged *M. c. capripneumoniae* strain 19/2 with several serological tests and PCR. While there was little evidence after infection of the infectious agent or clinical or pathological disease, apart from elevated temperatures and a transient cough in one goat, serological responses were detected by latex agglutination test and

competitive ELISA. Immunoglobulin G (IgG) immunodominant bands of 23, 40 and 44 kDa were seen by immunoblotting in experimentally infected animals as well as in some sera from a natural outbreak of CCPP in Eritrea which additionally showed bands of 62, 70 and 108 kDa (Nicholas, 2002).

2.8 Economic Importance

Contagious caprine pleuropneumonia is a highly contagious respiratory disease of goats characterized by severe sero-fibrinous pleuropneumonia with morbidity rate of 100 % and 60 –100 % mortality rate (Aiello and Mays, 1998; OIE, 2004b), imposing significant constraints on goat production owing to this high mortality and production losses. Although treatment of clinical cases is possible, due to the recurrent and highly contagious nature of the disease and presence of potential carriers, it is very expensive and consequently beyond the budget of many poor farmers to whom most goats belong. But there is no study which could indicate the annual economic loss attributed to CCPP in any of the countries where the disease is frequently reported.

2.9 The disease in Ethiopia

2.9.1 Outbreak reports

The monthly disease outbreak report data recorded in the Ministry of Agriculture (MoA) indicated that the disease is frequently reported every year at national level with higher number of outbreaks in SNNP (Southern Nation and Nationalities) followed by Afar region (Table 4).

Table 3 - Annual CCPP outbreak reports from 2000 - 2004 in Ethiopia

Year	No. of outbreak	Population at Risk	No. of Cases	No. of deaths
2000	5	21,926	575	163
2001	13	37,120	1,342	490
2002	23	236,988	11,976	2,101
2003	9	23,080	843	173
2004	21	18,670	1,680	525
Total	71	337,784	16,416	3,452

Source: MoA (2004): Monthly disease outbreak report

Table 4- CCPP monthly outbreak reports distributed in different regions of Ethiopia from 2000 – 2004

Region	2000	2001	2002	2003	2004	Total
SNNP	3	2	15	3	10	33
Afar	2	8	4	1	4	19
Amhara	-	1	1	-	1	3
Oromia	-	-	2	4	6	12
Benishangul	-	1	-	1	-	2
Dire Dawa	-	1	1	-	-	2
Total	5	13	23	9	21	71

Table 5- CCPP outbreak in Afar region from year 2000 - 2004

Zone	District	Population at risk	No. of outbreaks	Cases	Deaths
Five	Artuma ("Dalifagae"), Telalak, Fursi, Dewe	68,815	13	10,838	1,965
Two	Megale	35,000	4	350	108
One	Mille	1,514	2	1,108	110
Total		105,329	19	12,296	2,183

From these five years crude data (Table 5), it is possible to assume comparatively that CCPP is the major problem in the proposed study areas.

2.9.2. Prevalence study

In Ethiopia, different studies have indicated the wide distribution of CCPP. A study which was undertaken in nine *woredas* of the country, indicated a prevalence of 60%, 83%, and 87% using serological tests of CFT with MccP antigen, B-ELISA with F_{38} antigen, and CFT with MmmSC antigen, respectively (Sharew *et al.*, 2005). They also indicated wider distribution of CCPP in the country, with a prevalence of 52% to 100% in the lowlands and 77% to 87% in the highlands of the country as indicated in table 6. Other studies, which were conducted in

South western part of Ethiopia, showed a prevalence of 35% and 36%, (Bereket, 1995) and (Mekonen, 1996), respectively. Solomon (2005) has also reported a prevalence of 15.46% in South Omo pastoral areas.

The prevalences detected by CFT with MccP antigen (T1), B-ELISA with F38 antigen (T2), and CFT with MmmSC antigen (T3) in *woredas* where CCPP was not reported were 23%, 2% and 12%, respectively, where as, for *woredas* where CCPP was reported, the findings were 60%, 83% and 87%, respectively as indicated in table 6.

Table 6- Sera from nine *woredas* tested for CCPP by three tests: T1 (CFT-F38), T2 (B-ELISA-F38) and T3 (CFT-MmmSC).

No.	District	CCPP status	No. of sera	Percentage prevalence detected		
				T1	T2	T3
1	North Shoa	Not reported	29	17	0	17
2	Wollo	Not reported	96	29	4	7
3	Afar	Reported	72	58	64	78
4	Yabello	Reported	96	52	80	82
5	Metehara	Reported	161	62	81	88
6	Arsi	Reported	60	87	77	85
7	Borena	Reported	48	77	85	92
8	Konso	Reported	174	77	95	87
9	Awash	Reported	31	97	100	100

Source: Sharew *et al.*, 2005

In another study usingdot blot assay (Laikemariam *et al.*, 2004) in Omo, Borena, Tigray, Arbaminch and North Shoa have reported a prevalence of 86/163 (52.7%) for Mccp (CCPP agent), 31/163 (19%) for MmmLC, 15/163 (9.2%) for Mcc, 3/163 (1.8%) for M. agalactiae were found positive. MccP caused CCPP is prevalent in all the surveyed areas except North Shoa, but some samples had strains MmmLC and M. ovipneumoniae showing the presence of mixed infections, particularly in Omo and Borena areas. In Ethiopia, sheep are naturally resistant to clinical CCPP. The isolation of MccP, however, proves the role of sheep as reservoir of infection (Laikemariam *et al.*, 2004).

2.10 Diagnosis

2.10.1 Clinical signs and postmortem findings

The diagnosis of outbreaks of respiratory disease in goats, and of CCPP in particular, is complicated, especially where it is endemic. It must be differentiated from other similar clinico-pathological syndromes such as: peste des petits ruminants (PPR), to which sheep are also susceptible; pasteurellosis, which can be differentiated on the basis of distribution of gross lung lesions; and what has been called 'mastitis, arthritis, keratitis, pneumonia and septicaemia syndrome (also called 'MAKePS syndrome') or more often as contagious agalactiae syndrome caused by groups of other mycoplasmoses. As the longer name implies, the pneumonia is accompanied by prominent lesions in other organs in case of other mycoplasmoses (Thiaucourt and Bölske, 1996). Confusion is unlikely unless the strain has an unusual pulmonary tropism.

The disease caused by Mccp is readily contagious and fatal to susceptible goats of all ages and sexes, does not affect sheep or cattle, and is characterized histopathologically by an interstitial, intralobular edema of the lung (OIE, 2004b).

The localization of the disease in the lungs which characterizes CCPP may be masked by other infections. Diarrhea may co-exist because of parasitism, a common feature in flocks in developing countries. Other mycoplasmas may co-exist with MccF38 in flocks affected with CCPP. This may explain the frequent isolation of *M. mycoides subsp. capri* (Mmc) from CCPP affected flocks in Kenya and Turkey before the F38 strain was identified (Thiaucourt *et al.*, 1996).

A condition should only be termed as CCPP when the following criteria have been satisfied: *M. c. capripneumoniae* is isolated, or there is strong serological evidence of the mycoplasma, lesions are restricted to lung and pleura and consist of a pleuropneumonia, the condition is highly infectious with high levels of morbidity/mortality, and there is no enlargement of the interlobular septa of the lung (Thiaucourt and Bölske, 1996).

2.10.2 Collection of samples

The necropsy samples of choice are lung lesions, particularly from the interface between consolidated and unconsolidated areas, pleural fluid, and mediastinal lymph nodes (OIE, 2004a). One of the best samples is pleural fluid. At post-mortem, lung lesions together with pleuritic fluid should be collected. Blood samples of 10 ml should be collected aseptically from several animals in the affected flock in the acute phase of the disease for serological investigation (Thiaucourt *et al.*, 1996).

The best method is to sacrifice at least one animal of the flock that receives no antibiotic treatment. Other samples may be gathered from live animals by lower intra-thoracic puncture with needles (Thiaucourt and Bölske, 1996; OIE, 2004a).

Samples of hepatized lungs are also desirable. Square 3 cm sections should be taken from an area that borders normal lung tissue (Thiaucourt and Bölske, 1996). Duplicate specimens of active lung lesions of one set should be submitted fresh, the other collected into neutral buffered formalin for histopathology (OIE, 2004a).

Samples can be kept at +4°C, if transport to the laboratory does not take more than one or two days. Otherwise they can be deep frozen at -20°C. In the latter case, samples can be stored for months without loss of mycoplasma viability. For storage longer than 10 months it is recommended to keep the samples at -70°C. In the absence of cold chain, penicillin or ampicillin and amphoterecin B should be added to the sample to limit the contaminants growth of bacteria and fungi (Quinn *et al.*, 1994; Thiaucourt and Bölske, 1996; OIE, 2004a).

2.10.3 Microscopic examination of samples

Mycoplasma capricolum subsp. capripneumoniae shows a branching filamentous morphology *in vivo* that can be observed by dark-field microscopy in exudates or tissue suspensions from lesions or pleural fluid. Alternatively, smears made from cut lung lesions can be stained by the method of May–Grünwald–Giesma and examined by light microscopy. The other caprine *mycoplasmas* show a short filamentous or coccobacillary morphology. Neither of these techniques provides a definitive diagnosis (OIE, 2004a).

2.10.4 Isolation

Until recently, isolation was the only way to confirm the presence of CCPP. The success of isolation depends primarily on the attention that is given to the sample collection. The isolation of MccF38 may be a long and difficult process. Two difficulties can be described. First, MccF38 grows very poorly *in vitro* and secondly, samples are often contaminated and over grown by other mycoplasmas (Quinn *et al.*, 1994; OIE, 2004a).

2.10.4.1 Mycoplasma media

Mycoplasmas are very fastidious organisms, requiring specific growth factors, an isotonic medium and the absence of inhibitory substances for growth. The composition of the medium is very important and determines the rate of success as well as the size of the colonies. Horse serum permits good growth of MccF38. The serum should be added at a concentration of 20% to 30% by volume. Fresh yeast extract is also an important component of the medium (Quinn *et al.*, 1994).

The medium used by MacOwan & Minette (1977) to culture MccP organisms, is termed 'viande foie goat' (VFG), and included goat-meat liver broth and goat serum.

Alternative suitable media are WJ, modified Hayflick's, and modified Newing's tryptose. (A commercial medium called Mycoplasma Experience is also available from Mycoplasma Experience, Reigate, Surrey, UK.) Media enriched with 0.2% (or up to 0.8%) sodium pyruvate perform considerably better, both for primary isolation and antigen production of *Mccp*. This forms the basis for Thiaucourt's medium and modified Thiaucourt's medium (OIE, 2004a).

Certain medium components, particularly serum, yeast extract and deionised water, should be regularly monitored for growth-promoting capacity before incorporation into mycoplasma media. Low-passage field isolates should be used for this screening purpose (Quinn *et al.*, 1994; OIE, 2004a).

2.10.4.2 Cultural characteristics

Cultures are incubated at 37 °C. Plates are best incubated in a humid atmosphere of 5% CO_2, 95% air or N_2, or in a candle jar with a moisture source (Quinn *et al.*, 1994; OIE, 2004a). The growth of MccF38 usually takes about four to five days when first isolated, and the diameter of the colonies may be only 0.1 mm. These colonies have fried egg appearance and can be only seen by close observation with a binocular microscope. In a liquid medium, the turbidity is very faint and, should be compared with un-inoculated medium (Quinn *et al.*, 1994; OIE, 2004a).

2.10.4.3 Contaminants and overgrowth treatment

In the case of CCPP lesions, *M. ovipneumoniae* is often isolated. However where MccF38 *and M. ovipneumoniae* co-exist, the former is present in higher quantities, as has been demonstrated with a quantitative method, such as sodium dodecyl sulphate polyacrylamide electrophoresis (SDS- PAGE) (Thiaucourt *et al.*, 1996).

The major difficulties in growth of MccP lie in the presence of other bacterial contaminants in the samples and overgrowth on the media. Usually, the antibiotics, chiefly ampicillin and amphoterecin B, inhibit the classic bacteria and fungi respectively, that are incorporated into the primary culture. However, other mycoplasmas, such as *M. arginini* and *M. ovipneumoniae*, are often isolated which may overgrow. These two mycoplasmas species are natural colonizers of the upper respiratory tract of goats and can be regularly isolated from healthy lungs, as well as from diseased lungs (Thiaucourt *et al.*, 1996).

Once the MccF38 has been obtained in pure culture, there should be no difficulty in identifying it. The growth rate of this organism can be compared only with that of *M. ovipneumoniae*. All other members of the mycoides 'cluster' grow within 24 – 48 hours, producing bigger colonies (1-3 mm in diameter). *M. ovipneumoniae* can be suspected when the colonies lack the classic 'fried egg' appearance and do not stick to the agar surface. Finally there is no antigenic relatedness between these two mycoplasmas (Quinn *et al.*, 1994; Walker, 1999).

2.10.5 Identification

2.10.5.1 Biochemical tests

Wild strains should be passaged, and preferably cloned, three times before identification is attempted. Biochemical tests cannot identify an isolate unequivocally, which at present can only be done by serological or genetic means. Intra-species variation in some biochemical reactions is often considerable, but some tests perform a useful function both as a preliminary screening system and in providing supportive data for serological findings (Houshaimi *et al.*, 2002; Nicholas, 2002).

The tests most commonly used are glucose breakdown, arginine hydrolysis, 'film and spots' formation, reduction of tetrazolium chloride (aerobically and anaerobically), phosphatase activity, serum digestion and digitonin sensitivity (Quinn *et al.*, 1994; Walker, 1999; Nicholas, 2002). The first three of these tests are performed routinely in isolation and cultivation procedures.

The remaining biochemical tests require specific media or reagents. The test for tetrazolium reduction provides corroborative evidence of the mycoplasmal nature of *M. agalactiae* isolates, as this organism is neither glycolytic nor arginine-hydrolysing. Serum digestion distinguishes members of ruminant *mycoplasmas*, and phosphatase production separates *Mcc* from other members of this cluster. Digitonin sensitivity distinguishes members of the order Mycoplasmatales from those of the order Acholeplasmatales (Quinn *et al.* 1994; Nicholas, 2002; OIE, 2004a). Some of the biochemical properties of the different small ruminant mycoplasma species are indicated in table 7.

