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SERO-PREVALENCE INVESTIGATION AND ASSOCIATED RISK FACTORS OF CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN SELECTED DISTRICTS OF CENTRAL ETHIOPIA

MSc Thesis

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June 2017

Bishoftu, Ethiopia

SERO-PREVALENCE INVESTIGATION AND ASSOCIATED RISK FACTORS OF CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN SELECTED DISTRICTS OF CENTRAL ETHIOPIA

A Thesis submitted to College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Epidemiology

By

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DEDICATION

To my beloved late mom Nigat Habte Tegene, for her endless love

STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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LIST OF ABBREVATIONS

AAU	Addis Ababa University
AU-IABAR	African Union – Inter-African Bureau for Animal Resources
CCPP	Contagious caprine pleuropneumonia
cELISA	Competitive enzyme-linked immunosorbent assay
CFT	Compliment Fixation Test
CIRAD	International Cooperation Centre of Agricultural Research for Development
CVM	College of Veterinary Medicine and Agriculture
FAO	Food and Agriculture Organization of the United Nations
GIT	Growth inhibition test
LAT	Latex agglutination test
Mccp	*Mycoplasma capricolum* subsp. *capripneumoniae*
MoLF	Ministry of Livestock and Fisheries
NVI	National Veterinary Institute
OIE	World organization for animal health
PA	Peasant association
PCR	primary chain reaction
SAT	slide agglutination test
SNNP	South nation and nationalities of people
UNDP	United Nations Development Programme

ABSTRACT

Contagious caprine pleuropneumonia (CCPP) is a series respiratory disease of domestic goats and some wild ruminants caused by *M. capricolum* subsp. *Capripneumoniae* (Mccp). A cross-sectional study with a multistage sampling method was conducted to determine the sero-prevalence and associated risk factors of CCPP in the selected districts of central Ethiopia namely, Fentale and around Alage College from November 2016 to May 2017. Sera samples were collected and subjected to monoclonal antibody based competitive enzyme linked immunosorbent assay for the specific measurement of antibodies to Mccp. A semi-structured questionnaire was administered for 64 selected farmers to assess their perceptions and a 10 years (2007-2016) retrospective outbreaks data was analyzed to assess the national status of the disease. Furthermore, pathological lung tissue samples were collected from diseased goats showing respiratory sign and Mccp antigen was detected using species-specific primer of polymerase chain reaction (PCR). The PCR test showed an amplification of the Mccp antigen as an approximate size of 316bp. A total of 400 small ruminants (370 goat and 30 sheep) were sampled, and 14.9% and 3.3% sero-positivity was observed, respectively. At district level, the sero-prevalence of the disease in goats revealed 22.9% in Fentale and 6.2% around Alage area with statistically significant difference ($P< 0.05$). Among flock category >70 flock group significantly higher sero-prevalence were recorded in contrast to flock group <30 ($\chi 2= 9.73$; $P= 0.008$). Multivariate logistic regression analysis on the risk factors showed that production system was a significant factors for sero-positivity ($P= 0.002$; OR= 5.8; CI=1.9-17.1). From the questionnaire survey CCPP is known by the local name '*Sombesa'* in Fentale and they described the disease; however, around Alage College livestock keepers had no experience of the clinical disease. A total of 175 outbreaks were reported by 66 districts in the 10 year period. The lowland pastoral area was taken the large number of outbreaks report specifically Borana and Gamgofa zone. In conclusion, the findings indicated that CCPP is the major goat disease especially in the pastoral area which needs feasible measures to be put in place towards the control of the disease effectively.

Key words: *Alage ATVET College, CCPP, cELISA, Fentale, Mccp, sero-prevalnce*

1. INTRODUCTION

Small ruminants play an important role in the livelihoods and food security of poor families as a source of milk, meat and wool. The great Indian leader and freedom fighter M. K. Gandhi designated goats as "poor man's cow" (Chakraborty *et al.,* 2014) that emphasizing the importance of small ruminants in poor countries. They can survive in areas where a cow cannot and therefore, replace the cow in importance (DaMassa *et al.,* 1992) as it is the means of livelihood for many marginal farmers that contribute significantly to the nutrition and cash income of small farmers in Africa and Asia (Kumar *et al.,* 2014). Sheep and goats also play a critical role in the livelihoods of the traders, for instance in the horn of Africa the trade of small ruminants is geographically dispersed in Kenya, Somalia, Djibouti and Ethiopia. The trade of live animals extends into the Middle East and Arabian Peninsula, between 3 to 4 million live sheep and goats being exported every year (OIE and FAO, 2015).

Goats are among the major economically important livestock in Ethiopia. There are about 28.89 million sheep and 29.7 million goats in the country (CSA, 2016). Sheep and goats are generally owned by the poor sector of the community. The short generation interval coupled with high frequency of multiple births allows for rapid increase in animal numbers. However, it is facing diversified problem including diseases, feed scarcity, poor management, inadequate marketing infrastructure and Veterinary service (Hirpa and Abebe, 2008). Of these factors, diseases are widespread and have a significant impact on the performance of goat in Ethiopia (Mekuria *et al.,* 2008).

Contagious caprine pleuropneumonia (CCPP) is a devastating disease of goats first described in 1873 (Thomas, 1873 as cited in Lorenzon *et al.,* 2002). After a century, the causal agent was isolated in 1976 in Kenya from the lungs of goats (MacOwan and Minette, 1976). It is a listed disease of the World Organization for Animal Health (OIE) that causes a huge economic loss for goat producers (Manso-Silván *et al.,* 2011). *Mycpolasma capricolum* subsp. *capripneumoniae* (Mccp) is the causative agent of CCPP. Previously, this agent was known as *Mycoplasma sp. type F38* (Leach *et al*., 1993), a disease of domestic goats (Martin, 1983) and affect some wild

ruminants (Arif *et al*., 2007; El-Deeb *et al.*, 2017) is a highly contagious and serious respiratory disease. It is characterized by coughing with nasal discharge, severe respiratory distress, difficult to walk, high morbidity (80-100%) and the disease spreads inevitably to the whole flock. In the absence of treatment, mortality is also very high and may reach 60-80% (Rurangirwa and McGuire, 1996). The lesions at necropsy are mainly a fibrinous pleuropneumonia with unilateral hepatization, and accumulation of straw-colored pleural fluid (Thiaucourt *et al*., 1996; Arif *et al.,* 2007).

CCPP is a major threat to goat farming in parts of Africa and Asia (Wesonga *et al.,* 2004*)* that over 330 million poor people keep the livestock (OIE and FAO, 2015). Still the exact distribution of CCPP is not known and represents a significant threat to many disease-free countries (Manso-Silván *et al.,* 2011). There have been very few declarations of CCPP outbreaks to the OIE in the previous years due to lack of awareness of this disease and possible confusion with other diseases, such as peste des petits ruminants (PPR) and Pasteurellosis (Peyraud *et al.,* 2014). Mccp has been isolated in 20 countries and clinically described in nearly 40 countries globally (Nicholas and Churchward, 2012).

In Ethiopia, the presence of CCPP has been suspected for a long period especially in the remote region of Sudan and Kenya border. Since 1982 the outbreak were suspected later confirmed in 1990 by isolation and identification of the causative agent Mccp (Thiaucourt *et al*. 1992). Since then the disease has been known to be endemic in different regions of the country. A meta-analysis of CCPP conducted by Asmare *et al.* (2016) using twelve published and one unpublished articles indicated that the prevalence of the disease was 25.7% at the national level. In different years outbreaks of CCPP have been recorded from various regions (MOA-APHRD, 2010; AU-IBAR, 2013). Clinical diseases have been confirmed by some investigators especially in the pastoral area (Yigezu *et al*. 2004; Shiferaw *et al.,* 2006; Ayelet *et al.,* 2007a).

To diagnose CCPP a number of serological tests are available including CFT, LAT, GIT and cELISA. PCR method has radically improved the detection and identification of Mccp which do not grow easily in-vitro (Lorenzon *et al.,* 2002). It is the technique

of choice for the diagnosis of CCPP. However, isolation of Mccp remains the confirmatory diagnosis (Thiaucourt, 2012).

Despite CCPP outbreak report in Ethiopia almost certainly represents an underestimate as this disease is having a major socio-economic impact (Nicholas *et al*., 2008), even several fragmented work were done in the country (Thiaucourt *et al*. 1992; Shiferaw *et al*., 2006; Lakew *et al.*, 2014; Peyraud *et al*., 2014). Therefore, to clearly reveal the epidemiology of the disease additional epidemiological studies are needed to reduce the impact of the disease on the national economy and to support the control measures. Though the sero-prevalence works were undertaken in many districts of pastoral area of the Ethiopian lowland but there is limited information in the central Ethiopia. Due to paucity information on prevalence of CCPP, the present investigation was undertaken in Fentale and around Alage ATVET College of central Ethiopia.

General Objectives

The general objective of this research was to determine the sero-prevalence of contagious caprine pleuropneumonia and its associated risk factors in Fentale and around Alage ATVET College of central Ethiopia.

Specific Objectives

- To determine the sero-prevalence of contagious caprine pleuropneumonia
- To detect *M. capricolum* subsp. *capripneumoniae* antigen using PCR
- To assess the associated risk factors of contagious caprine pleuropneumonia.
- To assess the national status of the disease using a retrospective data.

2. LITERATURE REVIEW

2.1. The Disease Contagious Caprine Pleuropneumonia

CCPP is a highly fatal, contagious caprine disease firstly reported in Algeria in 1873 under the local name of 'bou frida' because, in the majority of diseased goats, only one lung was affected (McMartin *et al.* 1980 as cited in Nicholas *et al.,* 2008). Synonyms include: pleuropneumonie contagieuse caprine (France), bou-frida (Algeria) and abu-nini (Sudan) (OIE, 2014). The contagious nature of the disease was known after an outbreak in South Africa in 1881, which was traced back to the importation of infected goats from Turkey (Fischer *et al.,* 2012).

The causal agent was first isolated in 1976 from the lungs of goats with pleuropneumonia in Kenya and demonstrated to cause CCPP (MacOwan and Minette, 1976). Since 1993 the former name of the causative agent *Mycoplasma strain F38* changes in to the species name *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp). So far Mccp is the only mycoplasma that fulfils Kochs postulates for causing CCPP and F38-group mycoplasmas has been reclassified to Mccp (Leach *et al.,* 1993). It causing a severe disease of goats including in the list of notifiable disease of the World Organization for Animal Health (OIE) (Shahzad *et al*., 2016) due to the serious economic effects on livelihoods and has specific requirements for international trade (AU-IBR, 2015)**.**

2.2. Etiology

The *Mollicutes (mycoplasmas)* are members of the order *Mycoplasmatales,* class *Mollicutes* which is the smallest prokaryotic cells capable of self-replication. *Mollicutes* is the correct term to use when collectively referring to members in this order; however, the trivial name *mycoplasma* is also used for this purpose (Walker, 1999; Quinn *et al.,* 2011). Five distinct groups of Mollicutes have been identified by phylogenetic analysis of the 16S rRNA sequences, one of which the *Spiroplasma* group comprises four clusters represented by *Mycoplasma mycoides, Spiroplasma apis, Spiroplasma citri, and Spiroplasma ixodetis* (Heldtander *et al.,* 2001). Mccp

belongs to the classical Mycoplasma mycoides cluster, which contains six important ruminant mycoplasma that share many genotypic and phenotypic traits (Manso-Silva´n, *et al*., 2007) *M. capricolum* subsp. *capricolum*, *M. mycoides* subsp. *capri*, *M. mycoides* subsp. *mycoides type LC*, *M. mycoides* subsp. *mycoides type SC*, and *M*. sp. *bovine serogroup 7 of Leach* (Pettersson *et al.*, 1998; Heldtander *et al.*, 2001).

Mccp has a pleomorphic nonspiral cellular morphology, non motile, lacks a cell wall and bounded only by a single trilaminar cell membrane can be filtered through 450-nm-pore size membrane filters. Fastidious cultural requirements; it grow slowly requires 3-7 days incubation in a special media. Optimal growth occurs at 37°C an increased of an atmosphere of CO_2 with sterol requirement; produces small colonies on suitable solid media that difficult to visualize with unaided eye; the colonies have characteristic of fried-egg morphology (Leach *et al*., 1993; Walker, 1999).

2.3. Epidemiology

2.3.1. Host range

For a long time, CCPP has been reported to affect only the domestic goat (Martin, 1983). Now, it is a threat of wild ungulates both in their natural habitat and in captivity that raised concerns for zoos and for the conservation of some endangered species exposed to goats (Arif *et al*., 2007; Chaber *et al*., 2014; El-Deeb *et al*., 2017). Clinical disease and sero-positivity have been reported in sheep in contact with affected goats, but the role of sheep as reservoirs of infection is unclear (Thiaucourt, 2012).

Table 1**:** Mycoplasmas including Mccp, isolated from small ruminants

Mycoplasma	**Hosts**	**Primary site of isolation**	**Disease**	**Pathogenicity**
M. capricolum subsp. *capripneumoniae*	Goat, Sheep	Lungs	CCPP	High
M. mycoides subsp. *mycoides type LC*	Goat, Sheep, Cattle	Resp. tract, Udder, joints	Arthritis, mastitis, pleuropneumonia, conjunctivitis	Moderate
M. mycoides subsp. *Capri*	Goat, Sheep	Resp. tract, Joints	Arthritis, Pleuropneumonia, conjunctivitis	Moderate
M. capricolum subsp. *capricolum*	Goat, Sheep	Joints, udder, resp. tract	mastitis, arthritis, pleuropneumonia	High
M. ovipneumoniae	Sheep, Goat	Resp. tract	Pneumonia	Low
M. conjunctivae	Sheep, goat	Eyes	Keratoconjunctivitis	Moderate
M. agalactiae	Sheep, Goat	Udder , joints, eyes	pneumonia, mastitis, arthritis, keratoconjunctivitis	High
M. putrefaciens	Goat, Sheep	Udder, Joints	arthritis, mastitis	Moderate

(**Source:** Nicholas *et al*., 2008)

The experiment conducted by Litamoi *et al.* (1990) on calves, sheep and goats taken from CCPP outbreak experienced farm clinical sign of the respiratory disease were seen in 70% of the goats but in none of sheep and calves. All goats were developed thoracic lesion typical of CCPP. Mccp is isolated from both goats and sheep; the finding suggested that sheep may be the possible carrier of the causative agent. Some reports describing the isolation of Mccp from sheep with respiratory disease in Eritrea (Houshaymi *et al*., 2002) and from sick sheep mixed with goats in Uganda suffering from the disease (Bölske *et al.,* 1995). In East Turkey the causative agent Mccp has

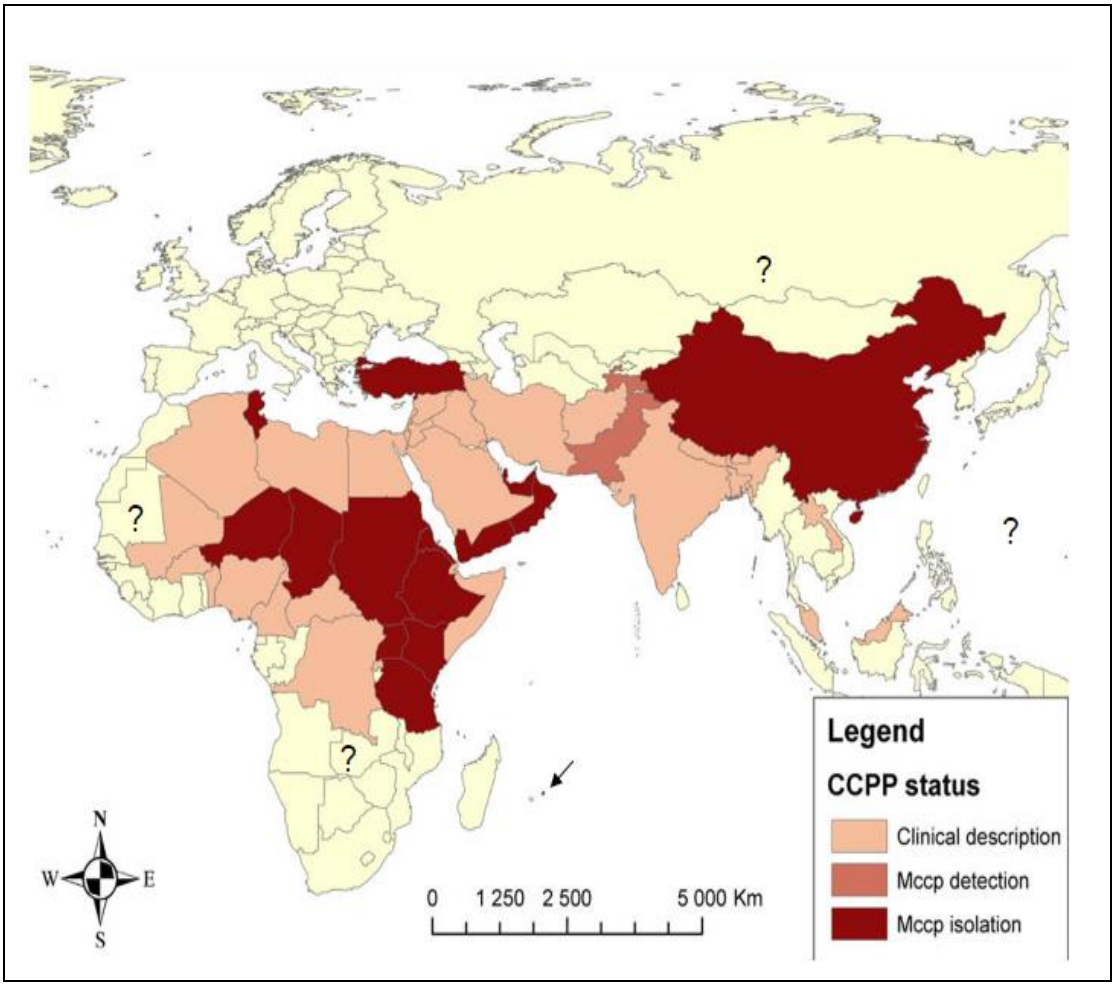
been isolated from the lung and nasal swab of pneumonic sheep (Çetinkaya *et al*., 2009).

