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**FIELD EVALUATION OF INACTIVATED WHOLE CULTURE OF
CONTAGIOUS CAPRINE PLEUROPNEUMONIA VACCINE FOR ITS
SAFETY AND IMMUNOGENICITY AT ADAMI TULU AGRICULTURAL
RESEARCH CENTER OROMIA REGION, ETHIOPIA**

MSc Thesis



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JUNE 2017
BISHOFTU, ETHIOPIA

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

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**JUNE 2017
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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluate the thesis prepared by: Behailu Tefera Haile entitled a study on “Field Evaluation of Inactivated Whole Culture of Contagious Caprine Pleuro-pneumonia Vaccine for its Safety and Immunogenicity at Adami Tulu Agricultural Research Center Oromia Region, Ethiopia” and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Science in Veterinary Microbiology.

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ABBREVIATIONS

APHRD	Animal and Plant Health Regulatory Directorate
ATARC	Adami Tulu Agricultural Research Center
AU-IBAR	African Union-InterAfrican Bureau for Animal Resources
CCPP	<i>Contagious caprine pleuro pneumonia</i>
Celisa	Competitive Enzyme Linked Immunosorbent Assay
CFT	Complement Fixation Test
CSA	Central Statistics Agency
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
G+C	Guanine + Cytosine
GIT	Growth Inhibition Test
HRPO	Horse radish Peroxidase
IFA	Indirect Fluorescent Antibody
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHA	Indirect Haemagglutination
LAT	Latex Agglutination Test
Mab	Monoclonal Antibody
MAKePS	Mastitis, Arthritis, Keratitis, Pneumonia and Septicemia
MCCP	<i>Mycoplasma Capricolum subsp Capri Pneumoniae</i>
Mmc	<i>Mycoplasma mycoides Subsp capri</i>
NVI	National Veterinary Institute
PCR	Polymerase Chain Reaction
PI	Percent Inhibition
SDS	Sodium Dodecylsulfate polyacrylamide gel electrophoresis
TMB	Tetra Methyl Benzoic Substrate
VFG	Viande Foie Goat
WSB	Working Seed Bank

ABSTRACT

This study was conducted between December 2016 and May 2017 in East Shewa Zone Oromia Regional State Adami Tulu Agricultural Research Center to determine field performance (seroconversion) of contagious caprine pleuropneumonia inactivated whole culture commercial vaccine, produced by the National Veterinary Institute in Ethiopia. Study animals were screened for absence of *Mycoplasma capricolum subsp capri pneumoniae* specific antibody and then tested for evidence of seroconversion after vaccination on day 28th post vaccination by using competitive enzyme linked immunosorbent assay and then percent sero positivity and the difference in percent inhibition between pre vaccination and post vaccination sera was analyzed. The result of the current study showed that out of 358 study animals 246 (68.7%), CI= 63.6-73.5, showed seropositivity on day 28 post vaccination. The comparison of percent inhibition of pre vaccination and post vaccination sera showed that the percent inhibition of day 28 post vaccination sera to be significantly higher ($p=0.0001$) than the percent inhibition of pre-vaccination sera. In conclusion, the present study indicated that field vaccination of goats by inactivated whole culture vaccine induced sero-positivity in majority of the vaccinated goats. However, the field immunogenicity test needs to be conducted broadly in different areas to see the performance of the vaccine in different field conditions. Moreover, future controlled experimental studies with challenge infection after vaccination need to be conducted for further evaluation of the vaccine efficacy.

Keywords: *Adami Tulu Agricultural Research Center, contagious caprine pleuropneumonia, Ethiopia, inactivated whole culture vaccine, Oromia, sero-conversion,*

1. INTRODUCTION

Contagious caprine pleuropneumonia (CCPP) is a highly infectious and devastating respiratory disease of goat caused by *Mycoplasma capricolum subspecies capripneumoniae* (*Mccp*). Clinically the disease is characterized by coughing, respiratory distress and very high morbidity and mortality rates (Thiaucourt and Bolske 1996). The disease is included in the list of notifiable diseases of World Organization for Animal Health as it threatens a significant number of goat populations throughout the world causing significant socioeconomic impact mainly in Africa and Asia. Infection of susceptible goats is mainly through inhalation of contaminated aerosols from infected goats. Due to the high sensitivity of *Mycoplasma species* to the external environment, close contact between infected and susceptible animals is essential for effective transmission of *Mccp* to take place (Thiaucourt *et al.*, 1996), and overcrowding and confinement have been known to favor close contact and circulation of the Mycoplasma. Stress factors such as malnutrition and movement over long distances have been documented to enhance spread and morbidity of the disease (Lefevre, *et al.*, 1987) (Mekuriya, and Asmare, 2010).

In Africa where extensive and traditional husbandry is practiced, pathogens have been reported to spread when animals meet at watering points and grazing areas. Latent carriers such as goats or sheep that recovered from the infection without becoming bacteriological sterile were reported responsible for the perpetuation of the disease in herds (Thiaucourt and Bolske 1996) (Wesonga *et al.*, 1993). Clinically, the disease is characterized by high fever, respiratory distress and high mortality between 60% and 100% in absence of antibiotic intervention (Thiaucourt and Bolske, 1996; Kaliner *et al.*, 1976; Jones and Wood, 1988).