Table 7- Major biochemical differences between *Mycoplasmas* of small ruminants

Mycoplasma spp.	Glucose fermentation	Arginine hydrolysis	Phosphatase activity	Film and spots	Casein digestion	Tetrazolium aerobic	Reduction anaerobic
M.c. capripneumoniae	+/-	-	-	-	+	varies	weak/+
M. mycoides LC	+	-	-	-	+	+	+
M. m. capri	+	-	-	-	+	+	+
M.c. capricolum	+	+	+	-	+	+	+
M. ovipneumoniae	+	-	-	-	-	varies	+
M. conjunctivae	+	-	-	-	-	-	+
M. agalactiae	-	-	+	+	-	+	+
M. putrefaciens	+	-	+	+	-	varies	+
M. arginini	-	+	-	-	-	-	+

Source: Nicholas, 2002

Substantial diversity is also reported in the metabolism of strains of *M. c. capripneumoniae*. Some strains including the type strain F38 oxidize organic acids but not glucose, while others, including the recently isolated Eritrean strains, metabolize glucose. Such biochemical diversity within a mycoplasma species is unique and may present diagnostic problems as glucose fermentation is a key characteristic in their preliminary identification. However, even with glucose-metabolizing strains, the addition of pyruvate to the medium leads to significantly higher yields *in vitro*. Thus it may be that organic acids are also important energy sources for glucose-oxidizing strains (Nicholas, 2002). Table 8 below illustrates the diversity of strains *M. c. capripneumoniae* in their substrate requirements

Table 8- Substrate utilisation by strains of *M. c. capripneumoniae* (MccP)

	Strain							
Substrate	19/2 Oman	T3 Eritrea	G183 Kenya	F38 Kenya	7/1a Turkey	G1943 Kenya	G94/83 Kenya	4/2 LC Oman
Lactate	+++	+++	+++	+++	+++	+++	+++	+++
Pyruvate	++	++	++	++	++	++	++	++
Glucose	+++	+++	+++	-	-	-	-	+++
Glycerol	-	+++	+++	-	+++	-	-	+++
2-oxybu-tyrate	-	-	-	+++	+++	+++	+++	-

Source: Nicholas, 2002

2.10.5.2 Polymerase chain reaction

Once the organism has been cultured, verification of *Mccp* can be achieved in 1 day by PCR. The test is based on amplification of a segment of the 16S rRNA gene. The amplified fragment is common to the mycoides cluster. However, when the amplicon is digested with endonuclease *Pst*I, a unique cleavage pattern of three fragments for MccP is observed when the enzyme digests are analyzed in agarose gel electrophoresis (AGE) and stained with ethidium bromide (OIE, 2004a).

In the case of CCPP, the primers were chosen specifically to amplify a fragment of specific gene for the mycoides 'cluster'. The identification of MccF38 is made in the second step, by digestion of the amplified products with an endonuclease: *PstI*. Three fragments are obtained with McF38 while only two are seen with other members of the mycoides 'cluster'. This difference indicates a single nucleotide substitution in one of the two that code for the 16S rRNA in the mycoplasmas; a difference, however, which is typical of MccF38. The suitability of PCR for the detection of CCPP has already been demonstrated in Uganda and Kenya, and also more recently in Chad (Thiaucourt *et al.*, 1996).

Recently, PCR and sequencing has been used to establish the molecular epidemiology of CCPP (Nicholas, 2002). These tests can be performed on dried samples, such as pleural fluid on filter papers. The sequencing allows a precise identification of the species (the cleavage site for the 16S rRNA and a specific detection for the 'locus H2') (Lorenzon *et al.*, 2002; OIE, 2004a; Woubit *et al.*, 2004). The test can be used directly on clinical materials such as lung tissue and pleural fluid (Bolske *et al.*, 1996).

2.10.6 Serological Tests

Mycoplasmal antigens used in hyper immune serum production are often contaminated with medium constituents. The antibodies stimulated by these contaminants can cause false-positive reactions in serological identification tests. This problem is avoided by absorption of the antiserum with the medium used to produce the antigen (10 mg lyophilized medium per ml of antiserum), or by growing the mycoplasmas to be used as antigens in medium containing homologous animal components, for instance growth in VFG medium to immunize goat (OIE, 2004a).

Because of the close serological relationships between members of the 'mycoides cluster', isolates from cases of CCPP should, preferably, be identified by comparative advantages tests described below (OIE, 2004a).

2.10.6.1 Growth inhibition test

The growth inhibition test (GIT) is the simplest and most specific, but the least sensitive, of the tests available. It depends on the direct inhibition of growth on solid medium by specific hyper immune serum, and detects primarily surface antigens (Quinn *et al.*, 1994; OIE, 2004a).

2.10.6.2 Growth precipitation test

The growth precipitation test detects soluble cytoplasmic and extramembraneous antigens released by growing cultures and allowed to diffuse through solid mycoplasma growth medium towards mycoplasma antiserum during growth. *From Mycoplasma capricolum subsp. capripneumoniae,* an antigenic polysaccharide is released to which a specific monoclonal antibody (MAb) (WM-25) has been produced. This MAb immunoprecipitates in agar gel with

the polysaccharide produced by MccP, and is used to identify the causative agent in cases of CCPP, particularly when tissue specimens are no longer suitable for culture because of deterioration during transit (OIE, 2004a).

2.10.6.3 Gel precipitin test

In the gel precipitin test, there are strong cross-reactions within the mycoides cluster. If growth inhibition is performed using MAb WM25, which is specific for Mccp, both specific inhibition and a growth precipitin line are achieved simultaneously (OIE, 2004a).

2.10.6.4 Indirect fluorescent antibody test

The direct and indirect fluorescent antibody tests are the most effective of the various serological methods for identifying most mycoplasmas. They are simple, rapid, and sensitive, yet economical in the use of antiserum. Several forms have been described, the most commonly used and perhaps best being the indirect fluorescent antibody (IFA) test applied to unfixed colonies on agar. Antiserum against a single strain is sufficient to identify field isolates of that species, and anti sera are diluted before use. Cultures do not have to be cloned, but the test is usually applied only after several passages have indicated whether the culture contains more than one species and the growth characteristics of the organism(s) present (Quinn *et al.*, 1994; OIE, 2004a).

All serological results that are obtained with a single antigen are prone to a lack of specificity and the presence of CCPP cannot be established in this way. Some serological cross-reactions can occur between two subspecies of the mycoides 'cluster': *M. capricolum subsp. capripneumoniae* and *M. sp.group 7*. However, the reactions with the homologous sera are always stronger. A precise identification always requires testing with a panel of anti sera from the whole mycoides 'cluster'. In regard to the subspecies capricolum, it is advisable to use a mixture of anti sera which have been prepared with different strains, as there are many variations within this species and some strains might not react with an antiserum prepared against a single strain. This may particularly be the case with sera prepared from 'California kid', the reference strain of the capricolum subspecies, which seems to be quite different from the other strains (Thiaucourt *et al.*, 1996).

In most cases, the interpretation of serological results is often difficult as these tests were not comprehensively validated. Precise information on sensitivity, specificity, precocity and persistence 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'. The fact that different mycoplasma species may co-exist within a goat population will confuse the picture of even further (Thiaucourt *et al.*, 1996).

2.10.6.5 Complement Fixation Test

The complement fixation test in various forms remains the most widely used serological test for diagnosis of contagious bovine pleuropneumonia. In CCPP, the CFT was used for detection of *Mccp* infection and it has been found to be more specific, though less sensitive, than the IHA test (OIE, 2004a).

2.10.6.6 Latex Agglutination Test

Latex beads sensitized with the polysaccharide produced by MccP and present in culture supernatant have been used in a slide agglutination test (Rurangirwa *et al.*, 1987; March *et al.*, 2000). This test is presently used routinely in Kenya.

In a comparative study, where two different tests were conducted on 763 sera from two different farms with outbreaks of classical contagious caprine pleuropneumonia, 63 percent were positive by the latex agglutination test and 23 per cent were positive to the complement-fixation test. This result indicated besides being more sensitive than complement fixation, the latex agglutination test can be performed in the field using undiluted serum or whole blood and result could be obtained within two minutes (Rurangirwa *et al.*, 1987).

Both CFT and IHAT findings emphasize the difficulties inherent in the serological diagnosis of CCPP when using whole cell or membrane preparations as antigen, whereas the use of latex agglutination test, with the more defined antigen, the polysaccharide elaborated by *Mccp*, provides greater specificity, as there is no cross-reactivity with sera against the other three principal caprine mycoplasmas (OIE, 2004a).

2.10.6.7 Enzyme Linked Immunosorbent Assay

Competitive enzyme linked immunosorbent assay (cELISA) is a newly developed test which permits the specific detection of antibodies in animals which have been affected by CCPP. This test is based on the use of monoclonal antibody (Mab), 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. A quantitative result is obtained with a single dilution of serum. This assay method permits the testing of numerous sera in large serological enquiries (Thiaucourt *et al.*, 1996; OIE, 2004a).

Antibodies detected by cELISA persist longer than those detected by the CFT. The latter vanish completely three months after the infection, while the former persist for more than six months. The exact persistence period is not yet established but may last much longer (Thiaucourt *et al.*, 1996).

2.10.6.8 Indirect haemagglutination

The indirect haemagglutination test (IHAT) is most commonly performed with RBCs that are either fresh and tanned, or treated with glutaraldehyde. The former are more sensitive but show greater variability between tests, and require sensitization of cells with antigen each time the test is performed. Glutaraldehyde treatment of RBCs reduces sensitivity but produces a much more useful diagnostic test, as sensitized RBCs remain effective for 1 year or more if kept refrigerated, and require little further manipulation before use in the test (OIE, 2004a).

However, all these tests exhibit certain limitations in specificity, sensitivity, and ease of application, cost, or the requirement for specialist equipment or expertise.

2.11 Treatment, Control and Prevention

In countries where vaccination program is not practiced, control method such as antibiotic treatments is commonly done. Although mycoplasmas are susceptible to a number of antibiotics *in vitro* treatment failures are common. The commonly used antimicrobial agents such as tylosin, tetracycline, lincomycin, erythromycin, terramycin or streptomycin can be used to treat clinical cases. Resistance to some of these antibiotics has been noted. Animals

that do not respond to treatment often become potential carriers (Walker, 1999; Nicholas, 2002).

Where an outbreak occurs in a previously free country, eradication should be attempted by slaughter of all infected and in-contact goats, and the implementation of quarantine and movement controls. But in enzootic areas, culling of infected animals and management changes to prevent dissemination are employed (Walker, 1999; Nicholas, 2002).

In general, because of the poor success in treating infected animals, culling of clinically ill animals is often employed as a control measure in infected population where test and slaughter is not feasible (Nicholas, 2002; Walker, 1999). Good hygiene and management practices are important in enzootic areas.

Protection against CCPP was shown to be possible more than a century ago when Hutcheon subcutaneously inoculated goats with lung extract from affected animals (Nicholas, 2002). Furthermore goats vaccinated with an attenuated broth culture of F38 did not succumb to contact infection. This clearly demonstrated control was possible (Nicholas, 2002).

Since then a number of different preparations have been produced which are reported to produce solid immunity even after one year. These include a vaccine composed of sonicated antigens emulsified with incomplete Freund's adjuvant and another in which lyophilized F38 is inactivated with saponin immediately before immunization. The latter vaccine has been in use in Kenya for the last few years but has been modified so that the mycoplasma is inactivated with saponin for at least 12 hours at 4^0C. Kids older than 10 weeks of age are vaccinated to avoid interference by maternal antibody (Walker, 1999; Nicholas, 2002).

3. MATERIALS AND METHODS

The study was conducted in Afar pastoralist area, particularly in four *woredas* (Afambo, Mille, Dalifagae and Semurobi) from October 2005 to April 2006. Data were collected using semi-structured questionnaire, participatory rural appraisal (PRA) methods and blood sample serological surveys. Secondary data on CAHWs activities was collected from locally working NGOs (FARM AFRICA, Action Contra la Faim) and government employees at region and *woreda* levels.

3.1 Description of the Study Areas

3.1.1 Afar National Regional State

The study was conducted in Afar National Regional State which is located in the north-eastern part of Ethiopia, at $8^0$40' to $14^0$47'N and $39^0$51' to $41^0$23'E, totally residing within the Rift Valley area. Topographically, the Afar Region is bordered with Eritrea in the north and north-east; Djibouti in the west; Tigray Region in the north-west; Amhara Region in the west; Oromiya Region in the south and Somali Region in the south-east (Fig. 1). Administratively the region, which covered a total of 85,410 Km^2, is divided into 5 zones, which are further subdivided into 29 *woredas*. The regional capital, 'Semera', is located some 600 Kms north-east of Addis Ababa in the main Addis – Djibouti tarmac road. The total population of the region is estimated at 1.3 million; of which over 90% are primarily livestock-dependent pastoralists while the remaining 10% are agro pastoralists.

The altitude ranges from -120 to1,800 m.a.s.l. Mean daily temperature varies widely ranging from 20 to 45°C. The region receives three rainy seasons. The main rain, *Karma* is from July to August, followed by rainy showers from November to December called *Dedaa*, and *Sugum*, the minor rainy season during March and April. Disruptions on the performance of any of the rainy seasons will have an impact on the availability of pasture and water as well as the overall food security of the pastoral and agro-pastoral communities. The rainfall distribution also varies with an erratic bimodal pattern with 200 – 650mm and being inconsistent in terms of coverage and amount. The study areas are characterized agro-

ecologically as lowlands with arid and semi-arid lands. According to precipitation classification system, semi-arid, upper arid and arid are areas which received mean annual rainfall of > 500 mm, 300 – 500 mm and < 300 mm, respectively.

The study area (Afar Region) was selected purposely because monthly disease out break reports for the last five years have shown CCPP to be tentatively endemic in all zones, however the disease has not been so far confirmed.

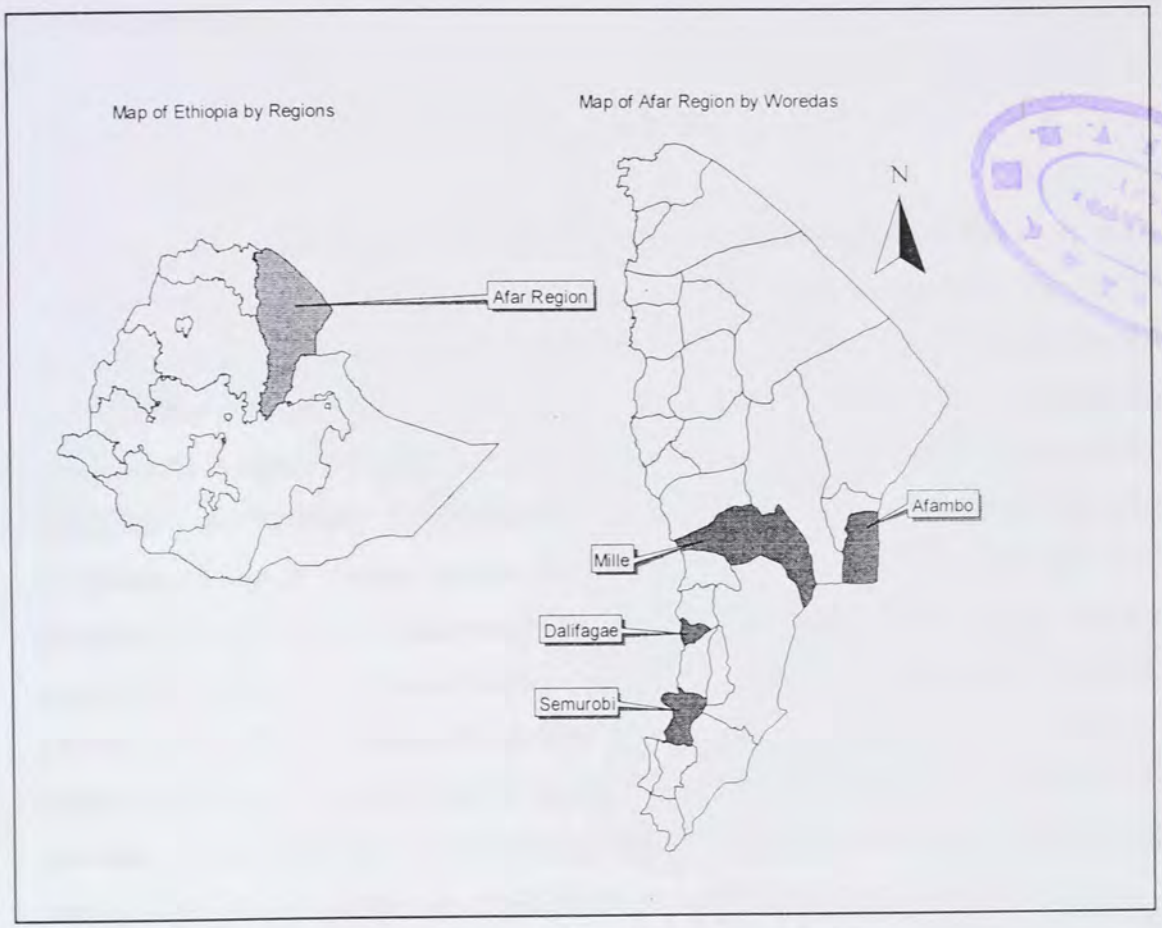

Figure 1- Map of Ethiopia by Regions and Afar Region

3.2 Livestock Management

Pastoral production system is the main agriculture system practiced by the community (over 90%) in the area. Cattle, goats, sheep and camel are the main livestock species kept in the area. The ANRS has owned large livestock resources that include 2,336,486 cattle, 2,463,632 sheep, 4,267,969 goats and 852,018 camels (CSA, 2004). Among the livestock, cattle are the most severely affected by recurrent drought and are highly mobile in search of pasture, but

camel, goats and sheep are less mobile and reared within or not far from their original settlement areas. Traditionally, goats are grouped and reared into three groups according to their reproductive stages (number of births given) as those less than one year, between one and three years and those greater than three years older. Flock sizes were also grouped into three based on the local wealth ranking system of the community as rich (< 50 goats), medium (51 – 150 goats) and poor (>150 goats).