In Ethiopia Afar region during the investigation of the acute respiratory disease outbreak of sheep and goat in 2002, the causative agent of CCPP has been isolated from goat but not sheep. The investigators suspected the organism might have been involved in an outbreak of sheep with their clinical appearance (Shiferaw *et al.,* 2006). However, outbreak investigation conducted by Yigezu *et al.* (2004) in Arbaminch, Ethiopian sheep did not show any clinical sign of CCPP but from apparently healthy sheep Mccp was isolated that may be proves the role of sheep as reservoir of infection. CCPP sero-positive sheep also reported from Tanzania (Mbyuzi *et al.,* 2014), India (Suryawanshi *et al.,* 2015) and Ethiopia (Ayelet *et al.,* 2007a, Hadush *et al.,* 2009).

CCPP is a threat of wild ruminants including wild goats (*Capra aegagrus*), Nubian Ibex (*Capra ibex nubiana*), Laristan mouflon (*Ovis orientalise laristanica*) and Gerenuk (*Litocranius walleri*) with significant morbidity and mortality (Arif *et al*., 2007). CCPP is also a substantial threat for the endangered Tibetan antelope (*Pantholops hodgsonii*) in China (Yu *et al*., 2013), Arabian oryx (Oryx leucoryx) in the United Arab Emirates (Chaber *et al*., 2014), captive Rhim, Dumani Gazelles and other deer species (Nicholas *et al*., 2008). In Kenya there was a report of Mccp antibody detected in Buffalo (Syncerus caffer), Impala (Aepyceros melampus) and camel (Camelus dromedaries) (paling *et al*., 1978).

2.3.2. Geographical distribution

Mccp has been isolated in 20 countries, mainly because of its fastidiousness in culture. However, clinical descriptions have been published in nearly 40 countries in Africa and Asia. The disease is present in the Arabian Peninsula, North, Central and East Africa and Asia, but its boundaries are still uncertain (Manso-Silván *et al.,* 2011, Nicholas and Churchward, 2012). When considered the contagious nature of the disease and the movements of pastoral goat flocks, it may be suggested a much wider distribution of CCPP from the known geographical area (Manso-Silván *et al.,* 2011; Quinn *et al.,* 2011).



(**Source**: Manso-Silván *et al*., 2011)

Figure 1: Probable distribution of CCPP: the countries in which the disease has been described, those in which the etiologic agent has been detected using molecular test and those in which it has been isolated are indicated. The arrow indicates the presence of the disease in Mauritius where Mccp isolated in 2009.

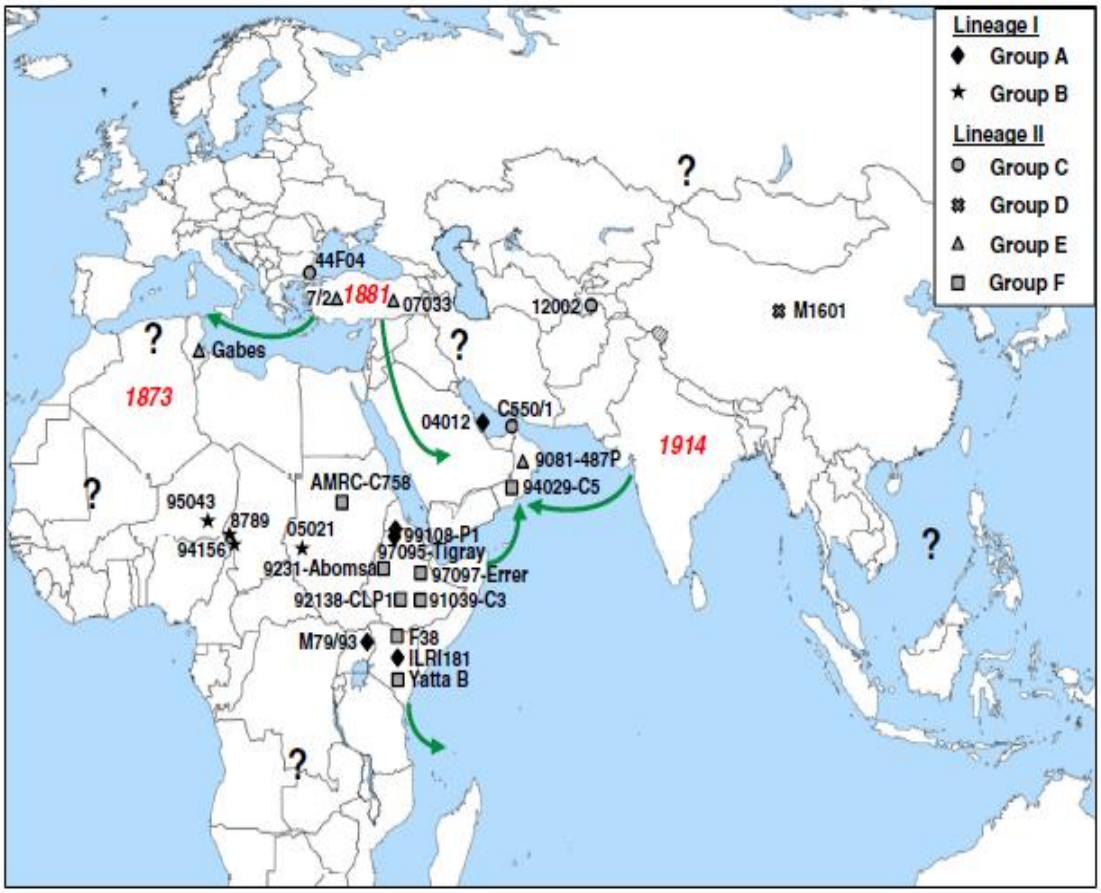
After the first isolation of the causative agent of CCPP in Kenya (MacOwan and Minette, 1976), Mccp has been isolated in the Sudan, Tunisia, Chad, Niger, Tanzania, the United Arab Emirates (OIE, 2008; AU-IBAR, 2013), Oman (Jones and Wood, 1988), Eritrea (Houshaymi *et al.*, 2002), Uganda (Bolske *et al.*, 1995), Ethiopia (Thiaucourt *et al.*, 1992) and Mauritius (Srivastava *et al.*, 2010). Furthermore, the presence of CCPP has been confirmed in Asia: Pakistan (Peyraud *et al.*, 2014), China (Chu *et al*., 2011), Tajikistan (Amirbekov *et al.*, 2010) and Turkey (Ozdemir *et al*., 2005). Although some reports suggest its presence in other countries in the region

including, Iraq and Kuwait based on clinical signs, postmortem findings and serology (El-Deeb *et al*., 2017). There have been no reports of the isolation of Mccp in the American continent (Nicholas *et al*., 2008).

2.3.3. Molecular Epidemiology of CCPP

A subsequent study on the molecular epidemiology of CCPP were conducted using different methods 16S rRNA gene locus sequencing (Pettersson *et al.,* 1998), based on H2 locus sequencing CCPP strain divided into four genotyping group were correlated to the geographical origin (Lorenzon *et al.*, 2002), Multi-Locus Sequence Analysis (MLSA) a method based on the analysis of several genetic markers and CCPP strain group in to five genotypic group respect to geographical origin (Manso-Silván *et al.,* 2011). Recently Dupuy *et al.* (2015) using a discriminatory genotyping system based on a large scale genomic approach or multi-gene approach by using 25 Mccp strains has been studied the evolution and molecular epidemiology to know the global diversity of Mccp (Figure 2). Mccp strains classified in to six genotyping groups, named group A to F which showed correlation with geographic origin. The six genotyping groups they formed two major lineages. Lineage I include group A and group B, Lineage II comprised groups (C, D, E, and F) and was divided into two sub-lineages. The first sub-lineage comprised groups C and D representing Asian strains, while group E (representing strains from the Mediterranean Basin) and group F (comprising mainly strains from East Africa).

Group A including homogeneous strains from East Africa and Arabian Peninsula and Qatar, Group B constitute strains from Central Africa, also showing little diversity. Group C including variable strains from the Mediterranean Basin, the Arabian Peninsula and Central Asia, Group D only include Chinese strain, Group E showed little variability originating from the Mediterranean Basin and a strain from the Arabian Peninsula and Group F, which is the most variable group, include strains from East Africa and Arabian Peninsula (Manso-Silván *et al.,* 2011; Dupuy *et al.*, 2015).



(**Source**: Dupuy *et al.*, 20415)

Figure 2: The worldwide distribution of CCPP lineages: Integration of genomic, geographic, historical and animal movement data to investigate the epidemiology of CCPP. The original location of each Mccp strain is indicated by a symbol, according to the corresponding genotyping group. The green arrows show Major CCPP diffusion routes. The dates of the first clinical description of the disease are indicated in red Italics. Question marks are place in area from where no recent data is available.

Lorenzon *et al. (*2002) conclude that Linage I strain originated from the horn of Africa (Kenya, Ethiopia, Eritrea, Sudan and Uganda). The presence of two extremely divergent groups (A, F) in East Africa suggests that Mccp emerged through trade and other animal movements between neighboring countries (Dupuy *et al.*, 2015).Various lineages found in the Arabian Peninsula, due to the fact that the frequent importing of small ruminants of various origins on the occasion of Muslim feasts. Strain of Turkish origin belongs to the same linage as the strain from North Africa might be accounted by Mediterranean trading routes. The excluding Lineage B result suggests that CCPP

has most probably been present in central Africa for a long period (Manso-Silván *et al.*, 2011). The identification of group C in Turkey reveals the great complexity of animal movements in this region (Dupuy *et al.*, 2015).

2.3.4. Prevalence of CCPP in Ethiopia

In Ethiopia, the circulation of CCPP has been suspected for a long period, especially in the remote regions those are bordering to the known infected countries with Mccp like Kenya and Sudan. In 1990 outbreak of CCPP were happen in Ogaden in eastern Ethiopia and in east Shoa province the causative agent were recovered in pure culture but Mccp isolated and characterized in the same year from the pleura fluid that collect during the outbreaks of Gojam in 1982 (Thiaucourt *et al*. 1992). Since then the disease has been known to be endemic in different regions of the country. Clinical diseases have been identified by various investigators especially in the pastoral area of the country Borana (Ayelet *et al.*, 2007a) and Afar (Shiferaw *et al.*, 2006). A meta-analysis of CCPP conducted by Asmare *et al.* (2016) using more than twelve published articles indicated that the sero-prevalence of the disease is 25.7%. Different scholars reported sero-prevalence of CCPP from different districts with various proportions (Mekuria *et al*., 2008; Hadush *et al*., 2009; Regassa et *al.*, 2010; Bekele, *et al.*, 2011; Lakew *et al.*, 2014; Peyraud *et al*., 2014). In different years outbreaks of CCPP have been reported from different regions for example from 1999 to 2003, 62 outbreaks (Eshetu *et al.*, 2007); 2007-2010, 94 suspected outbreaks but only one outbreak were confirmed (MOA-APHRD, 2010), and in 2011, 12 outbreaks have been reported (AU-IBAR, 2013).

Table 2: CCPP prevalence studies in different countries and districts of Ethiopia

Location	Sample size Lab Test	Prevalence	Reference
Africa			
Tanzania, Musoma	320-cELISA	64.4%-goat	Nyanja *et al., 2013*
NorthernTanzania	337-cELISA	9.2%-goat	Swai *et al.*, 2013
Tanzania	447-cELISA	52.1%-goat	Mbyuzi *et al.*, 2014
	30-cELISA	36.7%-sheep	
Mauritius	720-cELISA	8%-goat	Peyraud *et al*., 2014
Kenya,Turkana, Baringo and Kajiado	432-cELISA	47.2%-goat	Kipronoh *et al*., 2016
Uganda, Agago and Otuke	404-IELISA	20.8%-goat	Atim *et al.*, 2016
Asia			
India, Maharashtra	84-SAT	20.2%-goat	Suryawanshi *et al.*, 2015
	81-SAT	16%-sheep	
India, Madhya Pradesh	1427-SAT	10.6%-goat	Gupta *et al.*, 2016
Afghanistan	359-cELISA	0%-goat	Peyraud *et al*., 2014
Tajikistan			
Shuro-obod, Darv	197-cELISA	10%-goat	Peyraud *et al*., 2014
Ishkoshim, Murghob	199-cELISA	0%-goat	
Pakistan, Gilgit,	150-cELISA	2.7%-goat	Peyraud *et al*., 2014
Diamer	260-cELISA	44.2%-goat	
Pakistan, Khyber Pakhtunkhwa	384-cELISA	3.9%-goat	Wazir *et al., 2016*
Pakistan, Punjab	364-cELISA	8.5%-goat	Shahzad *et al.*, 2016
Eastern Saudi Arabia	23-PCR (Plural fluid)	12.1%-goat	El-Deeb *et al.*, 2017
	13-PCR (Lung tissue)	11.8%-goat	
East Turkey	32-PCR	37.5%-goat	Çetinkaya *et al.*, 2009
	13-PCR	15.4%-sheep	

Location	Sample size Lab Test	Prevalence and species	Reference
Ethiopia			
Export oriented abattoir, Bishoftu, Ethiopia	300-CFT	31%-goat	Eshetu *et al.,* 2007
Borana zone Yabello,	169-CFT	19.1%-goat	Ayelet *et al.,* 2007a
Dirre, Moyale and Liban	14-CFT	7.4%-sheep	
Southern Ethiopia Hammer and Benna-Tsemay	679-CFT	15.5%-goat	Mekuria *et al*., 2008
ELFORA export abattoir	704-cELISA	11.8% -goat	Gizaw *et al*., 2009
Tigray and Afar region	863-CFT	32.7% -goat 18.3% -sheep	Hadush *et al*., 2009
Afar Region Afambo, Assaita, Dubti, Mille, Gewane, Amibara, Dewe and Telalak	329-CFT	22.5%-goat	Regassa *et al.,* 2010
Southern Ethiopia Hammer, Benna-Tsemay, Arbaminch and Boreda	913-CFT	18.6%-goat	Mekuria and Asmare, 2010
Borana and Guji zone Liben, Teltale and Moyale	900-CFT	13.2%-goat	Bekele, *et al.,* 2011
Jijiga Zone Jijiga, Kebribayeh,Tuli Guled	334-CFT	32.6%-goat	Sherif *et al.,* 2012
Dire Dawa	244-CFT	4.92%-goat	Yousuf *et al.*, 2012
Borena pastoral area Arero, Dhas, Yabello districts	510-cELISA	31.6%-goat	Lakew *et al.,* 2014
Afar region Dubti and Hadar	1000-cELISA	14.6%-goat	Peyraud *et al*., 2014

2.3.5. Disease transmission

The disease is readily transmitted through aerosols (Quinn *et al.,* 2011). The transmission is direct, through droplets released during coughing. Very short periods of contact are sufficient to transmit the disease, but intimate contact is needed.