3.3 Study Design

3.3.1 Stratification of study areas and study population

The study areas and populations are stratified into two strata based on the presence or absence of community based animal health workers (CAHWs) based veterinary services in the selected *woredas*, since these factors are known to influence the prevalence of the disease. Two *woredas* were selected from each stratum. The term 'community animal health worker' encompasses a range of names for primary-level veterinary workers. Baumann (1990) has described it as "Nomadic Animal Health Auxiliaries (NAHA) is an independent, privately-practicing, informally trained person of pastoral origin who is not on the payroll of any government service or development project. He is an auxiliary in the sense of being a self-employed complement to official services, a person who lives a pastoral life himself." The CAHW is known and respected by the community, is recognized as a knowledgeable livestock keeper, and was selected for training by the community (Catley, 2002). The CAHW provides a preventive, diagnostic and curative service that is limited to important animal health problems, as identified by livestock keepers in the community concerned.

Stratum 1: *Woredas* which have got additional veterinary services to the existing government infrastructure and goats reared in those respective *woredas* where there are CAHWs supported by NGO's.

Stratum 2: *Woredas* which have not got any additional veterinary services other than the existing government infrastructure and goats reared in those respective *woredas* where there are no any CAHWs based animal health interventions.

The target population in this study was all goats estimated over 4,267,969 goats million which are found in 29 *woredas* of Afar Region. The type of study used was two stage stratified cross-sectional.

3.3.2 Sampling Frame

The sampling frame comprises of a list of all *woredas* under each stratum in the Region and Peasant Associations (PA's) found in selected *woredas*. *Woredas* and PA's are considered as primary and secondary sampling units, respectively. Thus two-stage stratified sampling was employed comprising of four *woredas*, i.e. two *woredas* from each stratum representing the presence and absence of CAHW. The sample frame is presented as follows:

I. Primary sampling units were a list of 27 *woredas* found in the region (excluding 2 *woredas*). To avoid possible seroconversions due to vaccination campaign undertaken in September 2005 and May 2005 in two *woredas* (Argoba special and Awash Fentale with CAHWs), they were excluded from the list. These *woredas* (27) were further subdivided into two strata as which have CAHWs (15 *woredas*) and without CAHWs (12 *woredas*). So in each stratum two *woredas* were randomly selected and included in the study.

II. Secondary sampling units comprised of a list of Pastoral Associations (PAs) on average having 14 PA's per district. Then 4 PA's from each selected district were selected randomly with a total of sixteen PAs. The total number of samples in each district and PAs were allocated with an equal proportion. The samples were taken randomly from goats above six months of age until the required sample size was obtained per flock (on average 15% of a flock).

3.3.3 Sample size determination

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

d = absolute precision

P_{exp} = expected prevalence

(at 95% confidence interval multiplier = 1.96).

A recent study of CCPP in goats in pastoralist areas of Southern Ethiopia using CFT has shown an overall prevalence of 15.46%. Thus the 16% was taken to determine the sample size by taking into account the same management system in this study. Hence, the 95% confidence interval level with expected prevalence of 16% at 5% absolute precision is used to determine sample size. The sample size obtained from the formula is 206. To compensate for errors in sampling design and increase precision, this number was multiplied by 3 to obtain the final sample size at each stratum. Thus the sample size for each stratum was 618 goats and a total of 1,236 samples for the two strata. This was divided among the four *woredas* and 16 PA's of both strata proportionally.

3.4 Study Methodology

3.4.1 Questionnaire Survey

Questionnaire survey was conducted in the selected *woredas* where sera samples were collected. The questionnaire was pre-tested in five groups of people before implementing the final survey. The questionnaire was semi-structured type and includes general description, which includes name of the owner, location, livestock owned, farming system, husbandry practices, their knowledge about the disease, movement of livestock and the purpose of keeping livestock (Annex 1). The other portion deals with veterinary services which includes major livestock diseases, measures taken against such important diseases, source of drugs or vaccine and their efficacy, cost of drugs, market value of study animals at their health and sick status and finally, traditional practices were interviewed what they have,.

3.4.2 Participatory Disease Search

Livestock owners were interviewed in-groups and individual interviews. Livestock owners were allowed to select their elders who can give information about the disease and eight to twelve persons were subjected to interview per group. To validate the knowledge of the

community about the disease, 40 groups interview (8 – 16 persons per group) was used during participatory disease search methods. Proportional piling, pair wise comparisons, seasonal calendars for diseases and movements, simple and matrix scoring techniques against indicators were used in the selected settlement areas.

To perform participatory disease search, the community or clan leaders and community members were identified and orientations were given about the objective of the study intended.

Among the communities, at least one translator was used for language translation. Groups of informants were selected from the herders. The program was started by the local culture. After a thorough discussion and information exchange among the local communities, they were directed towards issues related to their livestock in general and particularly the animal health problems they have. All problems were listed. Livestock health problems were then picked to discuss more. Then specific disease survey was conducted. For detailed and further survey, particular attention was given to the disease of interest. The participatory methods, approaches and procedures used were based on guidelines and manuals available (Mariner, 2001; Catley *et al*., 2002). The formats used were indicated in Annex 2.

1. Pair- wise ranking of the named items

- Lists of the major diseases were represented by locally available objects.
- The informants were checked for understanding the meaning of named objects.
- Two diseases were chosen at a time and compared.
- Questions like "which of these two diseases is most important?" was asked.
- "The reason why that disease was more important?" was asked to obtain indicators.
- The way how do these two diseases are different was asked?
- Then listing the indicators and repeating such question was continued until each disease was compared with every other disease.
- Take the counts of that specific disease appeared and give the first rank for a disease with a higher counts, and then for the others accordingly.

2. Matrix scoring of items with main indicators

- The disease names were placed using locally available objects in a row on the ground.
- The informants were checked for understanding the meaning of named objects.
- After collecting 30 stones, the informants were allowed to distribute such stones for one indicator against each disease.
- All stones were used during distribution.
- After checking the scoring; we told them if they wish to change the scoring.
- Then the proportions of stones allocated were recorded on the notebook.
- Then the procedure was repeated for the next indicators until we finished all the indicators listed.

3. Interviewing the matrix

We used the matrix on the ground to ask question and develop discussions. By physically pointing to the particular scores they did, we summarized all the indicators associated with a particular disease. Open, leading and probing questions were used to explore the knowledge of the informants.

4. Seasonal calendars

- One–year time line calendar was constructed.
- The local year calendar system was used.
- Horizontal line was drawn on the ground to represent one year.
- The line was at least one meter in length.
- Each season was represented using locally available materials.
- The informants were asked to show rainfall patterns using 30 stones and divided against seasons to show the pattern of rainfall throughout a typical year.
- The greater the rainfall in a particular season, the greater the number of stones assigned to it.
- The informants were asked to cite the season in which the disease and risk factors prevail using the same procedure.
- Seasonal mobility pattern of the herders were also asked.

- Then we summarized and presented and discuss to have clear understanding on the findings.

3.4.3 Serosurvey of CCPP

In this study serological test to detect antibodies from sera samples was done at National Veterinary Institute (NVI). The format was coded for computer analysis (Annex 3).

3.4.3.1 Serum Samples collection

Blood sample was collected by jugular venupuncture using sterile vacutainer tubes and needles. Up to 5 ml blood was collected from the jugular vein using sterile vacutainer tubes and needles. It was left to stand for a few hours (3-6 hrs). Removing the coagulated blood mass in the tube separated the serum. All samples were labeled (s/n, date, SA, age, sex, health status, CAHW, flock size) and transported in an icebox to the nearby veterinary clinic or laboratory where they were temporarily stored below 0°C. All samples were then deposited at Kombolcha regional laboratory at -20°C, until finally submitted to the National Veterinary Institute (NVI).

3.4.3.2 Complement Fixation Test (CFT)

This test was undertaken according to standard operting procedure set by CIRAD-EMVT. The antigen, inactivated one consisting of washed MccP cells, is produced by CIRAD-EMVT, was uded and kept at +4°C. It was diluted 1:40 to prepare working solution. The complement obtained freeze-dried (BioMerieux) was used. Fresh sheep red blood cells (SRBC) taken from sheep and kept at the research center was used. Equal volumes of SRBC and Alseve's solution were mixed-up at a time of collection. The SRBC was kept for two days at +4°C before use at 2% dilution. The haemolytic serum produced by (BioMerieux, France) was used at adilution of 1/700.

Test sera were decomplemented in water bath at 60°C and two fold dilutions was done except the last row for control. Antigen was added into each well, agitated and incubated for 30 minutes. Then complement was added in each well and kept overnight at +4°C. Then sheep

red blood cell washed with Verona buffer is prepared and then sensitized with haemolytic serum. The detailed procedures, validation and interpretation of the test are given in Annex 4.

3.4.3.3 Competitive ELISA (cELISA)

The competitive ELISA test was employed using a kit and it's obtained from CIRAD-EMVT, France. The detailed procedures of the test supplied with the kit are given in Annex 5.

Micro titer plates were coated with 1:5 diluted Mccp antigen incubated at 37°C for one hour with constant shaking. The plates were washed with water, followed by three washes with PBS Tween for three minutes and 50µl of competing antibody at a starting dilution of 1:25 was added to the first row. After incubation at 37°C for 30 minutes and washing as above, anti-goat conjugate was added. The conjugate was incubated as above, washed with PBS-T, drained and indicator solution (ABTS) added. The color development was stopped with a stop solution after 15 minutes and percent inhibition evaluated with an ELISA reader.

3.5 Data Analysis

All data were entered and stored in a separate database in both Ms-excel and Ms-access spreadsheet. Data were screened for proper coding and errors, and corrected prior to statistical analysis.

Seroprevalences of CCPP in areas where CAHWs are present or absent were calculated on the basis of CFT and cELISA positivity. Also flock and animal level seroprevalences were calculated by dividing the number of disease reactors by the total number of tested flocks and animals, respectively.

All data were transferred to Intercooled Stata 7.0 (Stata Corp.1984 – 2001) statistical software. After checking for multi-colinearity of variables by calculating the correlation coefficient, those factors not having strong linear relationship ($r < 0.4$) were used in the analysis. This enabled to exclude confounding effects among the factors. Univariate logistic regression analysis was employed to determine the associations of risk factors with seropositivity. A stepwise approach (forward selection and backward elimination) using

multiple logistic regressions was constructed to analyze those factors having putative effects on disease occurrence, based on a p-value < 0.2 as the significance threshold for entry or removal.

Logistic regression model for the multiple predictor variables is based on the formula:

$$\text{Log}\ \frac{p(X1, X2, \ldots Xp)}{1 - p(X1, X2, \ldots Xp)} = \beta 0 + \beta 1X1 + \beta 2X2 + \ldots + \beta pXp$$

The degree of association between putative risk factors and their serological status for those significantly associated factors was tested with their Odds Ratio (OR) values.

Questionnaire data were analyzed by descriptive statistics using Ms-Excel. Kinds of descriptive statistics to be used for were median, frequency and standard deviation.

Participatory epidemiology data were analyzed using different statistical tools. Comparative study was made on the selected *woredas* using non-CAHWs intervention areas with similar socio-economic and geographical situations with that of CAHW intervention areas which enable to see changes in goat's mortality and cumulative incidence of CCPP using MS-Excel like graphs, tables, medians and percentages. Agreements on preference of veterinary service providers and disease diagnosis of the herders were analyzed using SPSS 11.5.0 (2002) for the parameters Kendall's coefficient of concordance (W), medians and p-values. Kendall's coefficient of concordance (W) ranges from 0 to 1. The higher the value of W, the higher will be the agreement among the informants. Kappa ranges from 1 (complete agreement) to 0 (agreement is equal to that expected by chance), whereas negative values indicate agreement less than is expected by chance. Arbitrary, benchmarks for evaluating observed Kappa values are > 0.81 (almost perfect agreement), 0.61 – 0.80 (substantial agreement), 0.41 – 0.60 (moderate agreement), 0.21 – 0.40 (fair agreement), 0 – 0.20 (slight agreement), and 0 (poor agreement) (Thrusfield, 1995).

Data from participatory were analyzed using simple scoring, matrix scoring, pair wise comparison, and paired proportional piling methods. Graphs, medians, tables and percentages analysis were performed by Microsoft Excel version 2000. The software SPSS (2000) version 11.5.0 analyzed Kendall's Coefficient of Concordance (W), medians and "p-values". Kappa (agreement test) values in serological tests were analyzed by Win Episcope 2.0 (1998). All

statistical tools that were used in data analysis of this study with respective activities performed were shown in Table 9 below.

Table 9- Summary of the participatory assessment methods and analysis used

Issues discussed	Participatory method used	Statistical Analysis used
Comparative importance of Livestock	Proportional piling	Descriptive (tables, medians, range, graphs)
Flock composition of Goats	Proportional piling	Descriptive (tables, medians, range, graphs
Major problems of rearing goats	Pair wise	Ranking
Knowledge of Herders to Diseases	Matrix scoring	Concordance (Kendall's = W)
Major goat diseases in	Pair wise	Ranking
Morbidity rate due to CCCPP	Proportional piling	Descriptive(tables, medians, range, graphs
Mortality rate due to CCPP	Proportional piling	Descriptive (tables, medians, range, graphs
Measures taken during outbreak	Simple scoring	Ranking
Veterinary service providers	Matrix scoring	Concordance (Kendall's = W)
Cumulative incidence of CCPP and mobility	Disease calendars	Descriptive (tables, medians, range, graphs)

4. RESULT

4.1 Participatory Disease Search (PDS)

The questionnaire survey, which was conducted as a supplement to the PDS in every settlement areas during individual animal sampling indicated the occurrence of CCPP for more than ten years in the study areas. The participatory assessment was conducted in 40 settlement areas, forming 40 groups' interviews with a total of more than 450 respondents, on average 11 (8 – 16) respondents per group and the result has shown that CCPP was a common problem among the different diseases of goats in general, and the respiratory diseases next to pasteurellosis. The major findings of the participatory assessment are summarized and presented in Table 10.

Table 10- Summary of the participatory assessment findings

Rank order	Issues discussed with informants (herders)						
	Relative importance of livestock species	Livestock rearing problems	Major Diseases		Major sources and transmission	Measures taken during outbreak	Veterinary service providers
			Scientific	Vernacular (Local naming)			
1	Camel	Drought	Pasteurell-osis	*Suruatu*	Grazing /browsing	Treatment	CAHWs
2	Goats	Diseases	CCPP	*Gublu*	Watering	Isolation of cases	Public
3	Cattle	Water shortage	PPR	*Indahe (??)*	New cases introduced	Saling	Black market
4	Sheep	Conflict	Ectoparasite	*Agaraa*	Market	Slaughter	Traditional
5	-	Manage-ment and Genetic	Endoparasite	*Begu daria*	-	Vaccination (other vaccines)	Private

N.B Number of informant groups = 40 groups, average 11(8 – 16) persons per group

4.1.1 Proportional piling

Proportional piling method was used for the assessment of comparative importance of livestock species, morbidity and mortality rate of CCPP, and flock composition of goats. Among the different livestock species owned and kept by the herders, camel (36.7%) took the highest proportion and first rank followed by goats (27.0%), cattle (25.3%) and sheep (11.0%) according to their comparative importance.

With the goats' flock, the majorities (86%) were females and males constitute only 14%. Informants also have pointed out that the proportion of goats less than 1 year old, between one and three years and older than 3 years constitute about 28%, 37% and 35%, respectively. The overall distribution of age groups and between male and female goats within a flock is shown in Figure 2.

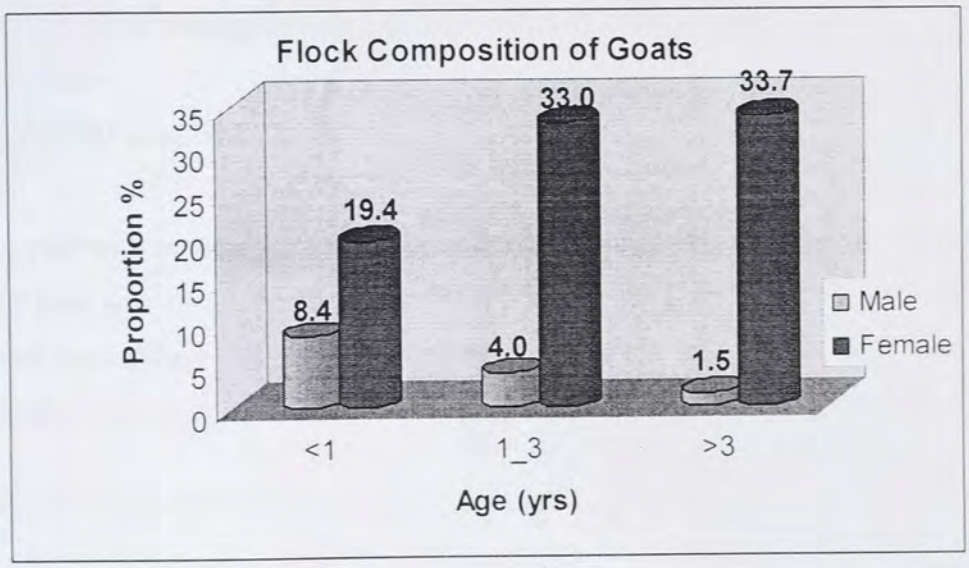

Figure 2- Flock composition of goats by age and sex

Through the use of proportional piling, the pastoralists were able to estimate the morbidity and mortality of goats due to CCPP when an outbreak occurred. They also indicated that the disease affects 80% of the flocks causing 70% mortality of all age and sex groups. Proportion of age groups affected by sex category is presented in Figure 3.

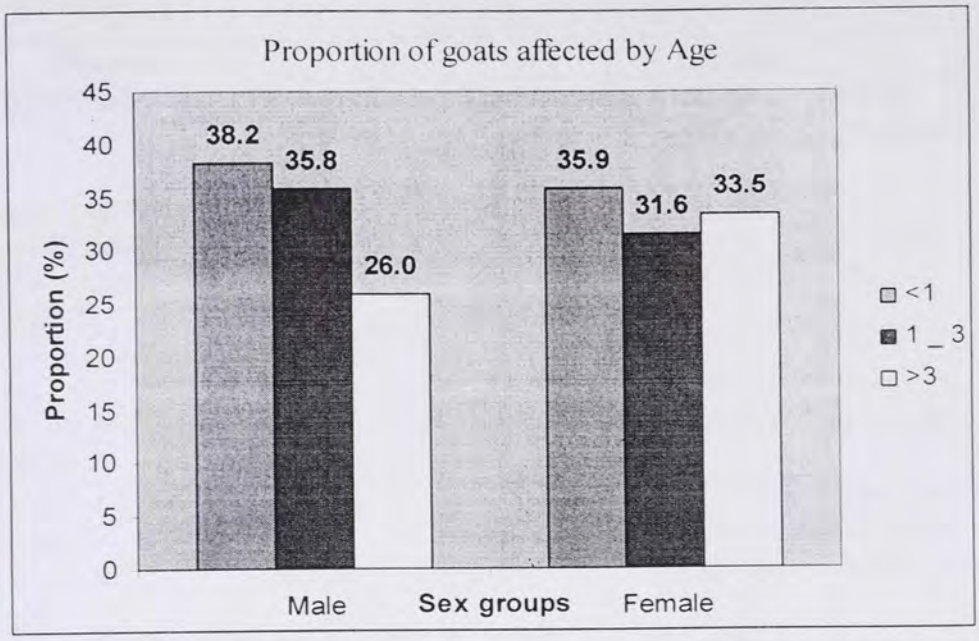

Figure 3- Morbidity of goats in different age groups of sex due to CCPP

From the focus group discussions, it was noted that recurrent drought was the first problem for livestock rearing followed by occurrence of different diseases.

4.1.2 Pair wise ranking

A pair wise comparison method was used to study and determine the most prevalent diseases of goat and livestock rearing problems. The result indicated that pasteurellosis was the first and most frequently occurring disease in the areas followed by CCPP and PPR (Table 10). Both of the latter diseases were introduced into the region during the last 12 years.

4.1.3 Matrix scoring

Matrix scoring was used for the assessment of scoring of disease signs for diseases of goats against their respective indicators among 16 groups of informants (1 group per PA). After the identification and prioritization of major diseases, informants also described such diseases accordingly against the different indicators mentioned during the pair wise comparisons. The result has shown the presence of high agreement among the informants ($p < 0.001$). The pastoralists' disease signs describing agreements about a specific disease was analyzed and presented pictorially below in Fig. 4.

	Disease signs/Syndromes	Diseases				
		Pasteurellosis ("*Suruatu*")	Ectoparasites ("*Agaraa*")	CCPP ("*Gublu*")	PPR ("*Indahee*")	Endoparasites ("*Begu Darya*")
1	Nasal discharge and Coughing (W = 0.952, p < 0.001)	•••••• 6 (3 - 11)	0 (0)	•••••••• •••••••• 16(10-19)	••••••• 7(6-10)	0 (0 - 1)
2	Chronic weight loss (W = 0.560, p < 0.001)	•• 2 (0 - 8)	•• 2 (0 - 6)	••• 3 (0 - 6)	•••• 4 (0 - 9)	•••••••••• •••••••• 17 (12 - 24)
3	Diarrhea / Enteritis (W = 0.910, p < 0.001)	0 (0 - 2)	0 (0)	•• 2 (0 - 6)	•••••••••• •••••••••• 20 (8 - 22)	•••••••• 8 (4 - 15)
4	Reduced milk yield (W = 0.668, p < 0.001)	•••••• 6 (1 - 9)	•••• 4 (1-5)	•••••••• 7 (6 - 12)	•••••••• 7 (6 - 9)	•••••• 6 (2 - 8)
5	Erosive stomatitis (W = 0.829, p < 0.001)	0 (0 - 1)	0 (0 - 3)	0 (0 - 3)	•••••••••• •••••••••• •••••••••• 30 (27- 30)	0 (0)
6	Tearing and lacrimation (W = 0.814, p < 0.001)	• 1 (0 - 5)	0 (0)	••• 3 (0 - 6)	•••••••••• •••••••••• ••••• 25 (20- 28)	0 (0)
7	Abortion (W = 0.899, p < 0.001)	•• 2 (0 - 3)	0 (0)	•••••••••• 10 (6 - 15)	•••••••••• ••••••• 16 (12 - 20)	••• 3 (0 - 4)
8	Highly contagious (W = 0.978, p < 0.001)	••••• 5 (0 - 6)	• 1 (0- 2)	•••••••••• ••••••• 16 (15 - 20)	•••••••• 8 (6- 10)	0 (0)
9	High mortality (W = 0.976, p < 0.001)	•••• 4 (1 - 7)	0 (0 - 1)	•••••••••• ••••••• 16 (14 - 20)	•••••••••• 10 (7 - 10)	• 1 (0 - 1)
10	High morbidity (W = 0.971, p < 0.001)	•••••• 6 (3 - 8)	•• 2 (0 - 3)	•••••••••• ••• 13 (12 - 16)	••••••••• 9 (7 - 10)	0 (0 - 2)
11	Acute/sudden death (W = 0.967, p < 0.001)	••••••• 7 (3 - 10)	0 (0)	•••••••••• •••• 14 (11- 16)	•••••••••• 10 (6 - 11)	0 (0)
12	Affect both goats and sheep (W = 0.888, p	•••••••••• •• 12 (7 - 12)	•••••••••• 10 (5 - 12)			•••••••• 8 (8 - 12)

	< 0.001)			0 (0)	0 (0)	
13	Affect only goats (W = 0.978, p < 0.001)	0 (0)	0 (0)	•••••••••• •••••••••• 20 (19 - 30)	•••••••••• 10 (0 - 10)	0 (0)
14	Pneumonic lung (W = 0.921, p < 0.001)	••••• 5 (0 - 5)	0 (0)	•••••••••• •••••••••• •• 22 (20 - 27)	••• 3 (2 - 7)	0 (0)
15	Marbling appearance of lung (W = 0.834, p < 0.001)	• 1 (0 - 3)	0 (0)	•••••••••• •••••••••• •••••••• 28 (24 - 30)	• 1 (0 - 3)	0 (0)
16	Serofibrinous deposits on lung (W = 0.957, p < 0.001)	••••••• 7 (2 - 7)	0 (0)	•••••••••• •••••••••• 20 (16 - 25)	••••• 5 (2 - 7)	0 (0)
17	Disease not treatable/ no recovery (W = 0.901, p < 0.001)	• 1 (0 - 5)	0 (0 - 1)	••••• 5 (0 - 7)	•••••••••• •••••••••• •••• 24 (20 - 30)	0 (0 - 1)
18	Has no vaccine /available/ (W = 0.921, p < 0.001)	0 (0)	•••••••••• •• 12 (0 - 16)	•••••• 6 (0 - 16)	0 (0)	•••••••••• •• 12 (9 - 15)

Figure 4- Matrix scoring of disease signs for diseases of goats

Number of informant groups = 16 (on average12 persons per group from four *Woredas* and 16 settlement areas). W = Kendall's Coefficient of concordance among informant groups. The black dots (•) represent the scores (number of stones) that were used during the matrix scoring. The median with numbers refers to the score and their respective minimum and maximum values in the parenthesis. A high number of dots indicate a relatively high association between a sign and a disease whereas a low number of dots indicate a weak association.

4.2. Seroprevalence of CCPP

4.2.1 Overall Seroprevalence using both CFT and cELISA tests

A total of 1,183 serum samples were collected proportionally at the *Woreda*, peasant associations (PA) and settlement areas or flock level based on their respective goat population (287 from Afambo, 276 from Mille, 306 from Dalifagae and 314 from Semurobi districts).

Hence, a total of 590 and 593 sera samples were tested for the presence of MccP infection, in the non-CAHWs (Mille and Semurobi) and CAHWs (Afambo and Dalifagae) intervention areas, respectively.

Using CFT and cELISA, a total of 497 (42%) animals were found to be seropositive to either of the tests. However, out of 1,183 sera samples tested 334 (29.08%) and 227 (19.19%) were seropositive to the MccP antigen using CFT and cELISA, respectively.

4.2.2 Seroprevalence using CFT

4.2.2.1 Overall seroprevalence

Among the four *woredas*, the seroprevalence for MccP infection was higher in Mille (40.22%), followed by 33.76%, 26.48% and 16.67% in Semurobi, Afambo and Dalifagae *woredas*, respectively (Table 11). There was a significant difference ($p < 0.001$) in the seroprevalence of CCPP among the four *woredas* using CFT.

Table 11- Seroprevalence of CCPP by *Woredas* using CFT

Woreda	*Sera*		*Seroprevalence (%)*	*95% Confidence Interval*
	Tested	*Positive*		
Afambo	287	76	26.48	21.27 – 31.69
Mille	276	111	40.22	34.32- 46.12
Dalifagae	306	51	16.67	12.41 – 20.93
Semurobi	314	106	33.76	28.42 – 39.10
Total	1,183	344	29.08	26.44 – 31.72

The seroprevalence of CCPP also varied among the different peasant associations (PA) ranging from 11.43% to 48.48%. Although not significant ($p > 0.05$), higher seroprevalences were observed in non-CAHWs areas than CAHWs areas as indicated in Table 12. Seroprevalence at flock level ranged from 0 to 52.63% in CAHWs areas and in non-CAHWs areas from 8.33% to 63.16%.

Table 12- Seroprevalence of CCPP at PA level using CFT

Woreda	*PAs*	*Sera*		*Seroprevalence (%)*	*95% Confidence Interval*
		Tested	*Positive*		
Afambo (CAHWs area)	Halisa Bolu	68	24	35.29	
	Humodeita	77	15	19.48	
	Mego	52	11	21.15	
	Deka	90	26	28.89	
	Total	287	76	26.48	21.27– 31.69
Mille (non-CAHWs area)	Intimegeyita	85	35	41.18	
	Bekeridar	65	23	35.38	
	Amibgus	60	21	35.00	
	Antimegeyita	66	32	48.48	
	Total	276	111	40.22	34.32 – 46.12
Dalifagae (CAHWs area)	Ataana Anbosi	70	8	11.43	
	Hadona Bidari	90	16	17.78	
	Biluna	79	19	24.05	
	Dermina				
	Gewanena	67	8	11.94	
	Fenhiru				
	Total	306	51	16.67	12.41 – 20.93
Semurobi (non-CAHWs area)	Kumaberi	102	33	32.35	
	Hare Hamo	76	27	35.53	
	Fentida	72	24	33.33	
	Asgefen	64	22	34.38	
	Total	314	106	33.76	26.44 – 31.72
Overall Total		1,183			

4.2.2.2 Univariate analysis of risk factors for seroprevalence using CFT

Univariate analysis using logistic regression was conducted for assumed factors which could have epidemiological importance. The effect of age, sex, flock size, CAHWs, and agro ecology on the seroprevalence of CCPP was analyzed and given in Table 13.