Introduction of infected or carrier goats into uninfected population accounts for dissemination of infection (Walker, 1999). For instance, the introduction of CCPP into the Cape Province in 1881 suggested that due to such a carrier in a group of animals from Turkey. These animals had been on the journey for more than seven weeks that is longer than the known incubation period. The stress of transportation might have reactivated a latent *Mycoplasma sp. F38* infection (Thiaucourt and Bölske, 1996). The introduction of CCPP into Mauritius was also related with the regular importing of goats from Kenya. The first recognized outbreaks were reported in 2009 after importing more than 2000 goats from Kenya (Srivastava *et al.*, 2010).

Although mycoplasmas are very fragile wall-less bacteria, but in case of a fatal case of CCPP infection in an Arabian oryx, the transmission suspected from a distance neighboring sand gazelles that the sequence type was identical to the Mccp strain previously identified in a sand gazelle from a nearby enclosure. The case shows that 'at a distance' transmission was possible even in the Emirate environment 28.8^{o}C - 42.8^{o}C (Chaber *et al.*, 2014).

2.3.6. Potential risk factors for CCPP transmission

The occurrence of CCPP is underpinned by risk factors related to environment, production system and immune status of the host population. Livestock mobility and presence of naïve populations in an infected area are major predisposing factors. The presence of chronically infected animals in close proximity with naive animals is also an important factor (AU-IBAR, 2015; Chaber *et al.*, 2014). In regions where CCPP already occurs, the severity of the disease may depend on the following factors: the proportion of immune animals, as an animal which has survived a previous infection is thought to be protected, the presence of co-existing viral infections like PPR which may favor the development of CCPP, poor climatic conditions, such as a large temperature difference between day and night or an abrupt change of climate, especially during the period between the dry and rainy seasons and stress due to movement over long distances are main factors (Thiaucourt and Bölske, 1996; Nicholas *et al.*, 2008).

Animal movement due to internal insecurity, informal trade, transhumance, watering, grazing and marketing accompanied by porous borders are key point for CCPP spreading (Nicholas and Churchward, 2012; AU-IBAR, 2015). In addition to these, infected goats remain carriers even when treated with the available antibiotics and are a potential risk to the healthy goats in the flock and those they come in contact at feeding areas, watering points and markets (Kipronoh *et al.*, 2016).

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2.4. Pathogenesis

As Tigga *et al*. (2014) reviewed that the exact mechanism of pathogenesis is still unclear. The mycoplasmas are extracellular pathogens that adhere to epithelial cell surfaces. Thus, adherence proteins are one of the major virulence factors. The adherence protein has been identified as a 168kD protein (P1). The P1 Adhesin localizes at tips of the bacterial cells and binds to sialic acid residues on host epithelial cells. Colonization of the respiratory tract results in the cessation of ciliary movement. The normal clearance mechanisms of the respiratory tract do not function, resulting in contamination of the respiratory tract and the development of a dry cough. The intimate association of the mycoplasma and the host cells provides an environment in which toxic metabolic products accumulate and damage host tissues. Both hydrogen peroxide and superoxide which are products of mycoplasma metabolism have been implicated in pathogenesis. Furthermore, the mycoplasmas have been shown to inhibit host cell catalase thereby increasing the peroxide concentrations.

2.5. Clinical Sign and Gross Pathological Lesions

All goats can be affected at any age or sex; with high morbidity (80-100%) and mortality (60-80%) rates (Rurangirwa and McGuire, 1996). the incubation period generally lasts on average 10 days but may vary between 2 and 28 days. The clinical findings are restricted to the respiratory system (Radostits *et al.*, 2006). The first signs are reluctance to walk and the onset of fever, typically 41-43°C (Thiaucourt and Bölske, 1996). After 2-3 days of high fever, respiration accelerates and becomes painful, grunt with violent coughing episodes. Animals stand with abducted limbs, neck extended and abortions in pregnant goats is occurred (Tigga *et al.*, 2014). There is continuous salivation and noses become blocked with a mucopurulent discharge. In

the terminal stages the goats are unable to move, mouth-breathing, tongue protrusion and frothy salivation and death follows quickly. In sub-acute or chronic forms, signs are milder with coughing usually noticeable only following exercise. High mortality can be seen in kids, where death is usually the result of septicemia (Ozdemir *et al.*, 2005; Shiferaw *et al.*, 2006; Nicholas *et al*., 2008).

The pathological lesions are confined in the thorax. In the early stages lung develop yellow nodules surrounded by zones of congestion. As the disease progresses these coalesce to include large areas of lung. The lesions at necropsy are mainly a fibrinous pleuropneumonia with increased straw-colored pleural fluid, unilateral hepatized and have a port wine color (Martin, 1983; Thiaucourt and Bölske, 1996; Ozdemir *et al*., 2005). A striking feature of CCPP is the tissue specificity of the causative agent, as lesions are produced in lungs. Although Mccp is present in high quantities in affected lungs, there is no dissemination to other organs. The specific reaction between lung tissue and mycoplasma component leads to an exacerbated inflammatory response (Thiaucourt and Bölske, 1996). Sequestrum is formed on the infected lung during chronic infection (Wesonga *et al.,* 1998; Shiferaw *et al.,* 2006) and after recovering from infection using antibiotic treatment (Ozdemir *et al*., 2006).

Clinical sign in wild ruminant are similar to goat, an abundant serous to mucopurulent nasal discharge, emaciation, reluctant to move and gross pathological lesion localized exclusively to the lung where severe pleuropneumonia with partial hepatization is reported. Affected antelope displayed a thickening of the interlobular septa, pleuritis, and an accumulation of straw-colored pleural fluid that solidified to form a gelatinous covering on the lung (Yu *et al*., 2013). In addition to this yellowish fibrin deposits and severe consolidation of the lung seen on Arabian oryx (Chaber *et al.*, 2014).

2.6. Economic Importance

Small ruminants particularly goats (also known as poor man's cow or the rural bank), contribute significantly to the nutrition and cash income of small farmers in Africa and Asia, the two regions occupied with the largest concentration (about 72.9%) of the poor peoples in the world (kumar *et al.,* 2014). Africa, the Middle East and Asia regions those are home to over 80% of the world's sheep and goat population, and for

more than 330 million of the world's poorest people play an important role in the livelihoods and food security (OIE & FAO, 2015). Goats are important commodities to a large segment of the world's population as a source of meat, milk, and skin. CCPP is a disease of major economic importance, posing a major constraint to goat production. The direct losses of the disease result from its high mortality, reduced milk and meat yield, cost of treatment, control, disease diagnosis and surveillance (AU-IBAR, 2013). In addition to this CCPP is a serious production disease with effect on livelihoods and has specific requirements for international trade (AU-IBAR, 2015).

2.7. Differential Diagnosis

CCPP is complicated by other pleuropneumonic disease causing similar syndromes (Rurangirwa and McGuire, 1996). PPR, Pasteurellosis and some other Mycoplasma mycoides cluster particularly *M. mycoides* subsp. *capri*, *M. mycoides* subsp. *mycoides LC* and *M. capricolum.* subsp. *capricolum* can cause a disease resembles CCPP (Tigga *et al.,* 2014) but may have extrapulmonary signs. That is known as 'MAKePS' syndrome, characterized by mastitis, arthritis, keratoconjunctivitis, pneumonia and septicemia in small ruminants (Manso-Silva´n *et al*., 2007).

2.8. Diagnosis of Contagious Caprine Pleuropneumonia

Tentative diagnosis is based on clinical signs, postmortem findings and demonstration of Mccp in pleural fluid by dark-field microscopy. Confirmatory diagnosis is mainly based on isolation and identification of the causative agent. The preferred samples for diagnosis are the pleural fluid and sections of hepatized lung, particularly from the interface between consolidated and unconsolidated area. The preferable method is to sacrifice the goat that did not receive any antibiotic treatment. If microbiological examination cannot be performed immediately, samples or whole lungs can be stored at –20°C for considerable periods (months) with little apparent loss of mycoplama viability (Thiaucourt *et al.*, 1996; Thiaucourt, 2012).

2.8.1. Identification of the causative agent

Definitive diagnosis requires culture of the causative organism from lung tissue samples or pleural fluid taken at post-mortem (Thiaucourt, 2012). After cloning and purification, it can be isolated by several biochemicals, immunological and molecular tests. Isolating the causative agent is a very difficult task for the mycoplasma diagnostic laboratory because of its highly fastidious nature, grows slowly in broth media, and produces only minute colonies on solid media. Furthermore, it is also frequently overgrown by other common mycoplasmas (Bölske *et al*., 1996).

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 hyperimmune serum, and detects primarily surface antigens. The GIT is particularly useful in identifying Mccp because they appear to be serologically homogeneous, and antiserum to the type strain produces wide inhibition zones (OIE, 2014).

Gel precipitin tests to detect antigen in tissue specimens: Mccp releases an antigenic poly-saccharide to which a specific monoclonal antibody (WM-25) has been produced this Mab immune precipitates in agar gel with the polysaccharide produced by Mccp, and is used to identify the causative agent in cases of CCPP, particularly when specimens are no longer suitable for culture because of deterioration during transportation (Thiaucourt, 2012).

Latex agglutination test (LAT): Antigen detection LAT provides earlier detection in affected animals before antibodies have appeared (March *et al.,* 2000). The test also provides a convenient means of monitoring growth of the mycoplasma in-vitro, which is often quite difficult to detect because of the small pH changes produced by some strains; the test requires just a few drops of culture fluid (Nicholas and Churchward, 2012). Cross reactions may occur as Mccp polysaccharides are similar to those produced by *M. leachii*. The test is more sensitive and easier to perform than the CFT (Rurangirwa *et al.,* 1987b).

Molecular diagnostic tests: PCR method has radically improved the detection and identification of microorganisms which do not grow easily in-vitro. The tests have been described and shown to be specific, sensitive and can be applied directly to clinical material, such as lung and pleural fluid, dried sample on filter paper and culture material (Lorenzon *et al,* 2002). Due to the difficulty of isolating Mccp, PCR is the technique of choice for the diagnosis of CCPP. However, isolation of Mccp remains the confirmatory test (Thiaucourt, 2012).

The PCR method which detects Mccp based on the amplification of a segment of the gene those codes for the 16S rRNA and analyzed by restriction enzyme cleavage with PstI to differentiate Mccp from the other species of Mycoplasma (Bascunana, *et al.,* 1994). This gene is well conserved in bacteria but also possesses regions that are variable enough to ensure a distinction between species or subspecies (Thiaucourt *et al.,* 1996). The other method is based on a specific amplification a DNA fragment is chosen as target sequence for the selection of a specific primer (Woubit *et al*., 2004). H2 locus DNA sequencing (Lorenzon *et al.,* 2002) and an improved resolution method, MLSA (multi-locus sequence analysis) based on the analysis of several genetic markers has also been used for the identification of Mccp (Manso-Silvan *et al*., 2011). PCR helps to establish the true geographical distribution of CCPP that detect in subacute or chronic cases were few or no living organisms are present in the tissues and using dried sample diagnose the disease in remote areas (Bölske et *al.,* 1996).

2.8.2. Serological tests

Complement fixation test (CFT): the prescribed test for international trade**,** demonstrating the potential limitations of antibody detection as a sole diagnostic technique. CFT expected to induce cross reaction to the mycoides cluster. Seroconversion of Mccp observed by CFT to start 7–9 days after the appearance of clinical signs, to peak between days 22 and 30, and to decline rapidly thereafter (OIE, 2014).

Latex agglutination test: LAT for antibodies detection uses a capsular polysaccharide specific antigen (CPS) coated beads extracted from Mccp, which agglutinate in the

presence of specific antibodies in the blood of affected goats (Rurangirwa *et al.*, 1987b). It can be run in two minutes on samples of whole blood or serum, without the need for any specialist training or sophisticated equipment and is adaptable to any laboratory or field conditions. The test is carried out by mixing a drop of the sensitized beads with a drop of blood or serum from the suspected animal on a glass slide for one minute and the results read visually and recorded as positive or negative (Rurangirwa and McGuire,1996).

Competitive enzyme-linked immunosorbent assay: It is a competitive enzyme-linked immunosorbent assay based on the monoclonal antibody Mab 4.52. Mabs were produced by immunizing mice with adjuved mycoplasma antigen, and standard fusion cloning procedures (Thiaucourt *et al.,* 1996). It is a strict competition assay of cut-off value, 55% percentage inhibition (PI), to obtain a strict specificity of 99.9% (OIE, 2014). It detects antibodies in sera of naturally infected or artificially immunized animals while it remained negative with hyperimmune sera to related strains (Thiaucourt *et al.,* 1994).

The high specificity of cELISA close to 100% was similar to the blocking ELISA. cELISA could be used as a surveillance tool in CCPP free regions at risk of disease introduction, including all the regions bordering infected zones. cELISA is likely to be more suitable for epidemiological surveillance and sero-prevalence studies. The high cutoff point, maximize the diagnostic specificity but it expected to decrease diagnostic sensitivity of the test (Peyraud *et al.,* 2014).

2.9. Control and Prevention

Treatment: the success of CCPP treatment largely depends on early diagnosis and intervention undertaken against the disease. The common active antibiotics belongs to the tetracycline group, macrolide family (such as spiramycin, lincosamine, erythromycin and tylosin) and some compounds belongings to the fluoroquinolones may also be active (such as enrofloxacin) (Thiaucourt *et al.,* 1996; Thiaucourt and Bölske, 1996). However, using treatment severity of the disease can be reduced but CCPP is refractory to commonly used antibiotics with development of carrier status, that capable of transmitting the infection (Hassan *et al.,* 1984; Radostits *et al.,* 2006).

Some antibiotics like dihydrostreptomycin sulphate used for completely recovered from CCPP infection (Rurangirwa *et al.,* 1981**)**.

Quarantine: apply quarantine measures as laboratory confirmation is awaited. Once CCPP is confirmed apply full quarantine in the identified area. Movement restrictions and slaughtering of infected and contact animals are recommended for newly infected countries or regions (Nicholas *et al*., 2008). Strict control on animal movements and a prohibition on the importation of live animals from infected regions (Thiaucourt *et al.,* 1996) in other words herd biosecurity is essential to prevent CCPP distribution (Radostits *et al.,* 2006).

Slaughtering infected animals is recommended for countries which are newly infected by the disease. This was demonstrated by Hutcheon in South Africa after the introduction of CCPP in 1881 in the country. Before anything was known of its real etiology, affected and in-contact animals were slaughtered to achieve eradication of the disease (Thiaucourt *et al.,* 1996).

Vaccination: The first immunization trial using Mccp was carried out by MacOwan and Minnette (MacOwan and Minnette, 1978 as cited Rurangirwa and McGuire, 1996). They inoculated intra tracheally with a high-passage culture of Mccp. The results provided an indication that goats could be protected against CCPP. Even though vaccination trail was begin a century ago when subcutaneously inoculated goats with lung extract from affected animals (Nicholas *et al*., 2008). Since then a number of different preparations have been produced which are reported to produce strong immunity even after one year. These include a vaccine composed of sonicated antigens emulsified with incomplete Freund's adjuvant and another in which lyophilized saponin killed Mccp vaccine is protect 100 percent against mortality (Rurangirwa *et al*., 1987a; Rurangirwa and McGuire, 1991; Litamoi *et al.,* 1989).

In Ethiopia, inactivated CCPP vaccine using F-38 Kenyan strain/Mccp is produced at NVI, Bishoftu. Inactivation of the bacteria is done by saponin which has also an adjuvant and each field dose contains minimum protein content 0.15mg/ml. Once the goat develop immunity, it protect for more than one year (http://www.nvi. com.et/ccpp. html).

Different preparation of vaccine trail were conducted at NVI, Ethiopia of which live vaccine trail carried out from local isolate strain of Mccp that reported 84.2% seroconversion where as inactivated vaccine develop 68.4% Mccp antibodies of vaccinated goats (Tarekegn *et al.,* 2012). At field trail of inactivated vaccine seroconversion of vaccinated goat were recorded 61.1% of various age groups of goats (Lakew *et al*., 2014). The other vaccine trail conducted by Tesgera *et al.* (2017) at the same organization by inactivating whole culture of the bacteria which was prepared from Mccp vaccinal seed grown in Mycoplasma specific hayflick media. The culture was inactivated by 37% formalin and adjuvanted by saponin results mean sero-positivity of 60.71% in the vaccinated goats. Furthermore vaccination trail using different preparation were attempted by Ayelet *et al. (*2007b) using inactivated CCPP vaccine adjuvated with saponin and ISA 50, and combined vaccine (CCPP + anthrax) with saponin adjuvat. All experimental trials promised the possibility of increasing the level of seroconversion to control and prevent the disease CCPP by a significant reduction.

Generally, during the application of vaccine coordination between neighboring geographical areas and countries in vaccination is very important to control the spread of disease across the region (AU-IBAR, 2015).

3. MATERIALS AND METHODS

3.1. Description of Study Area

The present study was conducted from November 2016 to May 2017 in selected districts of central Ethiopia which encompasses Fentale and around Alage ATVET College (Figure 3). The study areas were purposively selected due to large small ruminant population and to find non-vaccinated flocks for the last two years.

Fentale is one of the districts in East Shewa zone, Oromia region of Ethiopia located in the Great Rift Valley at 190kms East of Addis Ababa. The district lies on 8°44'59" N latitude, 39°49'59" E longitude, and an altitude of 900 to 1000 masl; Mount Fentale (2400 meters) is the highest point. It has an annual rainfall ranging from 560mm to 630mm and temperature ranging from 29°C to 38°C (Gebremedhin *et al.*, 2014). Awash and Germama river and Lake Beseka is an important water body in this district. A survey of the land in this district shows that 8.2% is arable or cultivable, 7.6% pasture, 28.8% forest, and the remaining 55.4% is considered degraded or otherwise unusable (UNDP, 2002). The area generally erratic rains are experienced. Fentale district has 20 PA, of which 18 rural and two urban PA: Metahara and Addis Katama. In 11 PA, the predominant agricultural practice is pastoral and the other 7 PA are Agro-pastoralist.

Camels, goats, sheep and cattle are the common livestock; goats and sheep are the important source of milk during the scarcity of feed and water for the cows and camels. According to Fentale Agricultural office there are about 381225 goat and 211746 sheep population in the district, migration to the neighboring areas and to the Metehara sugar factory for grazing is common, but in years of low rainfall herdsmen will migrate as far as Wonji, Arsi, Afar and west Harerge.

Alage ATVET College is situated at 217 kms south west of Addis Ababa at longitude of 38°24'55"E and latitude of 7°36′00"N with an altitude of 1600masl. It was established in 1981 during the Dergue Regime as an Orphan care center named as

'*HITSENAT AMBA'* since 2002 Alage is an Agricultural Technical and Vocational Education and Training (ATVET) College.

It triangulates three districts Alaba special district from Southern Nations and Nationalities regional state, Arsi Negele and Adami Tulu Jido Kombolcha districts from Oromia regional state. The area is characterized by mild sub-tropical climate with average minimum and maximum temperature of 11°C and 29°C respectively. There are two defined rainy seasons: short rainy season (March–April) and long rainy season (June–September). The mean annual rainfall of the area is 800mm (Addisu *et al.*, 2013).

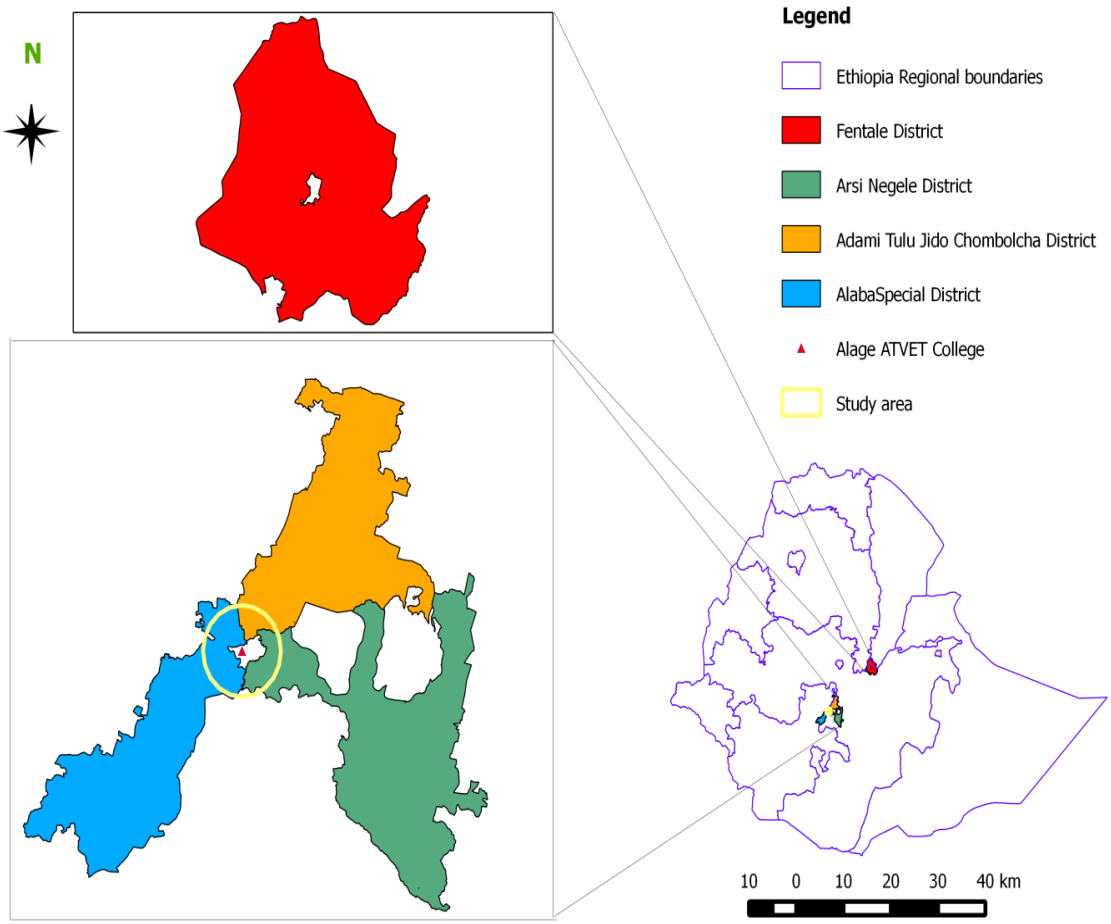

Figure 3: Study area map created by using QGIS version 2.0.1

3.2. Study Population

The study population was small ruminants which are reared on extensive husbandry system. Animals were allowed to graze freely at day time and kept in poorly constructed fenced barn or house at night. Sheep and goats with no history of vaccination for CCPP in the last two years and above 6 months of age were used as source of sera samples for this study. Vaccination history in the kebele (PAs) was determined from veterinary officials and by interviewing field veterinarians. Age of the animals was determined based on owner's information and dental eruption (DeLahunta and Habel, 1986); accordingly, animals were categorized into three age groups: > 6 months and ≤ 2 years, >2 years to ≤4 years and >4 years.

3.3. Study Design and Sampling Strategy

A cross-sectional study was employed to determine the sero-prevalence of CCPP and its associated risk factors. Multi-stage and systematic random sampling techniques were used to collect blood sample in this study. A semi structured questionnaire was administered for the livestock keepers to collect data regarding their perception of CCPP and the underlying risk factors.

3.4. Sampling Method

A systematic random sampling was adopted to select 370 goats. Therefore, three peasant associations (PAs) were selected randomly from each study area. In this study, a multi-stage sampling technique was used to select smallest administrative units (kebeles) and households. The following procedures were adapted during sampling.

- Primary sampling unit: PA's as the smallest administrative unit were used as primary sampling unit. Within the two study areas, three PA's for each study area (Fentale and around Alage College) were selected randomly from the PA's list.
- Secondary sampling unit: household or flocks were taken as secondary sampling unit. From each PA, flock of goats selected with a systematic

random sampling and include in the sero-prevalence study. The samples were disproportionally distributed in to six PAs.

- ❖ Tertiary sampling unit: from each household with a systematic random sampling method five goats were selected until the intended sample size was attained.
- ❖ Inclusion criteria animal should be above 6 month of age, none vaccinated for the previous two years. During sampling, history of animal whether the member of the flock or not was asked and newly recruited small ruminants replaced by existing goats to avoid the risk of including vaccinated animals.

3.5. Sample Size Determination

Sample size required for the study was determined, according to the formula described by Thrusfield (2005) with confidence interval 95%, precision 5% and expected prevalence 14.6% that previously studied by Peyraud *et al.* (2014) in Afar region Dubti and Hadar districts using monoclonal antibody-based cELISA test

.

$$n = \frac{1.96^2 X\, Pexp(1 - Pexp)}{d^2}$$

Where n= sample size

Pexp= expected prevalence

d = desired absolute precision

The required sample size was accordingly calculated as n = 191

Therefore to compute the two study areas Fentale and around Alage College a total of 382 sera samples were collected from the goats' population but only 370 were tested by cELISA due to discarding of 12 samples during transportation to the laboratory. Additionally 30 sheep sera samples were collected purposively from mixed population to detect sero-positive sheep.

3.6. Study Methods

3.6.1. Samples collection

To determine the sero-prevalence of CCPP a total of 400 sera samples (370-goats and 30-sheep) were collected approximately 5-7ml of blood sample from the jugular vein of each animal using plain vacutainer tubes. The samples were labeled accordingly to identify each animal and kept in a slanted position overnight to allow serum separation from clotted blood samples. Serum was aliquoted into a sterile 1.8ml cryovial and kept at -20^{o}C. Finally, the serum samples were transported in icebox to the National Veterinary Institute (NVI) at Bishoftu, Ethiopia where serological analysis was carried out. Sera in cryovial stored at -20°C until the cELISA were performed to detect antibodies against Mccp.

To perform antigen detection, a goat sero-positive to CCPP using cELISA and goats showing respiratory symptom was purchased from Fentale district then transported to the NVI, Bishoftu. The goat was slaughtered in the postmortem hall, using sterilized equipment and in aseptic condition. The pathological lung tissue was preserved at -20^{o}C until PCR procedure was run.

3.6.2. Laboratory analysis

3.6.2.1. Competitive enzyme-linked immunosorbent assay

Sera samples were examined for the presence of specific antibodies against Mccp by using cELISA at NVI serology laboratory. cELISA based on the monoclonal antibody (Mab 4.52) is developed by the CIRAD-EMVT (the Food and Agriculture Organization's world reference center for CCPP) (https://www.idexx.com/livestock /ruminant/ccpp.html). Test was conducted according to the test protocol supplied by the kit (Appendix 1). Mab based cELISA is used to assess presence of specific antibodies against Mccp (Peyraud *et al.,* 2014). The test has been evaluated and has a strict specificity of 99.9% (OIE, 2014).

The test sera were examined in 96-well flat bottom microplates coated with Mccp (*Mycoplasma F38*) antigen diluted with 0.01mol/L phosphate-buffered saline, pH 7.2–7.4. There were control sera of strong positive serum, weak positive serum, conjugate control and Mab control and negative control. Samples were pre-diluted on the preplate (uncoated plate) using dilution buffer. Then, serum sample was premixed with a specific Mab detection solution (Detection solution Mab) in a preplate and then transferred into the coated microplate. Any Mccp specific antibodies present in the sample was form an immune complex with Mccp antigen coated on the microplate, computing with the Mab in the detection solution for the specific epitopes. Cover the microplate and incubate at 37^{o}C for 1hour under gentle agitation, remove the solution and wash each well with wash solution 2 times. Tap each plate onto absorbent material after the final wash to remove any residual wash fluid. Then add conjugate in each well and incubate at 37^{o}C for 30minutes well with wash solution 3 times. Add TMB substrate in each well. Incubate at 37^{o}C for 20minutes. Dispense of stop solution per well. Finally, color development was observed and read at 450nm by ELISA reader to determine the optical density and percentage of inhibition was calculated. For the assay to be valid, results of internal quality control sera were first checked to make sure they are within the acceptable ranges. The result is expressed in "percentage of inhibition" by comparing the optical density in the test well with the optical densities in the Mab control wells. Finally, the percentage of inhibition (PI) for each sample was derived from the formula (Peyraud *et al.*, 2014).

PI = [(ODMab-ODTest)/ (ODMab-ODconjugate control)]*100
Negative: PI < 55%; Positive: PI >= 55%

3.6.2.2. Polymerase chain reaction

Antigen detection of Mccp: Conventional PCR was performed using species-specific primers (Woubit *et al.*, 2004). These primers targeted on the specific gene of Mccp with an amplicon size of 316bp. From lung tissue sample; around 1gm was chopped with scissors and finely minced with pestle and mortar dilute by 9ml of sterile PBS to disintegrate the cell. Then, the supernatant were subjected to DNA extraction.

DNA was extracted from the homogenized sample based on the protocol (Appendix 5) about 200µl of the supernatant was centrifuged at 12000rpm in a micro-centrifuge at 4^{o}C for 10minutes and add 200µl buffer AL. Treat with 20µl protienase K at 56^{o}C for 10minutes to digest cell. Add 200µl buffer AL, incubate at 56^{o}C for 10minutes. Add 200µl ethanol (96-100%). Pipette the mixture in to DNeasy Mini spin column placed in a 2ml collection tube. After centrifugation discard the flow-through and collection tube. Add 500µl buffer AW1, and centrifuge. Place the spin column in to a new 2ml collection tube, add 500µl buffer AW2, and centrifuge. Transfer the spin column to a new 1.5ml or 2ml micro-centrifuge tube. Elute the DNA by adding 80µl buffer AE to the center of the spin column membrane. Incubate for 1minute at room temperature (15-20^{o}C) and centrifuge. Finally, the extracted DNA was used as a DNA template in the PCR reaction mixture.

PCR amplification reactions was carried out in a final volume of 20µl containing DNA template 3µl and commercially available PCR IQ Super mix, RNase free water (3µl), Primer-Mccp-Spe-Fow-(5pM/µl) 5'-ATCATTTTTAATCCCTTCAAG-3'(2µl), Primer-Mccp-Spe-REV-(5pM/µl) 5'-TACTATGAGTAATTATAATATATGCAA-3' (2µl).

PCR was performed using the Mccp specific pair primers for an expected amplified product of 316bp long sequence. The amplification was carried out in a thermocycler under the following protocol initial denaturation at 94°C for 5minutes followed by 40 cycles of denaturation, annealing and elongation (95°C for 30sec, 50°C for 5sec, 72°C for 1minute respectively), and final elongation at 72°C for 7minutes followed by 4°C hold until machine off. The amplified products were detected by adding 4µl Gel red with Loading dye, 10µl PCR product and 10µl markers (Ladder), run Electrophoresis at 100V for 1hour in 1.5% agrose gel. Finally, read the result by using UV–light and camera. PCR products with a molecular size of 316bp were considered indicative for Mccp.

3.6.3. Questionnaire survey

A semi structured questionnaires (appendix 2) were administered to collect data from the herdsmen. The questionnaires were conducted with the local language (Afan

Oromo) using a translator. The questionnaire was pretested to ensure its validity and verbal consent was obtained from the respondent by explaining the objectives of the survey. Thereafter, respondents raised information on their perception of CCPP and the underlying risk factors of the disease that support serological results. Health status data was collected by recording important disease of goat, CCPP clinical signs, and flock management data including animal movement, watering, grazing, suspected source of the infection and awareness to control and prevent the disease.

3.6.4. Retrospective analysis of epidemiological data on CCPP outbreak

CCPP is a reportable disease in Ethiopia, on the present study a ten years period (2007-2016) retrospective data of CCPP outbreak was used to describe the situation of the disease in the country. The source of the data on the outbreaks and epidemiology of CCPP infection in various regions of the country was the Ministry of Livestock and Fisheries (MoLF), Epidemiology Directorate. The data were reported from the field veterinarians to the ministry through the officers of the districts concerned. Retrospective description of epidemiological data on number of CCPP outbreaks including morbidity, mortality, time (year and month) and place (regions, zone and districts) of outbreak and the vaccine doses used for control following the disease outbreak were considered in this study. A ten year CCPP vaccine production data (2007-2016) for domestic usage was taken from the NVI, plan and program office.

3.7. Data Management and Analysis

All the research data were stored in Microsoft Excel 2007 spread sheet. The analysis carried out by Stata version 13.0 software. Data collected through questionnaire were narrated and sero-prevalence of CCPP of cELISA result was summarized using descriptive statistics. Chi-square was used to evaluate the statistical significance association among different risk factors. Univariate logistic regression analysis was employed to assess the association of individual factors (independent variables) and the outcomes of interest. The degree of association between putative risk factors and CCPP sero-positive status was quantified by odds ratio (OR) calculations. On the final multivariate logistic regression model independent variables were analyzed without considering the multicollinear effect. For all analysis significance level $P< 0.05$ at 95%

confidence interval is considered as statistically significant. The retrospective data were described by line and bar graphs additionally the special distribution of CCPP were analyzed by quantum geographic information System software (QGIS 2.0.1).

3.8. Ethical Approval

This study was approved by Addis Ababa University, College of Veterinary Medicine and Agriculture Research Ethics Committee with reference number: VM/ERC/16/06/09/2017. Participants were provided with verbal consent to inform them the purpose of the study that participation was entirely voluntary, they were free to leave the study at any time and that all data would be kept securely. To ensure that the live animals subjected on the research were adopted a minimum pain and stress as a result of the research procedures. Every procedure was done under the ethical guideline with respecting the welfare of investigated animals.