Table 13- Summary of the univariate analysis of the different characters using CFT

Strata	Total sera tested	Positive	Seroprevalence (%) and 95% CI	P – value	Odds Ratio and 95% CI
Health intervention					
- Non-CAHWs	590	217	36.78 (32.81 – 40.75)	0.001	2.13 (1.65 - 2.76)
- CAHWs	593	127	21.42 (18.05 – 24.79)		
Flockgroup					
- < =50	82	19	23.17 (13.87 – 32.47)		
- 51–150 Vs <= 50	418	114	27.27 (22.91 – 31.63)	0.44	1.24 (0.71 – 2.17)
->=151Vs 51-150	683	211	30.89 (27.11 – 34.67)	0.15	1.48 (0.86 – 2.54)
Sex					
- Female	1049	317	30.22 (26.6 – 31.94)	0.017	1.71 (1.10 – 2.67)
- Male	134	27	20.15 (13.22 – 27.08)		
Age groups					
- < 1 yr	204	57	27.94 (21.66 – 34.22)		
- 1 – 3 yrs Vs 1 yr	500	147	29.40 (25.33 – 33.47)	0.67	1.07 (0.75 – 1.54)
- > 3 yrs Vs 1 yr	479	140	29.23 (25.07 – 33.39)	0.74	1.06 (0.74 – 1.53)
Agro ecology					
- Arid	563	187	33.21 (29.24–37.18)	0.003	1.47 (1.14 – 1.89)
- Semi-arid	620	157	25.32 (21.82 – 28.82)		
Total	1183	344	29.08 (26.44 – 31.72)		

The mean seroprevalence of MccP infection in non-CAHWs intervention areas was 36.78% and 21.42% in the CAHWs intervention areas as given in Table 13. There was a highly significant difference ($p < 0.001$) in seroprevalences between the two intervention areas.

The age group seroprevalences were 27.94%, 29.40% and 29.23% in age group less than 1 year (Group I), age group 1 – 3 years(Group II) and > 3 years older (Group III), respectively. The seroprevalence difference was not significant ($p > 0.1$) among the three age groups. Therefore, age has no association with seropositivity to MccP infection.

The average number of animals sampled per flock was considered as flock size. Considering at least one positive in a flock, which makes that flock positive, the overall seroprevalence at flock level with at least one seropositive animal per flock was 93.75% (4 out of 64), but prevalences by interventions were 100% and 87.50% (4 out of 32 flocks) in the non-CAHWs and CAHWs areas, respectively. The within flock level seropositivity varied considerably ($p < 0.001$) between each intervention area or flock as 8.33% up to 63.16% in non-CAHWs areas and 0 up to 52.63% in CAHWs intervention areas.

When flocks were grouped into three by flock size as <= 50 (Group I), 51 – 150 (Group II) and >150 (Group III), the respective seroprevalences were 23.17%, 27.27% and 30.89%. There was no significant difference ($p > 0.1$) in seroprevalence among the three flock groups.

The sex specific seroprevalence observed was 20.15% in males and 30.00% in females as shown in Table 13. Because of high male to female ratio (1:10) in a particular flock, the contribution of males to the total seropositivity was only 7.85% (27 out of 344). Females were 1.71 times more likely to be affected with MccP than males ($p < 0.005$).

Agro ecologically, higher seroprevalence has been observed in arid areas (33.21%) than semi-arid areas (25.32%). There is a significant difference between the two agro ecologies ($p < 0.05$) with an OR of 1.47 in arid areas.

Although sheep are not affected by CCPP, they are found to be seropositive for MccP infections with a prevalence of 20.00% (5 out of 25). The seropositivity of goats to MccP was 29.27%. But the ratio of sheep to goats in the sample was so small (1:50) that their contribution to the total seropositivity was only 1.45% (5 out of 344) and 0.4% (5 out of 1,183) in the seropositive and all test sera , respectively.

4.2.2.3 Multivariate analysis of risk factors for CFT seroprevalence

To fulfill the assumptions of independent variables, multi-colinearity of variables was checked initially by calculating the correlation coefficient. Those factors not having strong linear relationship ($r < 0.4$) were used in the analysis. These enabled to exclude confounding effects of the different intrinsic and extrinsic factors.

Table 14- Logistic regression of risk factors associated with seropositivity using CFT

Risk factors	*Odds Ratio*	*P-value*	*[95% Conf. Interval]*	
Agro ecology	1.295772	0.579	0.5182407	3.239858
CAHW	2.221852	0.002	1.33855	3.688043
Sex	1.728802	0.021	1.085513	2.753313
Age	.9842371	0.867	0.8173814	1.185154
Flock group	1.60255	0.020	1.077872	2.382626
Flock level	.9982712	0.902	0.9710571	1.026248

Odds Number of obs = 1183, LR $X^2(7) = 56.31$, Prob > $X^2 = 0.0000$, Pseudo $R^2 = .0395$

The summary of multivariate analysis of risk factors for CCPP seroprevalence is given in Table 14 and Table 15. Highly significant associations were obtained in the variables flock group, CAHWs and sex ($p < 0.005$).

Table 15- Regression coefficients of the assumed risk factors using CFT

Risk factors	*Coef.*	*P - value*	*[95% Conf. Interval]*	
Agro ecology	0.259107	0.579	-0.6573155	1.175529
CAHW	0.7983413	0.002	0.2915866	1.305096
Sex	0.5474288	0.021	0.0820527	1.012805
Age	-0.0158884	0.867	-0.2016494	0 .169872
Flock Groups	0.4715961	0.020	0.0749889	0.8682032
Flock level	-.0017303	0.902	-0.02937	0.0259094
_cons	-3.440638	0.000	-4.607021	-2.274254

Number of obs = 1183, LR $X^2(7) = 56.31$, Prob > $X^2 = 0.000$, Pseudo $R^2 = 0.0395$

After regression of those risk factors less than $p < 0.2$ again, major risk factors OR and their respective regression coefficients are obtained and presented in Table 16 and 17.

Table 16- Final multiple logistic regression analysis on seropositivity using CFT

Factor	OR	P-value	[95% Conf. Interval]	
Flock group	1.247956	0.042	1.007607	1.545637
CAHWs	2.134682	0.000	1.649473	2.762619
Sex	1.83021	0.008	1.168102	2.867618

Number of obs = 1183, LR $X^2(3) = 45.58$, Prob > $X^2 = 0.0000$, Pseudo $R^2 = 0.0320$

Table 17- Regression coefficients of the major risk factors on seropositivity using CFT

Risk factor	*Coef.*	*P-value*	*[95% Conf. Interval]*	
Flock group	0.2215072	0.042	0.0075783	0.4354362
CAHWs	0.7827826	0.000	0.5230496	1.042515
Sex	0.6044307	0.008	0.1553798	1.053482
_cons	-3.198377	0.000	-4.038105	-2.358648

Number of obs = 1183, LR $X^2(3) = 45.58$, Prob > $X^2 = 0.0000$, Pseudo $R^2 = 0.0320$

From the analysis, it was noted that flock group, CAHWs and sex are the major risk factors ($p < 0.05$) responsible for the occurrence of the disease. As there is no interaction observed among flock groups, CAHWS and sex, these three factors could be used to construct the regression model to predict the occurrence of the disease. The multivariate regression analysis on the three variables indicated the OR of 1.24, 1.83 and 2.13 in flock group, sex and health service, respectively.

$$\text{Log (P)} = 0.78\ \text{CAHW} + 0.22\ \text{Flock group} + 0.60\ \text{sex} - 3.20$$

4.2.3 Seroprevalence using cELISA test

According to the manufacturer (CIRAD-EMVT, France) of cELISA test kit, the cutoff point for seropositivity of CCPP was greater than 25% inhibition in optical density of the readings. Doubtful reactors i.e. showing 20 – 25% inhibition, were considered as seronegative.

4.2.3.1 Internal quality control

Plots of mean percent inhibition (PI) values for internal quality control sera categorized as strong positive (C^{++}), weak positive (C^{+}), negative control (Cn) and conjugate control (Cc) versus plate numbers are shown in Figure 5. From the figure, it was noted that the controls employed in the laboratory were within the upper and lower acceptable limits.

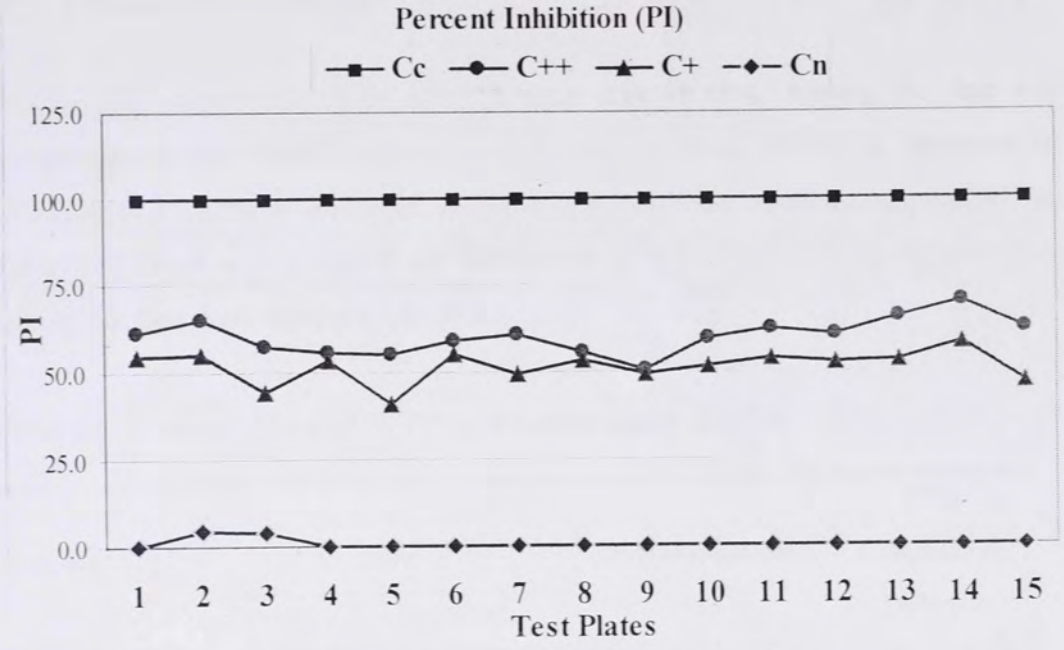

Figure 5- Percent inhibition of the controls

The optical density (OD) values of monoclonal and conjugate control for the entire plates were also within the acceptable ranges as indicated in Figure 6.

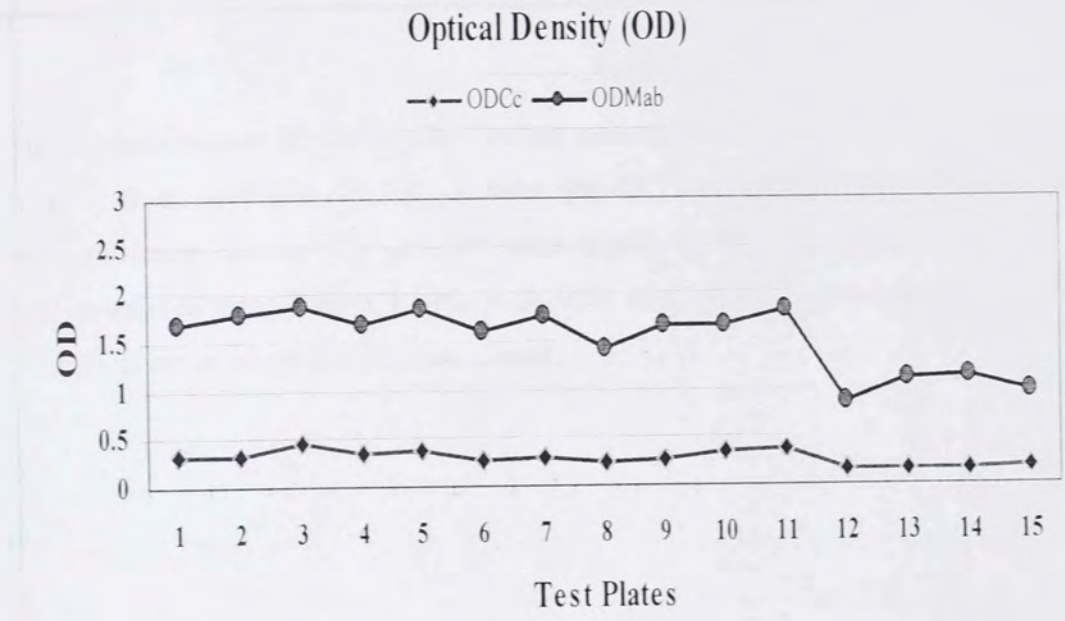

Figure 6- Optical Density of Monoclonal antibody and Conjugate control

4.2.3.2 Overall seroprevalence

The overall seroprevalence in cELISA tests was 19.19%. Among the four *woredas*, the seroprevalence for MccP antibody was higher in Mille (30.48%), followed by 18.15%, 15.33% and 13.73% in Semurobi, Afambo and Dalifagae *woredas*, respectively as shown in Table 18. There was a significant difference ($p < 0.001$) in the seroprevalence of CCPP among the four *woredas* using cELISA.

Table 18- Seroprevalence of CCPP by *Woredas* using cELISA

Woreda	*Sera*		*Seroprevalence (%)*	*95% Confidence Interval*
	Tested	**Positive**		
Afambo	287	44	15.33	11.08 – 19.58
Mille	276	84	30.48	24.94 – 36.02
Dalifagae	306	42	13.73	9.80 – 17.66
Semurobi	314	57	18.15	13.80 – 22.50
Total	1,183	227	19.19	16.90 – 21.48

The seroprevalence of CCPP also varied among the different peasant associations (PA) ranging from 1.49% to 77.27%. Unlike the CFT, a significant ($p < 0.05$) difference in seroprevalence among the peasant associations (PAs) was observed (Table 19). The seroprevalence ranged from 1.49% to 30.00% in CAHWs intervention areas and 1.96% to 77.27% in areas where CAHWs are absent.