4. RESULT

4.1. Prevalence of Mccp Antibodies using cELISA Test

4.1.1. Spatial distribution of Mccp antibodies in small ruminants

A total of 400 serum samples were obtained from small ruminants of apparently healthy, above six months old and with no history of vaccination against CCPP for the last two years. Out of the total number of the samples examined 56(14%) were positive for Mccp antibodies using cELISA kit. Of the goats (n=370) sampled, 192 were from Fentale district and 178 around Alage College, the sero-prevalence of CCPP was significantly different between districts Fentale (22.9%) and around Alage ATVET College (6.2%) ($\chi 2$= 20.45; P= 0.000) (Table 3).

Distribution of Mccp antibodies among sheep and goats are shown on Table 3. Of the 400 small ruminants sampled (370 goat and 30 sheep), 55(14.9%) and 1(3.3%) were found to be sero-positive, respectively.

Table 3: Magnitude of Mccp antibodies in small ruminants as compared in the two districts

District	**Species**	**Sample tested**	**Sero-positives**	**Sero-prevalence**	**95% CI**
Fentale	Goat	192	44	22.9%	17.2%- 29.5%
	Sheep	21	1	4.8%	0.1%- 23.8%
Around Alage	Goat	178	11	6.2%	3.1% - 10.8%
	Sheep	9	0	-	-
Total		400	56	14%	10.8% -17.8%

District level: Chi square ($\chi 2$) = 20.45, P= 0.000

4.1.2. Distribution of Mccp antibodies at PAs level

There were statistically significant difference observed among different PAs ($\chi 2$= 42.2; p= 0.000) (Table-4). The highest sero-prevalence was recorded at Tututi, Fentale district (34.4%) while the lowest was in Negele Wedesha around Alage area (4.9%).

Table 4: Distribution of Mccp antibodies at PA level

District	Peasant Association	Sample tested	Sero-positives	95% CI
Fentale	Lege Benti	74	6(8.1%)	3.03% - 16.8%
	Kobo	57	17(29.8%)	18.4 - 43.4%
	Tututi	61	21 (34.4%)	22.7- 47.7%
Around Alage	Naka	58	3(5.2%)	1.1% -14.4%
	Alge	59	5(8.5%)	2.8% -18.7%
	Negele Wedesha	61	3(4.9%)	1.02 -13.7%
Total		370	55(14.9%)	11.4% -18.9%

Chi square ($\chi 2$) = 42.17; P= 0.000

4.1.3. Prevalence of Mccp antibodies in goats with respect to sex

There was no statistically significant association between sex, whose sero-prevalence rate was 9.2% in male and 16.3% in female ($\chi 2$ = 2.42; P= 0.120) (Table 5).

Table 5: Prevalence of Mccp antibodies in goats with respect to sex

Sex	Sample tested	Sero-positive	Sero-Prevalence	95% CI	$\chi 2$	P-value
Male	76	7	9.2%	3.8% -18.1%		
Female	294	48	16.3%	12.3% - 21.05%	2.42	0.120
Total	370	55	14.9%	11.4% -18.9%		

4.1.4. Prevalence of Mccp antibodies among different flock category

Flocks of goat were classified in to three groups <30, 30-70 and >70. The result indicated that the prevalence of Mccp antibodies were 8.6%, 15.1% and 21.8%, respectively (Table 6). There was statistically significant difference among the flock category ($\chi 2$= 9.7; P= 0.008).

Table 6: Prevalence of Mccp antibodies among different flock category

Flock Category	**Sample tested**	**Sero-positives**	**95% CI**	**$\chi 2$**	**P-value**
<30	151	13(8.6%)	4.7% - 14.2%		
30 -70	86	13(15.1%)	8.3% - 24.5%	9.7347	0.008
>70	133	29(21.8%)	15.1% - 29.8%		
Total	370	55(14.9%)	11.4% -18.9%		

4.1.5. Distribution of Mccp antibodies in different age groups

The detail of sero-prevalence of CCPP tested by cELISA in different age groups is shown in Table 7. In this study, age was categorized into three distinct groups: 6months to 2years, 2 to 4years and over 4years. The result indicated that the prevalence of Mccp antibodies were 11.4%, 17.6% and 16.1%, respectively. The result revealed that the proportional prevalence of CCPP among age group were not statistically significant.

Table 7: Distribution of Mccp antibodies in different age groups of goats

Age	**Sample tested**	**Sero-positives**	**95% CI**	**$\chi 2$**	**P-value**
6month-2 years	149	17(11.4%)	6.8% - 17.6%		
2-4 years	165	29(17.6%)	12.1% - 24.3%	2.43	0.297
>4 years	56	9(16.1%)	7.6% - 28.3%		
Total	370	55(14.9%)	11.4% -18.9%		

4.1.6. Sero-prevalence of CCPP based on husbandry system

Regarding to the husbandry system, the sero-prevalence of CCPP in pastoral and mixed farming system were observed 22.9% and 6.2%, respectively. The difference was statistically significant ($\chi 2 = 20.45$; P= 0.000).

Table 8: Sero-prevalence of CCPP based on husbandry system

production system	**Sample tested**	**Sero-positives**	**Sero-Prevalence**	**95% CI**
Pastoral	192	44	22.9%	17.2% - 29.5%
Mixed farming	178	11	6.2%	3.1% - 10.8%
Total	370	55	14.9%	11.4% -18.9%

Chi square ($\chi 2$) = 20.45; P= 0.000

4.2. Risk Factors for CCPP Sero-positivity

The results of univariate and multivariate logistic regression analysis are summarized on Table 9. Production system and flock sizes are shown as potential risk factors for CCPP sero-positivity on univariate analysis. In the pastoral farming system goats were 4.5 times more likely to be sero-positive to CCPP than goats reared in the mixed production system (P= 0.00; OR= 4.5; CI= 2.2–9.1). Similarly, large flock size (>70) were at high risk of CCPP compared to small flock size (<30) (p= 0.002; OR= 6.1; CI= 2.8–13.6). Age and sex were not associated with the sero-positivity of CCPP in this study. All variables on univariate logestic regression analysis were subjected to multivariate logistic model. On the final model production system was found to be a risk factor for CCPP sero-positivity (P= 0.002; OR= 5.8 CI=1.9-17.1).

Table 9: Univariate and multivariate logistic regression of risk factors associated with CCPP sero-positivity

Variable	Sample tested	Sero-positives	Sero-prevalence	OR 95% CI	p-value
Univariate analysis					
production system					
Mixed farming	178	11	6.2%	Ref.	
Pastoral	192	44	22.9%	4.5(2.2 - 9.1)	0.000
Flock size					
<30	151	13	8.6%	Ref.	
30-70	86	13	15.1%	1.9(0.83- 4.3)	0.128
>70	133	29	21.8%	3.0(1.5- 6.0)	0.002
Age					
6month-2years	149	17	11.4%	Ref.	
2-4years	165	29	17.6%	1.7(0.8 -3.2)	0.125
>4years	56	9	16.1%	1.5 (0.6- 3.6)	0.374
Sex					
Male	76	7	9.2%	Ref.	
Female	294	48	16.3%	1.9 (0.8 - 4.4)	0.125
Passed multivariate analysis					
Production system					
Mixed farming	178	11	6.2%	Ref.	
Pastoral	192	44	22.9%	5.8 (1.9-17.1)	0.002

Ref-Reference group

4.3. Mccp Antigen Detection

From Fentale district a goat sero-positive with cELISA test and showing respiratory sign was sampled from the lung lesion to detect Mccp Antigen. A species-specific primer was employed directly to the field tissue samples for the detection of Mccp antigen. The conventional PCR showed an amplification of the antigen with an approximate band size of 316bp as shown on figure 4.

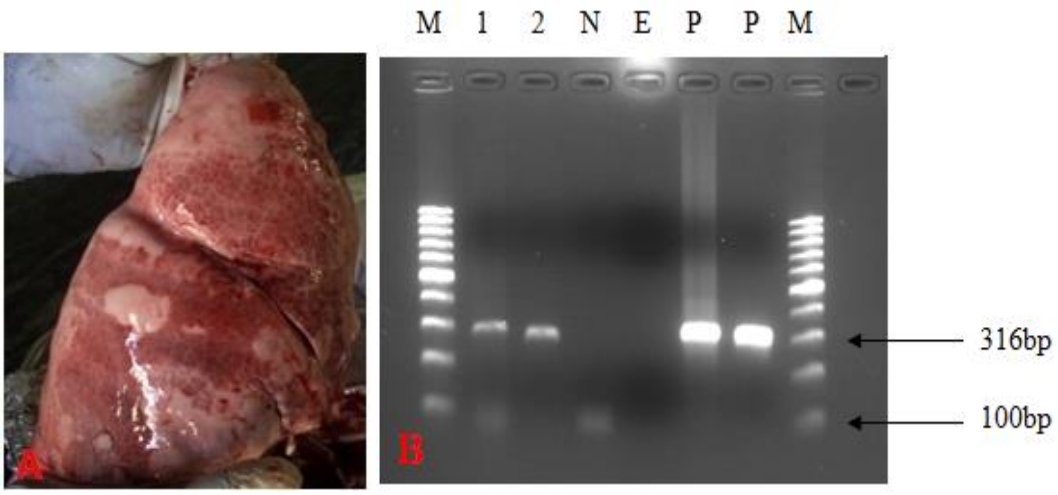

Figure 4: (A) Lung sample and (B) Agarose gel electrophoresis of Mccp specific PCR amplification product (316bp). Where Lane M: DNA molecular weight Ladder (100bp ladder, Feremantas); Lane 1and 2: tested goat lung tissue samples (positive result); Lane N: Non-template control; Lane E: Extraction control; Lanes P: Known Mccp two positive controls.

4.4. Questionnaire survey

A semi-structured questionnaires were administered to 64 participants from Fentale 48.4% (n=31) and around Alage College 51.6% (n=33) in order to retrieve information on the common disease of goat in the area and to assess the perception of the livestock keepers about CCPP. In Fentale, CCPP was known by the local name '*Sombesa*'. In this survey; goat pox, PPR and CCPP were the most important disease in Fentale district but around Alage area farmers had no experience about CCPP. Other diseases like pasteurellosis, anthrax, mineral deficiency and plant poisoning (*Prosopis juliflora* and others) were identified as a threat for small ruminant production (Table-10).

In Fentale, through open questionnaire the awareness of the respondents were assessed and 28(90.3%) described the clinical signs including: coughing, difficult breathing, nasal discharge, unable to walk, emaciation and stiffening of neck. 26(84%) express the route of transmission through inhalation with their own word through 'air' and 16% answer they did not know the route of transmission.

Interestingly some farmers (17.2%) described the gross pathological lesion, accumulation of yellow pleural fluid, hepatization, enlargement of the lung and adhesions to the pleura and fibrinous tissue coverage of lung were described in detail through open questionnaire.

In Fentale district most of the respondents were pastoralist, at dry season herdsmen move their flock to the neighboring districts from 10-90kms distance to look for water and pasture. In this area 61.3% of participants were not bought goats from the market for production whereas around Alage College 94% bought live animal from the local market for production purpose. But in both study area; goat that was not sold in the market could be brought back to the flock. Farmers mentioned factors associated with the occurrence of CCPP including; aggregation of goats at watering point (51%), grazing area (7%), not know (16%), and mixing of flock at watering point and grazing area (mineral soil licking) (26%) were identified as the main source of infection.

Table 10: Major goat diseases in the study area according to the farmers' perception

	Local name			**Around**
Disease	**Fentale (n=31)**	**Around Alage college (n=33)**	**Fentale**	**Alage college**
Goats pox	*Kodobo*	*Bega*	30(96.8%)	31(93.9%)
CCPP	*Sombesa*	-	28(90.3%)	-
PPR	*Desta*	*Likuche*	30(96.8%)	16(48.5%)
Anthrax	*Aba senga*	*Aba Senga*	1(3.2%)	9(27.3%)
ORF	-	-	10(32.3%)	6 (18.2%)
Pasturellosis	*Hirki*	-	13(41.9%)	7(21.2%)
Mineral deficiency	*Degemeka*	*Wuro*	6(19.4%)	15(45.5%)
Circling	*Azurit*	*Azurit*	9(29.1%)	15(45.5%)
External parasite	*Chito*	*Chito*	-	11(33.3%)
Plant poisoning (*Prosopis juliflora* and others)	*Yeweyane Zaf*	-	5(16.1%)	17(51.5%)

Seasonal occurrence of CCPP was specified by the farmers, 12(38.7%) respondents said to be CCPP is occurred commonly at winter season, 8(25.8%) at summer season, 8(25.8%) at any time and 3(9.7%) during spring season.

During the treatment of sick animals in Fentale district all herdsmen (100%) they purchased antibiotic from the market and human pharmacy then administer by themselves to the sick animals. In addition to this, 83% of the respondent use both market and government clinics. Previously, Fentale livestock keepers used the human preparation tetracycline, six capsules dissolve with water and drench for sick goat when suffered to CCPP. On the other hand, in Alage area most participant consult veterinary professionals and drugs purchased from the government clinics or from veterinary pharmacy. Livestock keepers were familiar to the importance of vaccination they were interested to vaccinate their animals by their own initiation. In Fentale livestock owners had excellent habit that they never brought diseased animals into the market. This might be important parameter to reduce the transmission of disease.

4.5. Retrospective Epidemiological Analysis of CCPP Outbreaks (2007-2016)

4.5.1. Temporal distribution of CCPP 2007-2016

Between 2007 and 2016, a total of 175 CCPP outbreaks were reported from different regions and districts to the Epidemiology Directorate of Ministry of Livestock and Fisheries (MoLF). The highest number of outbreak was reported in 2008 (16.6%) and the least report was recorded in 2016 (1.7%). The outbreak report trend gradually decreased year to year especially from 2012 toward 2016 (Figure-5).

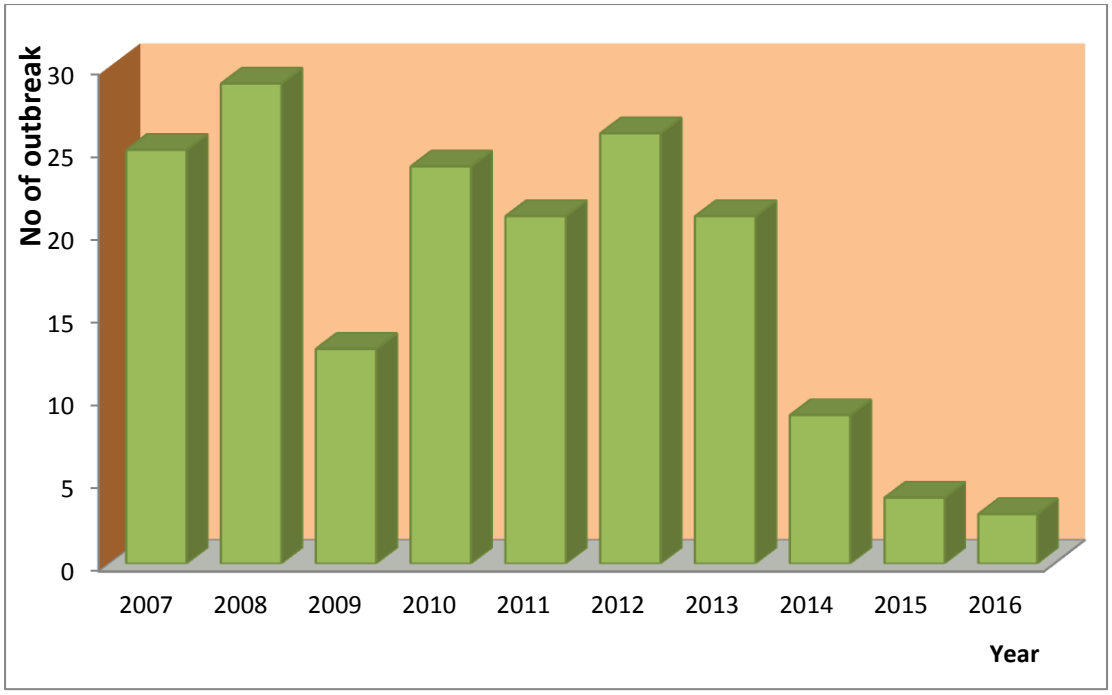

Figure 5: Yearly number of CCPP outbreaks from 2007-2016

The incidence of CCPP outbreaks increased gradually starting from December and with a peak in July. The lowest numbers of outbreak were seen in the month of December and January. The report showed clearly that the outbreak number gradually increased begins from the starting of dry season December up to the beginning of the main rainy season July (Figure-6).