Table 19- Seroprevalence of CCPP at PA level using cELISA

Woreda	*PA name*	*Sera*		*Seroprevalence (%)*	*95% Confidence Interval*
		Tested	**Positive**		
Afambo	Halisa Bolu	68	15	22.06	
	Humodeita	77	6	7.79	
	Mego	52	6	11.54	
	Deka	90	17	18.89	
	Total	287	44	15.33	11.08 – 19.98
Mille	Intimegeyita	85	10	11.76	
	Bekeridar	65	8	12.31	
	Amibgus	60	15	25.00	
	Antimegeyita	66	51	77.27	
	Total	276	84	30.48	24.94 – 36.02
Dalifagae	Ataana Anbosi	70	21	30.00	
	Hadona Bidari	90	16	17.78	
	Biluna Dermina	79	4	5.06	
	Gewanena Fenhiru	67	1	1.49	
	Total	306	42	13.73	9.80 – 17.66
Semurobi	Kumaberi	102	2	1.96	
	Hare Hamo	76	8	10.53	
	Fentida	72	45	62.50	
	Asgefen	64	2	3.13	
	Total	314	57	18.15	13.80 – 22.50
Overall Total		1,183	227	19.19	16.90 – 21.48

Seroprevalence within an individual flock level basis (settlement areas) showed that it ranged from 0 to 44.00% in CAHWs areas and 0 to 86.67% in non-CAHWs areas.

4.2.3.3 Univariate analysis of risk factors

Univariate analysis using logistic regression was conducted for assumed factors which have epidemiological importance. The effect of age, sex, flock group, CAHWs, agro ecology and flock group on the outcome variable was analyzed and presented below in Table 20.

Table 20- Summary of the univariate analysis of the different characters using cELISA

Strata	*Total sera tested*	*Positive*	*Seroprevalence (%)*		*Odds Ratio and 95% CI*
			and 95% CI	**P – value**	
Health intervention					
- Non-CAHWs	590	141	23.90(20.40 – 27.41)	0.001	1.85(1.37–2.49)
- CAHWs	593	86	14.50(11.60 – 17.40)		
Flockgroup					
- <=50	82	10	12.20(4.97 – 19.43)		
- 51–150 Vs <=50	418	94	22.49(18.41 – 26.57)	0.039	2.09 (1.04 – 4.20)
- >=151 Vs <=50	683	123	18.01(15.07 – 20.95)	0.19	1.58 (0.79 – 3.15)
Sex					
- Female	1049	204	19.45(17.00 – 21.90)	0.53	1.16 (0.73 – 1.87)
- Male	134	23	17.16(10.65 – 23.67)		
Age groups					
- < 1 yr	204	38	18.63(13.18 – 24.08)		
- 1–3 yrs Vs 1yr	500	99	19.80(16.24 – 23.36)	0.72	1.08 (0.71 – 1.63)
- > 3 yrs Vs 1 yr	479	90	18.79(15.22 – 22.36)	0.96	1.01 (0.66 – 1.54)
Agro ecology					
- Arid	563	128	22.74(19.21 – 26.27)	0.003	1.55 (1.16 – 2.07)
- Semi-arid	620	99	15.97(13.03 – 18.91)		
Total	1183	227	19.19(16.90 – 21.48)		

The mean seroprevalence of MccP infection in non-CAHWs intervention areas was 23.90% and 14.50% in the CAHWs intervention areas as given in Table 20. There was a significant difference ($p < 0.001$) in seroprevalence between the two intervention areas. The OR value for non-CAHWs compared to CAHWs was 1.85 which indicates the association of health intervention to CCPP.

The seroprevalences in different age groups were 18.63%, 19.80% and 18.79% in age groups less than 1 year (Group I), between 1 and 3 years (Group II) and greater than 3 years older (Group III) and the difference was not significant ($p > 0.5$). So like the CFT, age groups have no association to the occurrence of the CCPP.

Considering at least one positive animal in a flock, which makes that flock positive, in both intervention areas flocks were seropositive to MccP infection with 81.25% (26 out of 32 flockss or SA) in the non-CAHWs areas and also equally 81.25% (26 out of 32 flocks or SA) in the CAHWs areas with the same overall flock level prevalence of 81.25 %.

The within flock level seropositivity varied considerably ($p < 0.001$) between each intervention area as 0 up to 86.67% in non-CAHWs areas and 0 up to 44.00% in CAHWs intervention areas.

After grouping the flock size into three categories according to their flock size, the seroprevalences were 12.20%, 22.49% and 18.01% in flock groups of <=50 goats (Group I), group 51 – 150 (Group II) and flocks groups of >=151 (Group III). Unlike the CFT, there were significant differences ($p < 0.05$) in seroprevalences between Group II and Group I, but no difference ($p > 0.05$) has been observed in Group III versus Group I. The OR values for Group II compared to Group I, and Group III versus Group I were 2.09 and 1.58, respectively.

The sex specific seroprevalence was 17.16% in males and 19.45% in females (Table 20). Because of high male to female ratio (1:10) of the flocks owned by the pastoralists and the small contribution of males to the total seropositivity was 10.13% (23 out of 227). Unlike the CFT, the association of sex and seropositivity was not significant ($p < 0.05$).

Seroprevalence difference by agro ecology has been observed and it was higher in arid climatic conditions (22.74%) than semi-arid areas (15.97%). The difference observed between the two agro ecologies was significant ($p < 0.05$). Therefore, the seropositivity was associated to the climatic condition of the areas.

Although sheep are not clinically affected by CCPP, 6(24%) of 25 animals tested were seropositive for MccP infections. The seropositivity of goats to MccP was 19.08%. But the ratio of sheep to goats in the sample was so small (1:50) to compare.

4.2.3.4 Multivariate analysis using cELISA

The overall multivariate model adjusted for assumed risk factors like sex, age, flock size, flock group, and agro ecology was analyzed. Significant associations were seen with seropositivity to extrinsic factors (flock groups, flock size, agro ecology and CAHWs) using multivariate analysis (Table 21).

Table 21- Multiple logistic regression analysis of assumed risk factors associated with Seropositivity using cELISA

Risk factors	*Odds Ratio*	*P-value*	*[95% Conf. Interval]*	
Agro ecology	11.29657	0.000	3.87135	32.96328
Flock group	1.467347	0.101	0.928127	2.319841
CAHWs	0.6522032	0.150	0.3646513	1.166509
Sex	1.097282	0.716	0.6650495	1.810433
Age	0.9882092	0.913	0.7980426	1.223691
Flock level (SA)	1.067945	0.000	1.034103	1.102895

Number of obs = 1183, LR X^2 (6) = 48.00, Prob > X^2 = 0.0000, Pseudo R^2 = 0.0415

Table 22- Regression coefficients of the assumed risk factors using cELISA

Risk factors	*Coef.*	*P - value*	*[95% Conf. Interval]*	
Agro ecology	2.424499	0.000	1.353603	3.495394
CAHW	-0.4273992	0.150	-1.008814	0.1540153
Sex	0.0928361	0.716	-0.4078939	0.5935661
Age	-0.0118609	0.913	-0.2255933	0.2018716
Flock group	0.3834562	0.101	-0.0745863	0.8414987
Flock level	0.0657364	0.000	0.0335341	0.0979387
_cons	-4.613374	0.000	-5.934292	-3.292455

Number of obs =1183, LR X^2(6) = 48.00, Prob > X^2 = 0.000, Pseudo R^2 = 0.0415

The final model of the regression could be derived from the variables with $p < 0.001$ having strong association with the occurrence of the disease. Hence, agro ecology and individual flock were the choice of the variables on which the model could be constructed to predict the occurrence of the disease. These two variables are found to have no interaction to one another.

Those factors that would have p-value less than 0.2 should not be neglected as they could have some effect on the out come of interest. Therefore, after taking into account those factors which have $p < 0.2$ as indicated previously in the data analysis methodology part, CAHWs and flock groups were regressed again (Table 23) to determine the final model.

Table 23- Regression coefficients of major risk factors analysis using cELISA (p<0.2)

Risk factor	*Coef.*	*P-value*	*[95% Conf. Interval]*	
Agro ecology	2.611705	0.000	1.553982	3.669429
Flock level	0.0683388	0.000	0.0362363	0.1004413
CAHW	-0.4450106	0.134	-1.027742	0.1377205
Flock group	0.0010175	0.993	-0.2392023	0.2412373
_cons	-4.340563	0.000	-5.507438	-3.173687

Number of obs = 1183, LR $X^2(4) = 44.06$, Prob > $X^2 = 0.000$, Pseudo $R^2 = 0.0381$

As the flock group p – value is high (p- value = 0.993), it is excluded from the model and hence the final disease predicting risk factors are individual flock, agro ecology and health intervention. The correlation coefficient calculation also indicated that there is no interaction among these three factors.

The final model is constructed based on such predicting variables as:

$$\text{Log (P)} = 0.068 \text{ Flock level} + 2.61 \text{ Agro ecology} - 0.44 \text{ CAHW} - 4.34$$

4.2.4 Serological tests agreement

MccP seropositivity comparison of CFT and cELISA tests is given in Table 24. In the ELISA test the overall seropositive results was 19.19 % and 29.08% in the CFT test. There were discordant results between the two tests. One hundred and fifty three samples which were identified as negative by CFT were positive for ELISA while 270 samples identified as negative by ELISA were positive for CFT. In this study, the relative sensitivity (Se) and specificity (Sp) of both CFT and cELISA were not known.

Table 24- Contingency table for CFT and cELISA tests agreement on all test sera

		CFT test		
		Positive	**Negative**	Total
cELISA test	**Positive**	74	153	227
	Negative	270	686	956
	Total	344	839	1,183

Test agreements for CFT and cELISA based on the observed agreement (concordance) and the agreement beyond chance (kappa value) were performed on all sera samples. The mean concordance was 64.24% and kappa values for the overall sera samples were found to be and 0.036, respectively. So the high level of statistical agreement between cELISA and CFT was slight agreement on all 1,183 samples when tested in parallel (Table 25).

Table 25- Concordance and Kappa values of CFT and cELISA on all sera samples

Intervention Areas	*Woreda*	**No. of Sera tested**	**Positive sera**		**Concordance (%)**	**Kappa value**	**Agreement**
			CFT	**cELISA**			
CAHWs	Afambo	287	76	44	66.55	0.007	Slight
	Dalifagae	306	51	42	72.88	-0.066	Negative
Non-CAHWs	Mille	276	111	84	56.88	0.066	Slight
	Semurobi	314	106	57	60.19	-0.004	Negative
Overall Total		**1,183**	344	227	64.24	0.036	Slight

5. DISCUSSION

Participatory Epidemiology

In 1996 the term 'participatory epidemiology' was used in relation to field level investigation and searching for rinderpest in Africa (Mariner, 1996). The use of PRA methods to specifically to collect information from pastoralists on rinderpest was viewed as a key adjunct to laboratory-based epidemiology, particularly as serological surveys were difficult to conduct and interpret in remote pastoral areas (Catley, 1999).

The participatory disease search method has shown that there are many diseases affecting the goat population in Afar pastoralist areas and pastoralists were able to prioritize such diseases according to their severity and economic importance. In this study pastoralists have mentioned CCPP as their second problem of goats' rearing among the different diseases mentioned next to pasteurellosis. Additionally using the disease calendar technique, the most important seasons of the year for disease outbreaks were identified. Although the outbreak occurred at any time of the year, cold climate just beginning of rainy seasons and severe drought seasons are the most important predisposing factors for CCPP out break. This was in agreement with Solomon (2005) who reported similar findings in South Omo.

Among the different sources and transmissions of CCPP infection, grazing and watering points were identified as the two major sources followed by introduction of new cases and marketing areas. In extensive husbandry practices, communal grazing areas and watering points are the major sites for disease transmission (Seifert, 1996).

The disease calendar had showed that historically CCPP was introduced in the area through goats introduced due to different reasons for instance they have mentioned during restocking programs by local non-governmental organizations (NGOs) and other recovery and rehabilitating projects. When severe drought occurs, livestock are commonly affected; under such circumstances, NGOs restock the pastoralists' goats by purchasing from remote areas without regard to disease carrier status. The pastoralists have mentioned this and the researcher himself has observed the case.

The communities have scored the highest values for CAHWs during the matrix scoring followed by public service because of comparative advantages of CAHWs. The herders' preference to CAHWs was also reported by other studies (Mariner, 2001; Catley, 2002; Amare, 2004).

Communities were capable of describing features of a specific disease and a higher agreement among the herders has been noted. Such diseases and their descriptions were almost coinciding with the conventional textbooks. Comparison of NAHAs reports and serological survey on CCPP (locally called '*sambab*') in Somalia has indicated that the two sets of data were statistically dependent. Consequently, it was suggested that NAHAs could be a useful means of CCPP surveillance in the Somalia rangelands (Baumann, 1990).

Although it was mentioned that CCPP was the most important disease, active disease outbreak could not be identified. In Dalifagae *woreda* town, we made an interview with the local people who slaughter on contract basis for hotels and individuals, and they confirmed that no clinical CCPP cases despite CCPP like lesions at postmortem was observed in some animals. This indicted that despite the absence of clinical signs; the infection could cause unexpressed pneumonia and other pathological lesions.

Seroprevalence

The overall prevalences of CCPP using CFT and cELISA were 29.08% and 19.19%, respectively. Solomon (2005) reported a seroprevalence of 15.72% and 57.33% using CFT and cELISA tests, respectively. Other studies, which were conducted in Southwestern part of Ethiopia using B-ELISA showed that a prevalence of 35% (Bereket, 1995) and 36% (Mekonen, 1996). In outbreak areas, using the serological tests such as CFT and B-ELISA, Sharew *et al.* (2005) reported a prevalence of 52% to 100%. The differences in the prevalences observed due to a number of reasons. For instance some studies have focused on outbreak investigations (Mekonen, 1996; Bereket, 1995; Roger and Bereket, 1996) and while other works have focused on evaluating the sensitivity and specificity of different serological assays and their relative advantages (Sharew *et al.*, 2005; Roger and Bereket, 1996). The sampling techniques in most cases have thus used non-random sampling techniques.

Serological tests comparison

The present study has employed both CFT and cELISA in parallel on all sera samples and both tests indicated that CCPP is highly prevalent in Afar pastoralist areas. However, there was slight agreement between the two tests (kappa = 0.036) and concordance (64.24%). Solomon (2005) has even reported a negative agreement (kappa = -0.014) and concordance (44.88%), but he tested CFT positive goats serially with cELISA. The observed differences could be due to the fact that both tests detect different immunoglobulins (Tizard, 1996). Immunoglobulin M (IgM) is the first to appear and has little specificity which gives rise to pronounced cross-reactions and remains in the blood for short period. But IgG is produced lately and lasts much longer than IgM and more specific than IgM antibody isotypes (Staak *et al.*, 2001). Thiaucourt *et al.* (1996) reported that antibodies detected by cELISA persist in blood for more than six months while those detected by the CFT vanishes completely within three months after the infection. Thus cELISA could detect IgG that persists for longer period while CFT reacts to IgM, creating weak test agreement between them.