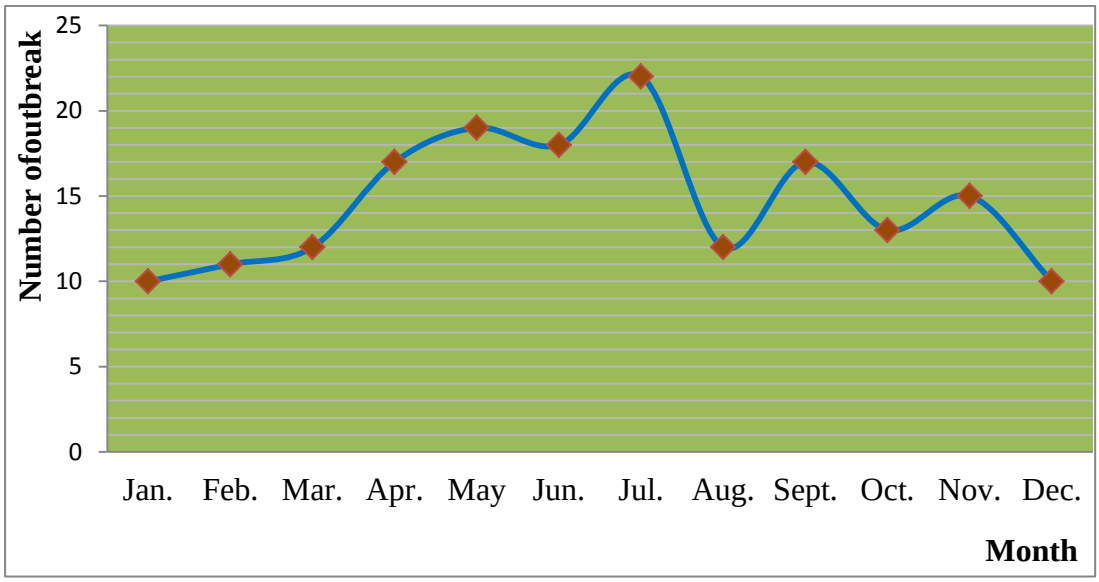

Figure 6: Monthly CCPP outbreaks 2007-2016

4.5.2. Epidemiological parameter estimates of CCPP and vaccine usage

An overall morbidity, mortality and case fatality rate of 3.3%, 0.82% and 26.8% were recorded, respectively, during the ten years period. The highest fatality rate of the disease was reported in 2009. From 2007 to 2011 there was high case fatality rate then after 2011 an outbreak number and case fatality rate gradually decreases toward 2016 (Figure-7). According to the NVI plan and program office in 2007 annual CCPP vaccine for domestic usage produced 69,585 doses and in 2016 the domestic usage reaches 3,320,100 doses (Appendix 3).

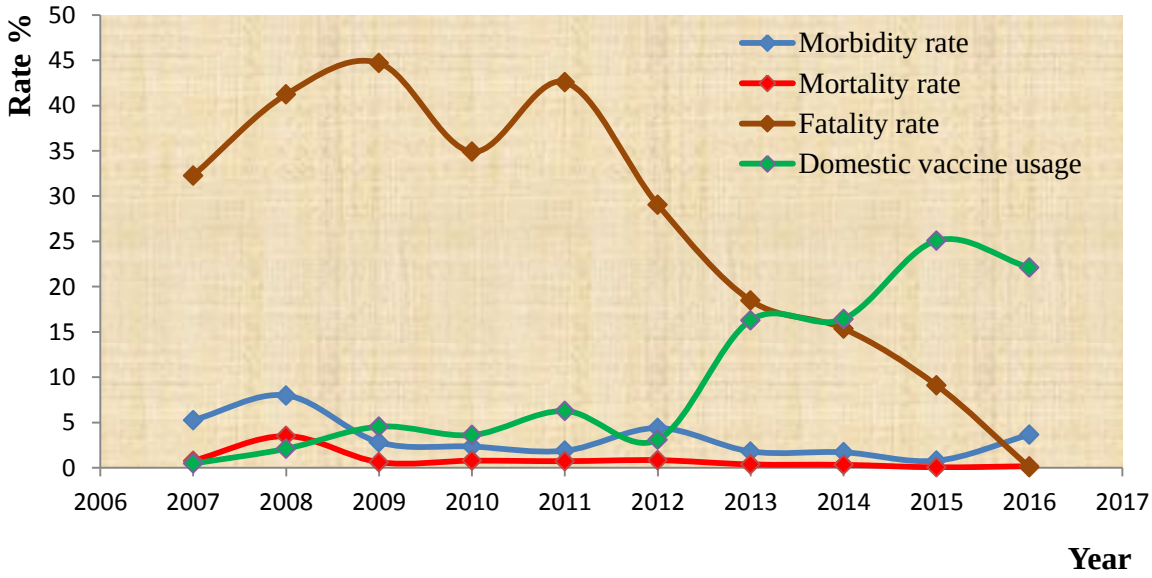

Figure 7: CCPP outbreaks and its effect in different parts of Ethiopia, 2007-2016

4.5.3. Spatial distribution of CCPP disease outbreaks in Ethiopia

The highest number of CCPP outbreaks were reported in Oromia region (40%) followed by SNNP (24%), Somali (9.7%), Afar (12.6%), Benishangul Gumuz (4.6%), Tigray (4.6%), Amhara (4%) and Gambella (0.6%) within the ten years period (Figure-8). 64% of the CCPP outbreaks were reported from the two regions Oromia and SNNP which have almost half of the country goat population 13,720,656 (46.2%) (CSA, 2016).

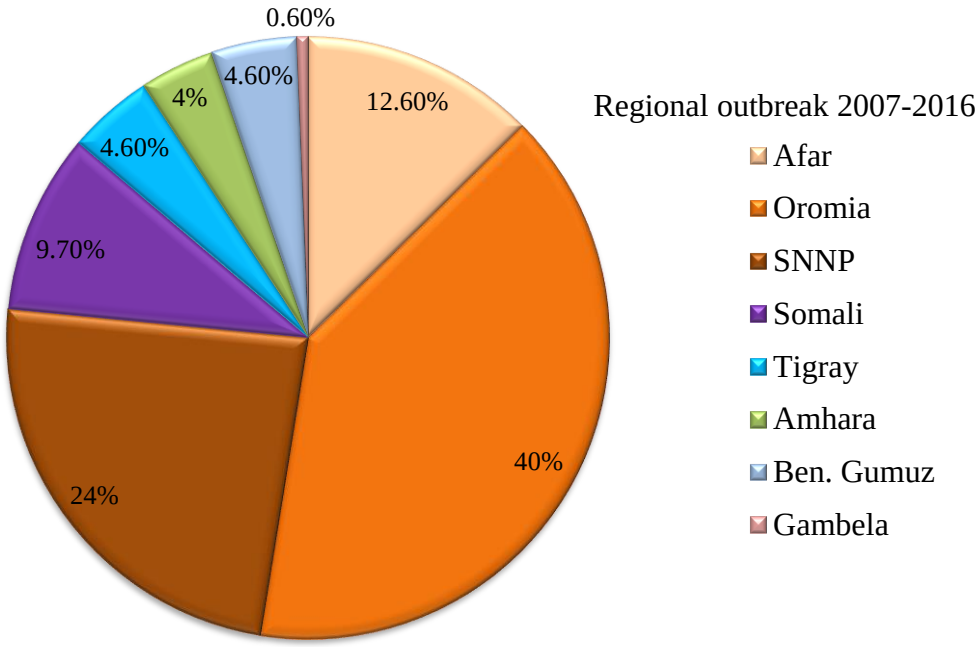

Figure 8: Proportion of CCPP outbreak reports across region 2007-2016

4.5.4. Spatial distribution of CCPP in Ethiopia at district level

From the total recorded outbreaks 62(36%) were reported from the southern part of the country districts like Arbaminch zuria, Dassa, Arero, Yabelo, Myo and Burji special district (Figure-9). As shown on the map in the west and central province of the country there was limited report in the previous ten years.

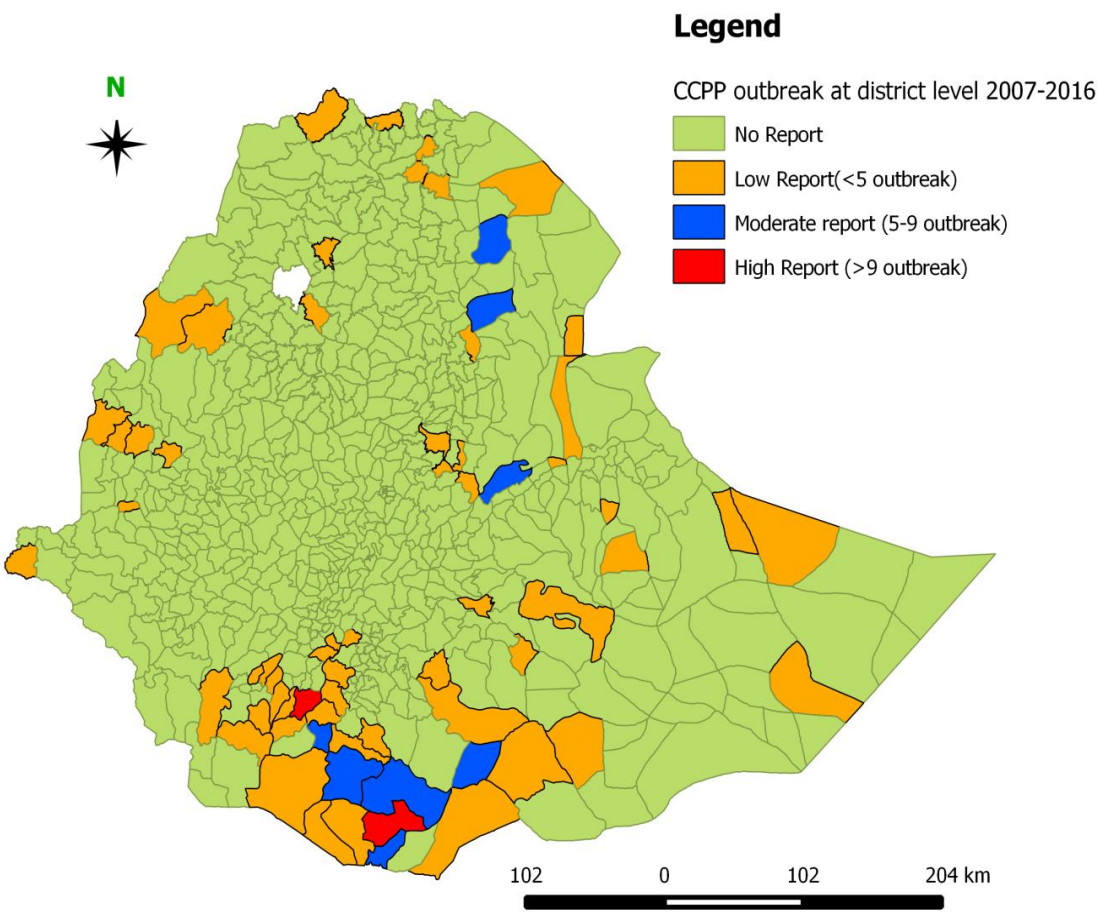

Figure 9: Map showing the distribution of CCPP outbreak at district level 2007-2016

4.5.5. Spatial distribution of CCPP across different Zone

CCPP endemicity in the lowland of Ethiopia was confirmed by various investigators and frequent outbreak reports were common especially in the Borana and Gamo Gofa zone. They had recorded 42.6% of the total national outbreak. In Afar Zone 1, Somali Liben, Burji and South Omo were the area of CCPP outbreaks frequently reported.

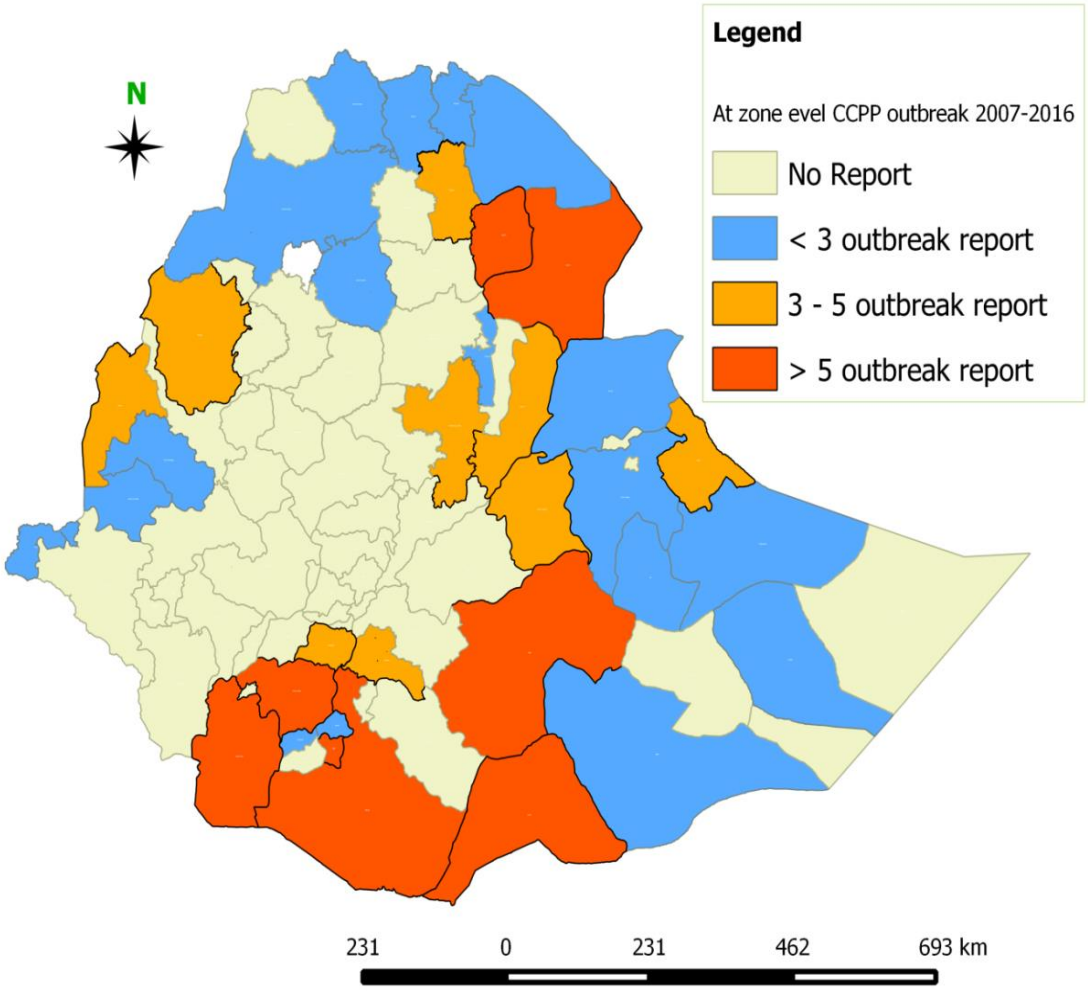

Figure 10: Zonal CCPP outbreak reports 2007-2016

4.5.6. Vaccine intervention following CCPP outbreaks

On these ten year retrospective data, Oromia region used the highest percentage (38.4%) of CCPP vaccine doses followed by SNNPR (25.8%), Amhara (17.7%), Somali (13.7%), Tigray (1.9%), Benshangul Gumuz (1.6%) and Gambella (0.9%) to control the outbreak. Majority of outbreaks as well as the large percentage of vaccine doses was used in Oromia region while the lowest vaccine usage was recorded in Gambella region. No vaccine intervention was recorded in Afar region despite 12.6% of CCPP outbreak were reported for the previous ten years.

5. DISCUSSION

5.1. Antigen Detection, Antibodies Prevalence and Risk Factors of Mccp

In the present study area, during feed scarcity for large animals (cows and camels), goats are the important livestock as the main source of milk for children. That is why goats are considered as "poor man's cow" that can replace the cow and camel values (DaMassa *et al.,* 1992). CCPP is one of the important diseases in the pastoral areas most herdsmen dread when observed respiratory symptoms on goats. Though it is necessary to implement epidemiological investigation for applying the appropriate control measures depend on the situation of the farmers to reduce the socioeconomic impact of the disease.

In this study, the circulation of Mccp antigen was confirmed in Fentale district using conventional PCR by a species-specific primer and observed the predicted molecular band of Mccp 316bp; previously, the disease was reported by antibody detection (Roger and Bereket, 1996; Sharew *et al.,* 2005).

The overall sero-prevalence of CCPP in the present study was 14.9% in goat using cELISA test. The finding is in line with the report of Peyraud *et al*. (2014) 14.6% in Afar region using the same test and nearly similar agro-ecological zone. However, our finding substantially different from the sero-prevalence of Arero, Dhas and Yabello districts in Borena pastoral area 31.6% (Lakew *et al.,* 2014), Turkana, Baringo and Kajiado in Kenya 47.2% (Kipronoh *et al*., 2016) tested by cELISA. The high sero-prevalence could be associated with the mobility of pastoral goat flock through the porous international border across this region. High sero-prevalence also reported from Musoma district Tanzania 64.4% (Nyanja *et al.,* 2013) and southern zone of Tanzania 52.1% (Mbyuzi *et al.,* 2014) using cELISA test.

Relatively low sero-prevalence of CCPP was reported from Mauritius 8% and Tajikistan 10% (Peyraud *et al*., 2014). In these countries the presence of the disease were confirmed in recent years. In Pakistan, Punjab province 8.52% was reported (Shahzad *et al.,* 2016) as the author suggests that relatively low serprevalence

detection could be associated with the low level of new animal introductions to the flock for expansion purposes that reduce the exposure for pathogen. In other area, using the same cELISA kit, low seroprevalence were recorded in Pakistan, Khyber Pakhtunkhwa 3.91% (Wazir *et al.,* 2016) and north Tanzania 9.6% (Swai *et al*., 2013).

Generally, the variation in the sero-prevalence of CCPP reported from different studies could be associated as a result of the temporal and spatial factors, the situation of the disease during the time of sampling like ongoing outbreaks of the disease (Lakew *et al.,* 2014) and the purpose of the study were some factors. Furthermore, climatic conditions of the area and stress favors for the wide prevalence of the disease at the sampling time.

The study has been shown 3.3% of Mccp antibodies in sheep. Sero-positive sheep were reported by different tests, Ayelet *et al.* (2007) 7.14%, and Hadush *et al.* (2009) 18.5% in Ethiopia using CFT test, Suryawanshi *et al.* (2015) 16% in India using LAT test and Mbyuzi *et al.* (2014) 36.7% in Tanzania using the same cELISA kit. Mccp is isolated from clinically diseased sheep (Bölske *et al.,* 1995), sheep raised with affected goat flock (Litamoi *et al.,* 1990; Houshaymi *et al*., 2002) and Mccp antigen detect using PCR from the pneumonic sheep (Çetinkaya *et al*., 2009) but the importance of sheep in the epidemiology of CCPP is under controversial. Some scholars stated that sheep act as a reservoir without developing clinical infection (Yigezu *et al.,* 2004). However there was no clear incident on the transmission and infection of CCPP in sheep (Thiaucourt, 2012).

In Fentale significantly a higher sero-positivity of CCPP was recorded in contrast to around Alage College which shares boundaries of the Afar region that reported outbreaks frequently to the MoLF. In addition to this, it could be associated with agro-ecology and production system. A similar observation was reported by Hadush *et al.* (2009) agro-ecology is a factor for the distribution of CCPP. Shahzad *et al.* (2016) were reported 3.7% to 33.3% sero-prevalence in various districts of Pakistan. Kipronoh *et al*. (2016) in Kenya 29.2% to 63.9% sero-prevalence were observed in different districts with a high sero-prevalence near to the international boundaries with South-Sudan, Tanzania and Uganda where as low sero-prevalence in the central part of the country.

The variation in the sero-prevalence of CCPP between districts could be attributed in pastoral area seasonal movement of flocks plays an important role in the transmission of CCPP. Regular mixing of flocks at watering points and communal grazing areas were common, which is likely to spread the infection between flocks (Ayelet *et al.*, 2007a). In Fentale high scarcity of feed and water is a challenge at the summer season and herdsmen migrate across the neighbor districts.

CCPP sero-prevalence significantly associated within different PAs (P< 0.05) with a higher sero-prevalence was recorded at Fentale, Tututi PA 34.4% and the lowest sero-prevalence was observed around Alage, Negele Wedesha PA 4.9%. The variations between locations were in agreement with the report (Mekuria and Asmare, 2010; Fasil *et al*., 2015). It could be associated with the presence of endemic foci in the localized area (Fasil *et al.*, 2015) and previous exposures (Kipronoh et al., 2016) are important source of infection. Goats recover from the previous infection may be developing subclinical infection.

The results obtained here revealed that there was no sex difference in sero-prevalence of CCPP. This scenario has also been reported in other studies Eshetu *et al.* (2007), Mekuria and Asmare (2010); Bekele *et al*. (2011) and Yousuf *et al.* (2012) in different parts of Ethiopia and Kipronoh *et al*. (2016) in Kenya which concluded that sex has no role in CCPP epidemiology. However, the results were contradicted to the findings by Regassa *et al*. (2010); Fasil *et al.* (2015) and Shahzad *et al.* (2016). This might be related with sex difference in the population mostly livestock owner not sold females and males reared in the flock commonly for breeding purpose.

With respect to age category, it was not a risk factors for the prevalence of CCPP (P=0.297). Due to the fact that the contagious nature of CCPP and the morbidity rate of the disease ranges 80-100% (Rurangirwa and McGuire, 1996) that means if one infected animals introduced to the susceptible flock, all flocks might be under the risk. Similar studies were reported by Eshetu *et al*. (2007); Hadush *et al.* (2009); Fasil *et al.*, (2015) where age is not a factor for the epidemiology of CCPP. But some studies reported old age has showed significantly higher sero-prevalence as compared to the young age (Bekele *et al.*, 2011; Regassa *et al*., 2010; Lakew *et al.*, 2014). These scholars were suggested that older animals have been at risk of exposure for a longer

period than young animals. Moreover, the goats are often repeatedly exposed to different stress conditions like malnutrition and movement over long distances to be intended a repeated infection.

Regarding on flock size significant variation ($p< 0.05$) was observed among different groups. In this study, flock size more than 70 goats were significantly sero-positive than the flock size lower than 30 goats. Similar finding were reported by (Bekele *et al.,* 2011; Yousuf *et al.,* 2012). This may be explained by the fact that the disease needs close proximity to source of infection and increasing number of susceptible population. The aggregation of goat flocks during watering and at rest times would favor the spread of the infection within the flock.

Using univariate logistic regression district, production system and flock size were a significant difference for CCPP sero-positivity. On the final multivariate logistic model production system is the main risk factors for CCPP infection (P= 0.002; OR= 5.8; CI=1.9-17.1). This could be be associated with pastoral mostly found at marginal area, environmental stress and malnutrition during dry season commonly appeared that favors CCPP infection.

5.2. Questionnaire Survey

The aim of this survey was to explore the awareness of the farmers and associated risk factors for the occurrence of CCPP in previously reported area of Fentale district and around Alage college where there is limited information regarding to the epidemiology of CCPP.

In Fentale area, CCPP is known by the local name '*Sombesa*' and around Alage area respondents did not know about the clinical disease. In other pastoral areas, CCPP has its own local name '*Sompo'* in Hammer and Benna-Tsemay (Mekuria *et al.,* 2008) and **'***Zom*' in Gambella region (Fasil *et al.,* 2015). These could be indicated the endmicity of the disease and the perception of pastoral in these area.

In this survey; goat Pox, PPR and CCPP were listed on the first line at Fentale district. CCPP also prioritize as major threat in pastoral area of Ethiopia (Mekuria and

Asmare, 2010; Fasil *et al.,* 2015), Uganda (Atim *et al.,* 2016) and Tanzania (Chengula *et al.,* 2013).

In Fentale, most of the respondent were pastoralist, they practice raising sheep and goats together. All respondents put their animals at night enclosure, with a fence from thorny woods. On the other hand around Alage College farmers built pen for night enclosure to protect from predators, and flocks rose together with other livestock. In both study area they used communal grazing area. In Fentale, during shortage of feed and water herdsmen move their flock long distance from 10-90kms toward the neighbor districts, Berehet, west Harerge, Afar, Metehara sugar factory and Awash national park. In the Park; they use the dam of the park as source of water. Probably this might be exposed the park wild ruminants like Abyssinian Oryx and Gazelle species for CCPP those species which are susceptible to the disease reported from Asian countries (Nicholas *et al*., 2008, Chaber *et al*., 2014) due to the close interaction of goats at watering point.

In this study, pastoralist described the clinical sign of CCPP in similar manner with scholars (Thiaucourt *et al.,* 1996) that shown the degree of perception about CCPP. Similar report was described by Swai and Neselle (2010) in Tanzania, Maasai pastoralists explained the clinical sign and epidemiological features of CCPP consistent with the typical descriptions of the disease in veterinary textbooks. Mekuria *et al.* (2008) also reported the Hammer and Benna-Tsemay pastoral in Ethiopia were knowledgeable to CCPP. Some respondent even they had a habit appreciating the gross pathological lesions similarly that described in veterinary books.

In Fentale, most respondent 61.3% were not bought goats from the market for production purpose. It was also noted during the questionnaire farmers pointed out mixing of flocks at watering point and farming practices are a risk factors for the occurrence of CCPP. During licking of mineral soil and at watering point the close proximity of large flock could be a risk for the prevalence of CCPP. This may be explained by the fact that a new CCPP infection needs proximity to an infected or latent carriers' goat (Quinn *et al.,* 2011).

During the treatment of sick animals in Fentale, all herdsmen they purchased antibiotic from the market and human pharmacy then administer by themselves to the sick animals. Similar situation was reported by Chengula *et al. (*2013) in Tanzania majority of pastoral and agro pastoral livestock keepers tends to treat their animals without the advising of Veterinarians.

5.3. Epidemiological Retrospective Data Analysis

CCPP cause a great economical impact on livestock production in fragile rural economies (Dupuy *et al.,* 2015). The fastidious nature of the causative agent to culture in vitro is a challenge for identification (Leach *et al*., 1993). Due to these reason most of the outbreak report being suspicion. The outbreaks of CCPP were reported from the various districts of the country to the MoLF that provided information about the endemicity of the disease in line with different studies.

From 2007 to 2016, a total of 175 CCPP outbreaks of goats were reported from 66 districts of 35 zones to the epidemiology unit of MoLF especially from the lowland areas those are known by goat rearing such as Borana, Gamo Gofa and south Omo zone. In central Ethiopia the outbreak report was very few comparing to districts located on the border of the country. Districts adjacent to the neighbor countries like Kenya had a highest number of outbreaks report. It was consistent with some studies that indicated the endemicity of the disease in the lowland of the country (Yigezu *et al.*, 2004; Sharew *et al.*, 2005; Shiferaw *et al.,* 2006; Bekele *et al.,* 2011).

The spatial distribution of CCPP shown that Oromia was reported the highest number of outbreak followed by SNNP. This might be related to size of goat population while the least case of the disease was reported from Gambela region. This might be attributed from poor disease reporting system, due to lack of trained manpower and seasonal movement of the pastorals which is difficult to monitor the status of the animals.

Frequent outbreaks were reported from the lowland of Ethiopia, 42% of the total ten years outbreaks reports were recorded from Borana and Gamo Gofa zone. In Afar Zone 1, Somali Liben, south Omo and Burji special districts were the area of CCPP

outbreaks repeatedly reported to the MoLF. In Borana zone for the previous ten years at least one outbreak was reported every year except in 2014. Interestingly, out of 70 outbreaks of Oromia region, 50(77%) were reported from Borana zone. Simultaneously, in SNNP, out of 42 outbreaks report, 20(48%) were coming from Gam Gofa zone. This could be shown that CCPP is endemic in a specific location and during some environmental stress the carrier animals become source of outbreak. The frequent occurrence of drought (Birhanu *et al*., 2015) in southern Ethiopia favors for a large number of outbreaks.

CCPP outbreaks have been associated with seasonal crisis. For instance in 2007 high outbreaks report were coming from SNNP (52%), in 2009 Afar region were the major area of outbreak that covers 77% of the national annual outbreak report, that might be related with the occurrence of drought (Bekele *et al.,* 2010). From 2011-2012 Oromia (Borana zone) on average (63%) of the national CCPP outbreaks were reported in each year. This situation might be linked to the stress of drought (ODA, 2011).

Furthermore, seasonal outbreaks could be associated with the contagious nature the disease that once outbreak occurred in a specified location due to the devastating nature of the disease it affects a wide geographical area. The seasonal movement of animal to look for feed and water suit mixing different flocks that enhance the distribution of the disease in to neighboring districts. The mobility of goats flock through porous border between the neighbors countries for instance during draught season Kenyan herdsmen that have come across to Borana Ethiopia and vice versa (personal communication). Endemicity of the disease in this area and carrier animals might be the main source of outbreak when flock immunity reduced by malnutrition.

In central province of Ethiopia, the frequencies of CCPP outbreaks were minimal. Most districts had no report in the previous ten years even though the presence of CCPP was confirmed in the central province. These probably due to the applying of better veterinary service and related to the awareness of the livestock keepers to follow up the health of their animal. Flock size and management system have its own impact. Similar situation were reported in Kenya which is high prevalence of CCPP in the border of the country compared to the central province (Kipronoh *et al.,* 2016).

Regarding on seasonal occurrence of CCPP the outbreak frequency gradually increase from January to July and decline from August to December. It could be associated with the mobility of flock to search feed and water herdsmen movement started at the beginning of summer season and return back to their village at the beginning of rainy season. Through this long walk goats were exposed for various stresses that could expose for infection and large outbreaks were reported at wet season. A participatory study and questionnaire survey conducted by Mekuria *et al.* (2008) and Fasil *et al.* (2015) revealed that the occurrence of CCPP is also associated with wet season. Extreme change of climate at the beginning of rainy season with the long period of stress prompts the infection. Furthermore the causative agent also survives outside the host for substantial period in moist and cool environments than dry and hot condition (Walker, 1999).

From 2007-2011 there was high case fatality rate, then after 2011, a frequency of outbreaks and case fatality rate gradually decreased toward 2016. It might be related with poor reporting system or truly reduce the disease impact through veterinary service, treatment and vaccination or livestock owners administered antibiotic as prophylaxis. This situation needs further detail retrospective analysis and active surveillance to modify the control strategy on the current status of the disease.

On these ten year retrospective data, Oromia region used the highest percentage (38.4%) of CCPP vaccine doses followed by SNNPR (25.8%) to control the outbreak of the disease. Majority of outbreaks as well as the larger percentage of vaccine doses was used in Oromia region while no vaccine intervention was recorded in Afar region despite 12.6% of CCPP outbreak were reported during the previous ten years. This might be due to the poor reporting system from regional and district officials and lack of awareness whether to report vaccination activities or not. According to the plan and program office of NVI, the annual domestic usage of CCPP vaccine production gradually increased in the previous ten years by more than tenfold.

6. CONCLUSION AND RECOMMENDATIONS

In conclusion, CCPP is OIE listed transboundary disease. It is a serious production disease with affecting the livelihoods and has specific requirements for international trade. The present study suggested that CCPP is perceived to be one of the important goat diseases in Fentale district and possibly other parts of Ethiopia. But farmers found around Alage College had no experience to the CCPP clinical disease. They had not supported the serological result of 6.2% through the questionnaire survey. CCPP outbreak were reported from all region of the country specifically high number of outbreak were reported from the lowland part of pastoral areas which are home of a huge goat flock.

CCPP prevalence has been a significance variation between pastoral and mixed farming system. The difference could be associated with the flock size, environmental factors and production system as risk factors. In the pastoral area we have observed a high willingness to reduce the socioeconomic impact of the disease this might be important to design control strategy by participating the concerned body.

Therefore, based on the above conclusive remarks the following recommendations are forwarded:

- Implementing annual vaccination regularly in endemic area prior to dry season is essential.
- Co-existing viral diseases favor the infection of CCPP, so it is important to control the disease like PPR, or it needs trying to produce a combined vaccine (CCPP and PPR) by the concerned institute.
- To reduce the geographical distribution of CCPP outbreaks, water point should be constructed at PA level or at the smallest administration unit.
- Further investigation needs to be clarified the importance of sheep on the epidemiology of CCPP.