The overall seroprevalence differences obtained in CFT (29.08%) and cELISA (19.19%) could be due to the fact that specificity of CFT is less as it could cross react with members of the 'mycoides cluster', a group of six mycoplasma species or subspecies which share many biochemical, antigenic and genotypic characteristics and hence their close relationship can lead to problems for diagnosis. The mycoides cluster contains six important ruminant mycoplasmas including *M. m. mycoides SC*, the cause of contagious bovine pleuropneumonia, *M. m. mycoides LC* and *M. m. capri*, *M. ovipneumoniae* and *Mycoplasma* species group 7 of Leach (Thiaucourt *et al.*, 1996; Thiaucourt and Bölske, 1996; Walker, 1999). Findings of CFT emphasizes the difficulties inherent in the serological diagnosis of CCPP when using whole cell or membrane preparations as antigen, as there is cross-reactivity with sera against the other three principal caprine mycoplasmas (OIE, 2004a). CFT test may show cross-reactions with other *Mycoplasma* spp. affecting small ruminants (Thiaucourt *et al.*, 1996). The cELISA specificity and suitability for large scale testing makes it an appropriate test for epidemiological investigations (OIE, 2004a).

Laikemariam (2004) reported that CCPP caused by Mccp was found to be prevalent in the surveyed areas (Omo, Borena, Tigray, Arbaminch) except North Shoa, but some samples had strains MmmLC and *M. ovipneumoniae* showing the presence of mixed infections,

particularly in Omo and Borena areas. Despite mixed infections are common in small ruminants, the cELISA permits the specific detection of antibodies since monoclonal antibody (Mab) specific to MccP is employed in the test. Thus, cELISA being highly specific, the seroprevalence result was less than that of CFT.

So the cELISA test has superior advantage and hence more of the conclusions of this study could be forwarded on the basis of its results keeping the CFT results complementary rather than undermine them. A quantitative result is obtained with a single dilution of serum. This assay method permits the testing of numerous sera in large serological enquiries (Thiaucourt *et al*., 1996; OIE, 2004a) and could detect antibodies after longer infection.

Seroprevalence differences of CCPP using CFT were observed between the two strata: prevalence was higher in *woredas* where no CAHWs are present (36.78%) than in *woredas* where CAHWs are present (21.42%). Such difference was also observed in the cELISA tests being higher in non-CAHWs areas (23.90%) than CAHWs areas (14.50%). The observed differences were highly significant in both tests ($p < 0.001$). The odds of exposure to the disease in non-CAHWS areas were 2.1 and 1.9 times more than that occurred in the CAHWs in CFT and cELISA tests, respectively. From the relatively lower seroprevalences observed where CAHWs were present may be due to the better animal health provision in the CAHWs areas. Community intervention program is contributing a significant increment on the livelihoods of the societies as has been reported by EPIACT (2002). Kenyi (2001) who stated that changes in livestock benefits have been caused by the primary animal health program. Mortality of livestock species has decreased in CAHWs intervention programs (Amare, 2004). Mc Corkle (2003) noticed that livestock mortality in selective CAHWs intervention areas of Tanzania, Kenya and Philippines is very less compared with similar areas in non-CAHWs. EPIACT (2002) also reported that sheep and goat diseases have been reduced by 50% in CAHWs intervention areas, Amibara *woreda* of Afar region.

Non-governmental organization like FARM AFRICA was working in Dalifagae for more than two years in the area on Food Security Programs through strengthening animal health service. This NGO has trained and organized 18 CAHWs, which give basic veterinary services and vaccinations. The local NGO has given a special attention to such groups and made a routine follow up and hence they gave any necessary support like regular vaccinations and even drugs free of charge at time of problems to the whole population of goats in the *woreda*. FARM

AFRICA had supported CAHWs in Dalifagae *woreda* and established their own association and drug shop in the respective *woreda* town, Dalifagae. The establishment of the drug shop enabled them to have easy access to replenishment and recycling their capital and to give the health service in a better condition and hence indicated the strength of the association of CAHWs.

The numbers of CCPP outbreak reports in Dalifagae *woreda* in Afar region were 13 from year 2000 – 2004 (MOA, 2004), however no such occurrence was observed since then. This could be associated to the existing better and additional veterinary service in the area through NGOs' supported CAHWs. The CFT and c ELISA tests indicated that the seroprevalences in Dalifagae *woreda* were 16.67% and 13.73%, respectively, which were much lower than any other *woredas*.

Comparison of seroprevalences using CFT and cELISA among flock groups indicated a prevalence of 23.17% and 12.20% (less than 50 goats), 27.27% and 22.49% (51 – 150 goats) and 30.89% and 18.01% (greater than 150 goats), respectively. No significant difference ($p > 0.05$) was observed among flock groups using CFT, but it was significant ($p < 0.05$) using cELISA. Solomon (2005) had reported that larger flock size groups were more affected with CCPP than smaller flock sizes. This may be explained by the fact that MccP infection needs proximity to source of infection and increasing number of susceptible population (Lefèvre *et al.*, 1987). Goat flocks naturally and behaviorally are highly aggregated during grazing and watering time besides their resting time, and hence the disease can easily be spread within that flock.

As the number of CCPP cases increases then the probability of its transmission within a flock would be higher due to frequent contact and the presence of susceptible animals. Comparable seroprevalence was observed in those flock groups of less than 50 goats per flock which is attributed to the disease affected the flock before and those remaining goats are carriers of the disease. In pastoral areas livestock are not sold in market due to market inaccessibility and traditions.

Using CFT, the overall flock level seroprevalence was 93.75%, which was 100% and 87.50% in non-CAHWs and CAHWs areas. Whereas in those sera tested using the cELISA, the mean seroprevalence at individual flock level was relatively lower (81.25%) and did not vary

between CAHW and non-CAHWs areas. In this study relatively higher seroprevalence was observed in CFT than cELISA test. Solomon (2005) has reported a mean flock level prevalence of 93.15% using CFT. The wide range of prevalence among flocks could be attributed to the differences among the husbandry practices in the different *woredas*. Those traditional measures are treatments, isolation and culling clinical and suspected in contact animals. Once a single case is introduced into the flock, the disease could spread and affect the majority of the flock. Also there could be differences among herders themselves to react and take measures against occurrence of diseases within their flocks.

The participatory assessment findings and both serological tests have indicated that the occurrence of the disease in all age groups. Results obtained in different age groups using CFT and cELISA have also shown a prevalence of 27.94% and 18.63%, 29.40% and 19.80%, and 29.23% and 18.79% in those age groups less than 1 year, 1 – 3 years and greater than three 3 years of goats, respectively. There are no significant differences ($p > 0.05$) in prevalence among such age groups. The participatory part also strongly supported the serological results that all age groups of goats were susceptible to the disease with an average proportion of 33%, 33.55% and 34.45% in age groups less than 1 year, between 1 and 3 years and greater than 3 years, respectively. This result is in agreement with Dawit (1996), Teshome (1997) and Solomon (2005). It might indicate that humoral immunity to MccP is not be influenced by age. The disease caused by Mccp is highly contagious and fatal to susceptible goats of all ages (OIE, 2004a). But the result of the present study somehow contradicts the one that described by other authors that stated young animals are very susceptible to infection and generally develop more severe disease than adult animals (Aiello and Mays, 1998; Walker, 1999).

Higher seroprevalence was observed in females (30.22% and 19.45%) than males (20.15% and 17.16%) in CFT and cELISA tests, respectively. In the CFT result, the difference observed was not significant ($p > 0.05$). But it was significant ($p < 0.05$) in the cELISA-tested animals. Other studies have shown that there are no significant ($p>0.05$) in differences in CCPP seroprevalence between male and female goats (Dawit, 1996; Teshome, 1997; Beyene, 2003; Zenebe, 2004, Solomon, 2005). The disease caused by MccP is highly contagious and fatal to susceptible goats of both sexes (OIE, 2004a).

Animals in highly stressed conditions are more likely exposed to diseases like CCPP, pasteurellosis, and endoparasites. Because such diseases are naturally stress associated and needs predisposing factors. The present study also ensured goats from arid areas have a higher seroprevalence (33.21%) than from the semiarid areas (25.32%) where their difference in prevalence was significant ($p < 0.05$) using CFT. The cELISA results also have indicated where higher prevalence was observed in arid areas (22.74%) than semi-arid areas (15.97%). Goats reared in arid areas are found to be 1.5 and 1.6 times more likely to contract infection than goats in semi-arid areas using CFT and cELISA tests, respectively ($p < 0.001$). This could be explained by the fact that goats reared in arid areas are exposed to severe stresses like recurrent drought and frequent climatic changes. Carrier animals may shed more organisms after times of stress and changes in climate. CCPP spreads to new areas by the movement of infected or carrier goats to susceptible goat population (Thiaucourt *et al.*, 1996). A difference between humid and dry climate has been also reported for mycolplasmal infection (Lefèvre *et al.*, 1987). The present finding was also in agreement with other reports (Roger and Bereket, 1996; Teshome, 1997; Beyene, 2003) that reported the significant differences in the occurrence of CCPP among the different agro ecological zones.

In the present study, as sheep are kept and reared with goats, they were included in the sample just to evaluate if sheep could be infected. In the CFT, prevalence in goats was 20% compared to 29.7% in goats. Similarly, the cELISA has also shown higher prevalence (24%) compared to 19.2% in goats. Analysis could not be done to compare the two species, because few sheep (25) were tested. Goats are the only clinically affected species known (Aiello and Mays, 1998; Walker, 1999; OIE, 2004a; OIE, 2004b). They may contract the infection being kept and reared as part of the flock of goats. Serological and bacteriological studies on sheep have indicated the presence of subclinical infection by Mccp. In two reports, sheep have been shown to harbour MccP in the nares and lungs (Litamoi *et al.*, 1990; Bölske *et al.*, 1995). In Ethiopia, sheep are naturally resistant to clinical CCPP. The isolation of Mccp, however, proves the role of sheep as reservoir of infection (Laikemariam *et al.*, 2004). However, the pathogenicity of the ovine isolate to caprine and the role of sheep in the epidemiology of the disease have not yet been determined (Mekonen, 1996). The detection of MccP specific antibodies in sheep kept together with flocks of goats could possibly be due to lateral passage of the MccP organisms to this species. Similarly MccP antibody in sheep has been described from CCPP outbreak areas in sheep (Muriu *et al.*, 1990; Roger and Bereket, 1996).

Among the different risk factors considered on the multiple regression analysis, the major risk factors identified in CFT were flock group; CAHW and sex, which could be responsible for the disease outcome. Whereas in the cELISA test, agro ecology, CAHWs and individual flocks (flock found in a single settlement area) were found to be important among the different epidemiologically assumed risk factors. Therefore in both cases extrinsic risk factors except sex are significantly associated and responsible for the disease occurrence. This could be explained by the fact that the animal health service would contribute in decreasing the disease occurrence in its part. On the other hand, the association of the disease to flock level might be attributed to once the MccP infection is introduced within the flock then it would circulate in that specific flock causing higher disease prevalence. Frequent and adverse climatic changes would predispose the susceptible population to stressed associated diseases like CCPP, pasteurellosis, and others and transmission occurred predominantly by spread from animal to animal through direct contact and through inhalation of respiratory secretions (Walker, 1999) affecting the whole flock.

6. CONCLUSION AND RECOMMENDATIONS

The participatory disease search, questionnaire survey and serological findings agreed and indicated that CCPP is one of the major goat health problems in Afar pastoral areas, in North-eastern part of Ethiopia.

In the participatory assessment, CCPP, PPR and pasteurellosis are complained as the major cause of morbidity and mortality of goats in the study areas. The disease, CCPP is introduced in the area mainly during restocking of animals after severe drought had affected livestock.

Although official reports and clinical cases of CCPP were not observed during the study period, the serological studies have shown that CCPP is endemic and exists in sub clinical level in the study areas. In both serological tests conducted despite differences in seroprevalnces, they are suggestive of the wide occurrence and distribution of MccP infection in both intervention areas. The presence of CAHWs had contributed a lot in reducing the occurrence of CCPP through its additional veterinary service impact.

Factors such as the absence of community animal health workers, arid climatic conditions, and individual flock and flock sizes have contributed to the occurrence and distribution of the disease. These variables were responsible for predicting the disease outcome and hence to be included in the final model construction.

Comparison of serological tests between CFT and cELISA has shown that there was slight agreement between them, though both are known to be used as diagnostic tests for CCPP. The use of CFT and cELISA at the same time increases the probability of detecting infection status of CCPP instead of either of them. Therefore, these tests could be used in parallel and complementarily to each other instead of one being used as a better test for another because of their existing comparative advantages.

In the present study sheep were highly positive despite no clinical disease has been detected or observed in the participatory disease search (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:

- Contagious caprine pleuropneumonia was identified as one of the most economically important diseases in the Afar pastoral areas.

- Participatory disease search (PDS) could be used in the disease diagnosis and investigation process. It breaks the barrier and acts as a bridge between the community and the veterinary professionals. The PDS is a powerful tool and cost effective, and could be used complementarily with the modern way of approach in disease investigation in the pastoral production system.

- Strengthening of the community animal health workers system is very highly recommended since they are the ones who live with the community problems in far remote areas and even could take immediate actions on disease outbreaks through notifications, prevention and control.

- The identified major epidemiological risk factors need to be further studied to be included in the disease prevention and control schemes.

- Further studies should be conducted on CFT and cELISA tests in known CCPP infected and free animals to evaluate their specificity and sensitivity.

- The epidemiological importance and role of sheep in CCPP needs to be further studied as it could be the source and reservoir of infection for MccP. Therefore sheep should be included in the CCPP control and prevention programs design and implementation stages.

- An integrated control and prevention CCPP should be conducted through multi directional approaches which include minimizing stress factors, isolation of clinical cases, and care during restocking, vaccination in disease free areas and early treatment of clinical cases by the government and other supporting agencies. The traditional flocking strategy towards disease prevention should also need to strengthen.

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ANNEXES

Annex 1: Questionnaire for CCPP survey

PA name ---------- Village name---------- Date sample collected----------

1. Owner's name ----------, Sex---------- , Age---------- ---, Ethnic/clan---------
2. Address: Region -------- Zone ------- District ---------- Village----------
3. Type of livestock owned: Cattle -------- Sheep ------- Goats --------- Camel --------
4. Comparative use/ importance/ keeping animal species (1 to 4)

	Cattle	goat	sheep	camel
Disease resistance	----------	----------	----------	----------
Drought resistance	----------	----------	----------	----------
Market preference	----------	----------	----------	----------
Economic/social value	----------	----------	----------	--------
Milk/meat use	----------	----------	----------	----------

5. Flock composition of goats owned/ flock(s).

Age pattern	Male % (0)	Female % (1)
< 1 yrs (1)		
1 -3 yrs (2)		
> 3 yrs (3)		

6. Objective of goats keeping: Dairy ------- Cash income ------- Meat------ Others-------
7. Production system: Pastoral/ Mixed production
8. Feeding system. Communal grazing/other
9. Do goats move far from the locality? No (0) ------- Yes (1) ---------

   If yes, for grazing-------- watering ------- trade --------- cultural exchange------

10. What problem encountered due to movement?

    Lost -----, Disease contract-------, Preyed---------, Others----------

11. What are the major goat productivity and production constraints? Mention them in accordance with their priority and economic importance (giving 1 to the highest priority).

Nutrition-----, Diseases----, water shortage----, Management-----, others---

12. What are the major diseases in goats according to their decreasing order of importance? (giving 1 to the highest priority)

1----------, 2----------, 3 ----------, 4----------, 5----------.