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8. APPENDIXES

Appendix 1: cELISA procedure

1. Obtain the required number of coated micro plates and uncoated microplates for sample preparation and record the position of each sample
2. Dispense the dilution buffer N.24 , controls, samples, and detection solution in the uncoated micropltes
    a. Dispense 100µL of dilution buffer N.24 into each well of the uncoated microplates
    b. Dispense another 110µL of dilution buffer N.24 into two appropriate wells(CC)

       Note: Total volume of Dilution Buffer N.24 in CC Wells: 210µL
    c. Dispense 11µL of undiluted strong positive control in four appropriate wells

       Note: The supplied SPC can be replaced in two wells by your own IRC
    d. Dispense 11µL of undiluted positive control in tow or four appropriate wells
    e. Dispense 11µL of undiluted negative control in two appropriate wells
    f. Dispense 11µL of undiluted sample per well into remaining wells of the preplates
    g. Dispense 110µL of diluted detection solution into each well of the preplate except in CC
3. Homogenize the contents of the wells of the uncoated microplate and transfer100µL from each well to the appropriate wells of the Microplate
4. Cover the microplate and incubate 1 hour (±5 min) at37^{o}C (±3^{o}C) under gentle agitation, avoiding desiccation of the plates.
5. Remove the solution and wash each well with approximately 300µL of wash solution 2 times. Avoid plate drying between plate washings and prior to the addition of the next reagent. Tap each plate onto absorbent material after the final wash to remove any residual wash fluid.
6. Add 100µL DILUTED conjugate in each well.

7. Cover the microplate and incubate 30 minutes (±3 minutes) at 37°c (±3°C) under gentle agitation, avoiding desiccation of the plates.
8. Repeat step five but this time washing three times.
9. Add 100µL of TMB substrate N.9 in each well.
10. Incubate 20 minutes (±3 minutes) at 37°C (±3°C) away from direct light.
11. Dispense 100µL of stop solution N.3 per well.
12. Measure and record the absorbance values of samples and controls at 450 nm.
13. Calculation

    Control

    Calculate conjugate control mean absorbance ($CC\bar{X}$) and Mab control mean absorbance ($MabC\bar{X}$).

    $CC\bar{X}$ = (CC1 A (450) + CC2 A (450))/2

    $MabC\bar{X}$ = MabC1 A (450) + MabC2 A (450) + MabC3 A (450) + MabC4 A (450)/4

    Sample and controls

    Calculate the percentage of inhibition (SPI) for each sample and control.

    SPI% = 100*(mab-$C\bar{X}$ - S A (450)) / ($MabC\bar{X}$ - $CC\bar{X}$)

    Validation criteria

    0.500 < = $MabC\bar{X}$ < = 2.000

    $CC\bar{X}$ < = 0.300

    Mean NC PI < = 0.35%

    50% < = Mean PC PI< = 80%

    60% < = Mean SPC PI < = 90%

    Interpretation

    Negative: S PI < 55%

    Positive: S PI > = 55%

cELISA test layout on 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									20
C	CP++	CP++	21									30
D	CP+	CP+	31									40
E	CP+	CP+	41									50
F	Cm	Cm	51									60
G	Cm	Cm	61									70
H	Cn	Cn	71									80

- Cc: Conjugate control (without serum, Mab=100% inhibition)
- Cm: Mab control (without serum= 100% inhibition)
- CP++: Strong positive serum control
- CP+: Weak serum positive control
- Cn: Negative serum control
- 1…80: serum sample to be control

Appendix 2: Conventional PCR for CCPP identification protocol

DNA extraction

The Dneasy Blood and Tissue kit (cat.nos.69504 and 69506) can be stored at room temperature (15-25^{o}C) for up to one year

Notes before starting

- ✓ Perform all centrifugation steps at room temperature (15-25^{o}C)
- ✓ Redisolve any precipitates in Buffer Al and buffer ATL
- ✓ Add ethanol to Buffer AW1 and buffer AW2 concentrates
- ✓ Equilibrate frozen tissue or cell pellets to room temperature
- ✓ Preheat an incubator to 56^{o}C
- ✓ Refer to the hand book for pretreatment of fixed tissue, insect, bacterial, or other material.

1a. Tissue: cut tissue (<=10 mg spleen or <=25 mg other tissue) into small pieces, and place in a 1.5ml micro-centrifuge tube. For rodent tail, use 1(rat) or 2 (mouse) 0.4-0.6 cm length of tail. Add 180µl Buffer ATL. Add 20µl protienase K, mix by vortexing, and incubate at 56^{o}C until completely lysed. Vortex occasionally during incubation. Vortex 15s directly before proceeding to step 2.

1b. Nonnucleated blood: pipet 20µl protienase K into a 1.5ml or 2ml microcentrifuge tube. Add 50-100 µl anticoagulant-treated blood. Adjust volume to 220µl with PBS. Proceed to step2.

1c. nucleated blood: pipet 20 µl protienase K into a 1.5ml or 2ml microcentrifuge tube. Add 5-10µl anticoagulant-treated blood. Adjust volume to 220µl with PBS. Proceed to step 2.

1d. Cultured cells: centrifuge a maximum of 5*10^{6} cells for 5 minutes at 300* g (190rpm). Resuspend in 200µl PBS. Add 20 µl protienase K. Proceed to step 2.

2. Add 200µl Buffer AL. Mix thoroughly by vortexing. Incubate blood samples at 56^{o}C for 10minutes.
3. Add 200µl ethanol (96-100%). Mix thoroughly by vortexing.
4. Pipet the mixture in to DNeasy Mini spin column placed in a 2ml collection tube. Centrifuge at >=6000*g. Discard the flow through and collection tube.

5. Place the spin column in a new 2ml collection tube. Add 500µl Buffer AW2, and centrifuge for 3minutes at 20,000*g (14,000 rpm). Discard the flow through and collection tube.
6. Place the spin column in to a new 2ml collection tube, add 500µl Buffer AW2, and centrifuge for 3min at 20,000*g (14,000rpm). Discard the flow through and collection tube.
7. Transfer the spin column to a new 1.5ml or 2ml microcentrifuge tube.
8. Elute the DNA by adding 200µl Buffer AE to the center of the spin column membrane. Incubate for 1 minute at room temperature (15-20^{o}C). Centrifuge for 1minute at >=6000*g.
9. Optional: repeat step * for increase DNA yield.

Protocol on preparation and use of gel Red

1. For PCR product

   95µl loading buffer–(960)

   25µl Gel red–(40)

   Mix by vortex and 4µl for 20µl of PCR product, mix by pipetting and load 10µl into the Gel
2. For DNA ladder

   475µl loading buffer
   25µl Gel red
   } Mix by vortex

   Then prepare the following again:

   25µl PCR water

   59µl pre-prepared (From No 2 above)

   27µl DNA ladder

   Mix by vortex, and use 10µl to load into the Gel as molecular marker

Date: - 23/05/2017

Conventional PCR for CCPP antigen detection and identity test procedure

1-Master mix preparation

SI No	Type of reagent	For one reaction	Total for 10 reaction	Remark
1	RNase free water	3µl	30 µl	
2	Primer- Mccp-Spe-Fow-5pM/µl 5'-ATCATTTTTAATCCCTTCAAG-3'	2 µl	20 µl	
3	Primer-Mccp-Spe-REV-5pM/µl 5'TACTATGAGTAATTATAATATATGCAA-3'	2 µl	20 µl	
4	IQ Super mix	10 µl	100 µl	
5	Template (DNA)	3 µl		
	Total volume/PCR tube	20 µl		

2-Run PCR Reaction

	Temperature	Time	Cycle	Remark
Initial Denaturation	95^{o}C	5 minutes	1-Cycle	
Denaturation	95^{o}C	30 Sec	40 Cycles	
Annealing	50^{o}C	30 Sec		
Elongation	72^{o}C	1 minute		
Final Elongation	72^{o}C	7 minutes	1-Cycle	
Put at	4^{o}C	Until machine off		

3-Agrose gel preparation and gel electrophoresis

- Prepare 1.5% Ag agrose gel
- Add 4µl Gel red with Loading dye, 10PCR product and 10µl markers (Ladder)
- Run Electrophoresis for 1hour at 100V
- Read the result using Gel documentation
- It is around band size of 316bp positive result.

Appendix 3: Annual domestic usage of CCPP vaccination in Ethiopia (Source: NVI plan and program office)

Year	**CCPP vaccine doses**
2006/07	69585
2007/08	315400
2008/09	683000
2009/10	543312
2010/11	942100
2011/12	464900
2012/13	2448000
2013/14	2469900
2014/15	3767850
2015/16	3320100

Appendix 4: Questionnaire format

ADDIS ABABA UNVERSITY

COLLEGE OF VETERINARY MEDICINE

QUESTIONNAIRE SURVEY

***Title*: SERO-PREVALENCE INVESTIGATION AND ASSOCIATED RISK FACTORS OF CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN SELECTED DISTRICTS OF CENTRAL ETHIOPIA**

FOR MSc THESIS RESEARCH

Dinberu Mamuye

I. Study Location and Interviewee Detail

Date of collector name……..

Code No…….

1. Region……Zone……District…Kebele……....
   pecific name of place……
2. Owner's Name……□ Male □ Female
3. Geo-reference of Kebele Long……Lat..…….Alt…….……
4. Flock structure

Species	**Young <2 year**	**Adult2-4 year**	**Old >4years old**	**Remark**
Sheep				
Goat				

II. CCPP Occurrence

1. List important health problems of goat mortality in your area? /It could be in local language/ ……
2. Have you had respiratory syndrome in goat coughing abduction of leg nasal discharge Yes □ No□
3. Have you had CCPP (Local name??) in your goat? Yes □No□ Name……

4. Could you list some of the sign of CCPP?

   ……

5. If the animal is once infected what did you take the measure

   □slaughtering □ Treatment □ isolation from the herd □Not take any measure

   ➢ If you were slaughter the goat, have you seen any lesion on the lung

   Please prescribe it……

6. When did the disease commence in the area (Kebele)?

   □Summer □winter □spring □autumn other ……

7. What measures are taken to prevent the above listed health problems?

   □Traditional treatment □ Modern treatment

   □Vaccination □ No treatment □Other……

8. What problems do you face when treating or vaccinating goat in your area (rank them)?

   □Lack of modern services/clinics □ Lack of drugs and vaccines

   □Transport/distance □other……

III. Flock Management

9. Do you move your shoats to other place for grazing seasonally? □Yes □No

   If yes, when……, where……, how long did you keep them there……

10. Grazing and watering resource managements

    □Communal □Private □Both

11. How do you raise your sheep and goat?

    □Sheep and goat grazing separately

    □ Sheep and goat grazing together

    □ Sheep and goat grazing with other livestock

    □ Sheep and goat tethered feeding at home

    □Other……

12. Housing: □ Fenced stable □ House barn

13. Did you vaccinate your goat for CCPP? □Yes □ No

14. Did you encounter any critical season of feed and water shortage?

    □Yes □ No, If yes in which season……

IV. Control and prevention Measure

15. What are the possible sources of CCPP?

□ Water point □ Grazing point □ New introduction from marketing.

16. Risk factors

□ herd sizes □ farming practices □ source of breeding stock

17. What measures do you take to combat the disease?

□ treatment □destocking □vaccination □isolation □slaughtering

Appendix 5: Serum sample collection format

Region……Zone……

District……

PA……..

Village……Code…….

Date ……Geo ref, long……..Lat……..alt….....

No	Owners name	District	PA	Sex	Spp	Age	Last vacc of CCPP	Remark
1								
2								
3								
4								
5								

Appendix 6: Age determination based on teeth eruption of small ruminants (DeLahunta and Habel, 1986)

No	Teeth	Ages
1	Eight sharp incisors/ No permanent teeth/	under one years
2	2 permanent teeth	2years
3	4 permanent teeth	3 years
4	6 permanent teeth	4 years
5	8 permanent teeth	5 years
6	Worn teeth and some missing	5 years and above

Appendix 7: cELISA test PI value

	1	2	3	4	5	6	7	8	9	10	11	12
A	CCAVE	0.06	7.85	14.73	36.31	24.26	19.89	11.42	20.16	27.44	29.96	33.80
B			51.41	45.98	54.19	43.33	54.45	35.12	45.05	90.73	20.56	7.71
C	PPAVE	0.27	47.43	36.18	17.38	47.43	55.38	27.97	26.38	34.86	12.61	35.78
D			86.63	51.94	33.66	47.70	29.43	9.04	31.94	32.74	35.39	30.75
E	PAVE	0.40	32.61	23.60	53.00	27.84	15.66	41.08	30.75	18.44	31.41	27.71
F			47.17	29.56	9.57	12.61	18.04	37.90	-7.51	22.41	35.65	47.70
G	MCAVE	0.81	50.88	37.50	8.90	38.43	26.51	41.21	50.22	32.08	36.84	25.32
H	NCAVE	0.77	46.11	49.55	30.35	27.44	37.11	24.79	16.45	16.85	29.56	4.14
	1	2	3	4	5	6	7	8	9	10	11	12
A	CCAVE	0.06	42.04	54.22	43.99	39.85	37.90	37.53	53.49	36.19	57.50	82.71
B			53.85	53.36	53.61	59.70	65.30	90.02	57.50	58.36	64.57	43.50
C	PPAVE	0.28	84.66	59.09	70.90	62.62	77.35	60.67	74.79	72.12	53.61	48.98
D			48.13	49.95	45.69	58.72	50.44	92.69	49.83	42.28	90.62	47.40
E	PAVE	0.42	84.66	71.87	77.60	20.97	61.28	39.00	45.69	41.43	28.40	73.46
F			58.72	57.14	38.14	37.17	55.31	44.60	65.05	55.56	78.45	49.59
G	MCAVE	0.88	52.15	41.67	56.41	50.08	48.37	55.19	46.30	42.89	51.17	23.77
H	NCAVE	0.88	41.43	39.48	49.10	33.27	42.53	40.46	39.24	46.67	35.71	79.42
	1	2	3	4	5	6	7	8	9	10	11	12
A	CCAVE	0.07	42.54	47.70	54.33	47.11	30.31	52.12	91.31	21.47	30.90	21.47
B			59.93	49.76	43.87	44.60	30.02	54.48	58.45	35.62	30.61	25.89
C	PPAVE	0.26	40.33	34.29	44.01	41.07	39.30	37.97	40.48	52.85	50.79	49.47
D			26.78	38.56	37.09	36.50	35.17	27.66	35.91	35.17	38.42	39.01
E	PAVE	0.39	56.39	24.27	44.75	34.00	47.85	37.97	43.28	40.92	54.18	32.38
F			45.64	46.08	32.82	42.69	87.03	21.92	21.18	22.36	22.36	39.30
G	MCAVE	0.74	29.13	42.10	33.85	33.26	31.79	45.93	41.36	27.96	43.43	23.68
H	NCAVE	0.85	37.53	46.37	35.91	27.96	26.19	26.78	66.26	22.95	35.03	19.12
	1	2	3	4	5	6	7	8	9	10	11	12
A	CCAVE	0.11	16.47	11.52	25.09	32.16	33.14	18.45	9.68	30.18	20.71	34.28
B			47.99	57.17	34.84	46.15	49.96	43.75	42.05	36.25	27.49	25.23
C	PPAVE	0.28	42.90	31.17	28.34	44.45	35.83	43.89	46.29	23.82	32.86	48.13
D			35.69	35.69	38.37	28.34	19.01	37.10	37.53	55.19	55.05	37.39
E	PAVE	0.42	13.78	22.54	29.33	17.74	-2.90	25.65	40.78	12.79	59.29	32.86
F			20.14	33.71	27.21	30.32	11.10	25.51	17.60	56.47	32.01	23.39
G	MCAVE	0.82	47.70	35.55	47.84	26.64	36.54	40.21	59.15	37.39	53.07	35.69
H	NCAVE	0.81	41.20	48.27	42.05	10.95	41.77	33.85	58.16	51.24	32.58	15.34
	1	2	3	4	5	6	7	8	9	10	11	12
A	CCAVE	0.06	38.14	92.59	34.73	46.93	23.19	38.01	65.82	28.17	31.58	81.83
B			29.62	54.67	49.29	37.49	46.67	61.23	49.43	71.07	40.37	25.94
C	PPAVE	0.25	45.88	47.85	35.26	42.21	44.97	65.96	32.24	41.95	36.57	34.60
D			47.06	37.09	38.01	34.34	41.82	42.08	34.86	37.23	39.85	56.90
E	PAVE	0.38	47.33	22.01	14.14	20.30	72.12	29.09	29.22	20.70	32.37	26.21
F			29.62	31.85	32.90	14.53	8.10	1.94	19.25	44.44	51.79	16.89
G	MCAVE	0.83	32.90	24.50	26.60	29.88	20.17	31.72	32.90	48.38	30.53	23.58
H	NCAVE	0.81	39.46	42.34	24.11	31.32	23.84	39.32	37.62	26.21	28.04	21.09

Appendix 8: Different images taken during field work

Typical Flock (A) Fentale district (B) Around Alage College district

Housing system C. Fentale simple fenced barn D. around Alage common type of pen

Drug sellers displaying antibiotics and anthelmintics on open air at Metahara

With Fentale pastoral