13. What are the respiratory diseases of goats? 1----------2----------,

3 ----------, 4----------, 5----------.

No.	Type of Respiratory Diseases (Scientific name)					
		1.	2.	3.	4.	5.
1.	Vernacular name					
	Symptoms - Clinical - Post mortem					
2	Occurrence					
3	Frequency					
4	Seasonality					
5	Gravity					
6	Prognosis					

14. Do these diseases involve sheep? Which ones?

15. For how long do CCPP exist in the area: Months ---------- Years ---------

16. Is the disease present in your / neighboring flocks / currently? Yes (1) --- No (0) ---

17. Which age and sex groups were more affected?

Age group	Male % (0)	Female% (1)
< 1 yrs		
1 – 3 yrs		
> 3 yrs		

18. What are the possible reasons for the source of the disease outbreak?

Watering points ---, Grazing point----, New flock introduced---, Gift---, Purchased--

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

1. Destocking/ saling ----- 2. Vaccination ---- 3. Treatment -----

4. Isolation of affected ones ---- 5. Slaughter ---- 6. Others-------

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

Oxy tetracycline------ Is it one injection ----cost/ Rx

Penicillin--------- Is it two times ----------cost/Rx

Streptomycin----- Is it three times ----------cost/ Rx

21. Where is the source of treatment (service provider)?

Public service----- Private service-----Local trader ---- Traditional---- Others---

22. Is the vaccination you are using helpful? Yes (1)/ No (0)

23. Market values of goats and sheep

Age	Healthy				Sick			
	Goats		Sheep		Goats		Sheep	
	F	M	F	M	F	M	F	M
< 1year								
1-3 years								
> 3 years								

24: Was there any traditional treatment used? Yes/No

25. If yes, how much could it be effective?

26. Do you have the mechanism to follow up the source of infection? Yes / No

If yes, how could it be?

Annex 2. Participatory Disease Search Methods

Relative importance of livestock species using Matrix scoring method

Indicators	Cattle	Goats	Sheep	Camel
Total				

Pair wise comparison of goats' production constraints

Constraints	Recurrent Drought	Mana-gement	Diseases	Genetic	Feed	Watering	Scoring	Rank
Recurrent Drought	-----	-----	-----	----	----	---		
Management		-----	-----	-----	----	----		
Diseases			-----	----	----	----		
Genetic				-----	----	----		
Feed					------	----		
Watering						-----		

Pair wise comparison of diseases of goats

Diseases	Mange	Pasteurellosis	CCPP	PPR	Endo-parasites	Scoring	Rank
Mange	----	----	-----	----	----		
Pasteurellosis		----	----	----	-----		
CCPP			----	----			
PPR				----	----		
Endo-parasites					----		

Matrix scoring of disease signs for diseases of goats

Disease signs	Pasteurellosis	Endo-parasites	CCPP	PPR	Mange mites

Matrix scoring of veterinary service providers in CAHW intervention areas

Indicators	Public service	Traditional	CAHWs	Private	Black market

Annex 3: Record sheet format

Record Sheet N° _____ Date _____

Test Tube No.	District	PA s	SA	Flock size	Agr. ecology	Age (months)	Sex M(0) F(1)	CAHW	CFT	cELISA	Remark

Annex 4- Complement Fixation Test for CCPP

Principle of the test

Activation of classical complement system by antibody bound to antigen results in generation of membrane attack complexes capable of disrupting cell membranes. If the antibody is bound to erythrocyte surfaces, the erythrocyte membranes are disrupted and haemolysis occurs. It is possible to use this reaction to measure serum antibody levels and this test is known a complement fixation test (CFT).

The principle of the test is that if complement is fixed by antigen-antibody immune complex, it is unavailable to lyse the target cells in the indicator system. In the absence of antibody, the complement remains unfixed and is unavailable to lyse the target cells in the indicator system

In case of negative sera samples, the unbound complement react the indicator (SRBC and Ambocepter) result in lyses of SRBC by activation of complement.

Materials

- Water bath, incubator, and agitator, Distilled water
- U-shaped micro plates,Trough. Syringe
- Multichannel and single micropipettes, tips
- Male guinea pigs (complement), Male sheep
- VCM buffer for dilution, Alsever's solution
- Amboceptor, MccP antigen
- Arranged test sera, positive and negative control sera and sheets of plate lay out for record

Preparation of red blood cells (SRBC)

1. Draw blood from the jugular vein of male sheep freely flowing into a syringe containing Alsever's solution, take 75 ml sheep blood in 125 ml Alsever's solution and if more blood is required, increase the volume in the same proportion.

2. Add small amount of crystalline penicillin to avoid bacterial contaminants.
3. Store at +4°C. The blood can be used for about 2 weeks.
4. Sheep blood for CFT should be at least one day old.

Preparation of haemolytic system

1. The sheep blood is washed three times at a dilution of 1/10 by adding Veronal buffer (VCM) at pH 7.2 and centrifugation at 2,500 rpm for 5 minutes. Discard the supplement.
2. Re-suspend the SRBC in VCM, mix gently and centrifuge as above. Repeat this step three times
3. Take a tube of identical size and hold it next to the centrifuge tube and measure packed cell volume of SRBC. Add water to related tube until you have reached the meniscus of the SRBC. Add water to related tube until you have reached the meniscus of the SRBC (volume of SRBC in ml).
4. Dilute the SRBC in VTM to 2% (e.g. 2 ml packed cell volume of SRBC are made up to 98 ml VCM).
5. Reconstitute the freeze dried Ambocepter with 1 ml distilled water and keep at +4°C. The working dilution of the Amboceptor is 1:1000. (Always draw the Amboceptor sterile from the bottle). Mix the diluted Amboceptor properly with an equal amount of 2% SRBC V/V and mix with constant gentle agitation (sensitization) during incubation for 30 minutes at room temperature. The final concentration of the SRBC will be 1%.

Evaluation of Complement

1. Dispense 25µl VCM into each well.
2. Add 25µl of complement at a starting solution of 1:2 into the first wells of row A_1, B_1, C_1, D_1
3. Make two fold dilutions by transferring 25µl of the complement to the other wells until A_{12}, B_{12}, C_{12}, D_{12}.
4. Distribute 25 µ of the haemolytic system (Amboceptor + SRBC) per well and incubate at 37°C with constant agitation for 30 minutes.
5. Read and record the last dilution's column showing complete haemolysis and greater than or equal to 50% haemolysis of SRBC. Take the medium as one international unit. Evaluate complement daily.

The Test proper

The sera are tested at three dilutions 1/10, 1/20, 1/40 to final volume of 25µl. Before dilution, the test sera are decomplemented by heating in a water bath set at 58°C for 30 minutes. The sera are distributed to the wells and heating is done in a micro plate sealed by a micro plate sealer. For this purpose pre-plate is used after which the sera are transferred to the test plate at different dilutions (1/10, 1/20, 1/40).

The test is conducted on a 96 well U-shaped micro-titre plate and the procedure is as follows:

1. One-tenth dilution of serum to be tested is prepared by transferring 20µl of sera into 180 µl of VCM (Veronal buffer with Calcium and Magnesium) in a microplate.
2. 25 µl VCM is added into the wells of rows A, C, D, E, G and H and this serves as a diluent.
3. 25 µl of diluted sera is added to the wells of A, B, C, E, F and G. Good homogenization is made in the wells of rows C and G with a multichannel pipette and 25µl is transferred to the next rows D and G from which 25µl is discarded after good homogenization.
4. 25µl of diluted antigen is added to the wells of rows B, C, D, F, G and H.
5. 25 µl of diluted complement is added into the wells of rows A, B, C, D, E, F, G and H excluding complement and haemolytic system wells.
6. The plates are diluted at 37°C for 30 minutes covered by micro plateµµ sealer to prevent evaporation.
7. 25µl haemolytic system is added to all the wells, including control wells and incubated at 37°C for 30 minutes at constant shaking using incubator with a shaker.
8. The micro plates are centrifuged at 2,500 rpm for four minutes using sigma centrifuge or put in a refrigerator overnight and the results are read.

Volumes used:-

1. Serum	= 25 µl
2. Ag	= 25µl
3. Complement	= 25 µl
4. Haemolytic system	= 25 µl
Total amount	= 100 µl

We must have controls for all the plates. Make the controls the same volume as test sera by adding equal volume of dilution buffer, VCM.

1. **The serum control**:- each sample will have control.
   25μVCM, 25 μl serum, 25 μl complement and 25 μl haemolytic system
2. **The Ag control**
   25 μl VCM, 25 μl Ag, 25 μl complement and 25 μl haemolytic system
3. **The haemolytic system control:-**
   75 μl VCM and 25 μl haemolytic system
4. **The complement control**
   50 μl VCM, 25 μμl complement and 25 μl haemolytic system

Validation and Results

Validation

1. Positive serum control: inhibition of haemolysis should occur up to the expected dilution
2. Antigen control: complete haemolysis
3. Complement controls: complete haelolysis in the first two wells (pure and 1/2), partial haemolysis in the third well (1/4), and traces of haemolysis in the fourth well (1/8).
4. Haemolytic system control – no haemolysis

Results

Anticomplementary control: there should be a complete haemolysis in this well. If not, the results can not be read.

The degree of haemolysis is observed for each serum dilution. The results vary from 0 to ++++, from no inhibition of haemolysis to a complete haemolysis.

Interpretation

In case of an acute outbreak of CCPP, large proportion of sera would completely inhibit the haemolysis at dilutions above 1/40. In CCPP free areas, some sera may exhibit false positive results. Usually the percentage of false positives never exceeds 2% and these sera are seldom positive at high titers.

A complete inhibition of haemolysis at a dilution of 1/10 should be considered as positive result. This type of reaction may occur at the beginning of the outbreak or when there are carrier animals. Full haemolysis indicates negative result. Sedimentation of SRBC indicates positive reactions. Partial haemolysis at dilution 1/10 should be considered as doubtful.

CFT layout on a micro plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	AC	Ac	Ac	Ac
B	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
C	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20
D	1/40	1/40	1/40	1/40	1/40	1/40	1/40	1/40	1/40	1/40	1/40	1/40
E	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	S	S	S	S
F	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	Ag	Ag	Ag	Ag
G	1/20	1/20	1/20	1/20	1/20	1/20	1/20	1/20	C	C	C	C
H	1/40	1/40	1/40	1/40	1/40	1/40	1/40	1/40	HS	HS	HS	HS

S – serum control

Ag – Antigen control

C – Complement control

HS – Haemolytic system control

Ac – Anticomplementary (no antigen added)

Annex 5- Competitive ELISA (cELISA) Test

Principle of the test

The wells of the polystyrene micro plate are coated with MccP lysate. The serum samples to be tested are diluted and incubated with specific monoclonal antibody in a pre-plate. This mixture is transferred into the MccP coated micro plate. Any antibody specific to MccP in the serum forms MccP/goat antibody immune complex, which is effectively masks the MccP sites. In this case, the Mab cannot bind to the corresponding epitope.

After washing, anti-mouse IgG antibody coupled to peroxidase is incubated in the wells. In the presence of specific MccP antibodies in the serum to analyze, the monoclonal antibody is not fixed in the plate and the conjugate can not bind in the wells. On the contrary, the conjugate can bind to the monoclonal antibody.

After washing, the enzyme substrate is added to the conjugate forming a blue compound becoming yellow after blocking. The intensity of the colour is an inverse measure of the proportion of anti-MccP antibodies in the serum sample to test.

Test procedure

The manufacturer CIRAD-EMVT supplied operating procedure with the kit.
The procedures were as follows:

1. 100 µl of dilution buffer 24 (Phosphate buffered saline with Tween 20) was dispensed in all wells of the micro plate used as a pre-plate.
2. 11µl of the three controls: strong positive control (CP^{++}) in B_1, B_2, C_1, C_2, weak positive control (CP^{+}), in D_1, D_2, E_1, E_2 and negative control (Cn) in H_1, H_2 were dispensed into respective wells.
3. 11µl dilution of buffer 24 was dispensed in to wells A_1 and A_2.
4. 11 µl of test samples were dispensed in to wells A_3 to A_{12}.
5. Monoclonal antibody was reconstituted in 1 ml of distilled water. This reconstituted antibody was diluted 1/20 in a solution buffer 24.
6. Additional 110 µl dilution buffer was dispensed into wells A_1 and A_2.

7. 110 µl diluted Mab was dispensed in to wells except A1 and A2 which were later called conjugate control (Cc).
8. 100 µl of the test sample and Mab mixture was transferred from the pre-plate to the coated test provided by the manufacturer using a multi channel pipette.
9. The plates were incubated for 1 hour at 37°C under gentle agitation.
10. Wash solution was prepared by diluting a vial of wash concentrate in 1900 ml of distilled water as required. Washing was undertaken two times manually by empting and refilling the micro wells.
11. The conjugate was diluted 1/100 in dilution buffer 24 and 100 µl was dispensed to all wells.
12. The plates were covered with aluminium foil, incubated for 30 minutes at 37°C under gentle agitation using incubator with agitator.
13. The plates were emptied, washed 3 times and properly tapped on clean towel.
14. 100 µl substrate was added to the plates and the plates were covered with aluminium foil and incubated for 30 minutes at 37°C.
15. 100 µl of stop solution was added to all wells and the plates were gently shaken until the colored solution was homogenized.
16. The results were read at optical density (OD) of 450 nm after the plates were dried thoroughly with clean towel and photometer was blanked first on air.

Reading

- Read the optical densities at 450 nm (OD.450). The photometer must first be blanked on air. Calculate the mean value of the Cm (0% inhibition) and Cc controls (100% inhibition).

$$PI = 100 \times \frac{(ODCm - ODTs)}{ODCm - ODCc}$$

where, PI – Percent Inhibition
ODCm – Optical Density of Monoclonal control
ODTs – Optical Density of Test serum
ODCc – Optical Density of Conjugate control

Validation criteria

The reaction is considered valid when the following criteria are obtained:

- ✓ The OD Cm must be between 0.5 and 2.0 (preferably near1.0)
- ✓ The OD Cc must be inferior to 0.3
- ✓ The PI of Cn must be inferior to 25%
- ✓ The PI of CP^{+} must be between 25 and 55%
- ✓ The PI of CP^{++} must be between 55 and 85%

Interpretation

- ❖ Sera with an inhibition percentage lower than 20% are considered negative.
- ❖ Sera with an inhibition percentage between 20 – 25% are considered doubtful.
- ❖ Sera with an inhibition percentage greater than or equal to 25% are considered to be positive.

cELISA Test Layout on a Micro plate

	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									
C	CP^{++}	CP^{++}	21									
D	CP^{+}	CP^{+}	31									
E	CP^{+}	CP^{+}	41									
F	Cm	Cm	51									
G	Cm	Cm	61									
H	Cn	Cn	71									80*

Cc: Conjugate control (without serum, without Mab = 100% inhibition)

Cm: Monoclonal control (without serum = 0% inhibition)

CP^{++}: Strong positive serum control

CP+: Weak positive serum control

Cn: Negative serum control

* : 1, 2, …, 80 sera samples to be tested.

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Work experience

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SIGNED DECLARATION SHEET

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

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

Dr. Berhe G/Egziabeher _____

Dr. Ademe Zerihun _____

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