The pathological lesions are characteristic presenting interstitial fibrinous pleuropneumonia, interlobular oedema and hepatization of the lung. This involvement is often unilateral, and the affected lungs are known to be port-wine coloured with possible total hepatization (Thiaucourt and Bolske 1996) (Thiaucourt *et al.*, 1996). There is involvement of the pleura with the pleural cavity containing straw-colored exudate with fibrin flocculation's (Kaliner *et al.*, 1976) (Wesonga *et al.*, 1993). CCPP is a disease of major economic

importance in Asia and Africa, causing major constraint to goat production because of high mortalities (Rurangirwa *et al.*, 1984) but the presence of CCPP in Ethiopia had been suspected since 1983 and was confirmed later in 1990 by isolation and identification of *Mccp* following outbreak of CCPP in Ogaden, eastern Ethiopia (Thiaucourt *et al.*, 1992). Since then the disease has been known to be endemic in different regions of the country (Sharew *et al.*, 2005). Outbreak of CCPP has been reported from almost all regions of the country especially from low land areas which are known in goat rearing regions (APHRD, 2010). Vaccine against CCPP is currently produced from *Mccp* (F 38 strain) which is Kenyan source by the NVI in Ethiopia. The vaccine is inactivated by 0.05% formaldehyde and adjuvated with 0.3% saponin. This vaccine is extensively used for the control of disease in endemic area of the country (APHRD, 2010).

CCPP is a highly contagious disease; hence, control of the disease is one of the priority areas of the country. Like most African nations, vaccination remains the most cost effective strategy to control animal disease in Ethiopia. To realize success in vaccination campaigns, use of effective vaccine is crucial (AU-IBAR, 2013). According to previous studies conducted in Kenya, inactivated CCPP vaccines consisting of saponized organisms have been shown to be protective but the quality and efficacy may be variable (Rurangirwa *et al.*, 1987). For long period inactivated CCPP vaccine has been produced by either centrifugation or concentration techniques at the National Veterinary Institute (NVI) in Ethiopia, using saponin as an adjuvant. Recently, since 2016 the NVI shifted the production of vaccine to inactivated whole culture vaccine. The recent experimental study made by the Institute (Tesgera *et al.*, 2015) proved a relatively improved the safety and immunogenicity of the inactivated whole culture CCPP vaccine as compared to the previous vaccine production technique. Hence, the institute shifted its CCPP vaccine production technique to this inactivated whole culture technique.

However, the immunogenicity of inactivated whole culture *Mccp* vaccine under field conditions has not been investigated and well documented with detail studies. Therefore, the present study was conducted in Adami Tulu Agricultural Research Center (ATARC) East Shewa Zone Oromia Regional State to evaluate the safety and immunogenicity of the existing inactivated whole culture vaccine.

Hence, the objectives of this study were:

- To evaluate safety of inactivated whole culture vaccine at field level.
- To evaluate the immunogenicity (seroconversion) of the vaccine at field level.

2. LITERATURE REVIEW

2.1. Economic Importance of Goats

Ethiopia is home, excluding some pastoral areas of Afar and Somali regions, to approximately 24 million goats (CSA 2013). Though the population density of goats in mid- and low-altitude areas is high, they are produced across the country from the arid lowlands to the coolest highland areas.

Goats are amongst the commonest farm animal species, which sustain the livelihoods of smallholder farmers, pastoralists and agro pastoralists alike. They fulfill various functions such as generating cash income, serving as household security, accumulating capital, and fulfilling cultural obligations (Workneh and Peacock 1993; Workneh 2000; Grum 2010; Dhaba *et al.*, 2012; Feki, 2013).

Goats are also a source of other non-food products such as skin and manure. No recognized use of fiber has been reported from Ethiopian goats though some goat breeds (e.g. Arsi-Bale goats of Bale highlands) are known to have long hair. Goats also significantly contribute to the live animal and meat export trade.

They play an important role in smallholder mixed, agro pastoral and pastoral production systems due to their low initial capital investment, ability to produce multiple products (meat, milk, skin, manure etc.) at low input costs, high rates of reproduction (multiple births are not uncommon), and high turnover rates due to the short time they take to attain maturity.

The current contribution of goats to the country's economy and producers' livelihoods is however still below the total potential production capacity (Girma *et al.* 2000).

2.2. CCPP

CCPP is a disease affecting goats and some wild ruminant species, caused by Mccp. In goats, it is manifested by anorexia, fever and respiratory signs such as dyspnoea, polypnea, cough and nasal discharges. The acute and subacute disease is characterised by

unilateral sero-fibrinous pleuropneumonia with severe pleural effusion. Diagnosis is carried out by clinical and necropsy observations that should be confirmed by laboratory tests. As the isolation of *Mccp* is difficult, molecular techniques should be the methods of choice for laboratory confirmations (OIE, 2014).

2.2.1. Etiology

The causative agent of CCPP has recently been named *Mccp* (Leach *et al.*, 1993) previously, this agent was known as *Mycoplasma* spp. type F38, as the taxonomic position had not been clearly determined, each isolate has been compared with one of the strains that had been isolated in Kenya: the F38 strain. The authors used the abbreviation F38 for these strains, in an attempt to link the new name with the former denomination. It is important to remember that *Mccp* was characterized only in 1976, meaning that all previous publications were to be analyzed with care, since *Mycoplasma mycoides subspp. Capri (Mmc)* was considered responsible for CCPP before this finding (Edward, 1953; Gee, 1977; Jonas and Barber, 1969; Little Johns and Cottew., 1977; Longley, 1951; Macowan and Minette, 1976 and Moulton, 1980). The etiological role of *Mccp* cannot be questioned now. Numerous writers have successfully reported the disease with the causative agent (Macowan and Minette, 1976). Since diagnostic tools have been improved, *Mccp* is regularly demonstrated in clinical cases in which there is a strong suspicion of CCPP (Bolske *et al.*, 1996). Therefore, CCPP and *Mccp* fulfill all Koch postulates, as these have been revisited (Evans, 1976).

2.3. Mycoplasma species in goats

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

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

goats but mastitis induced experimentally. It is believed that *Mycoplasma mycoides sub species capri* has a clear tropism for lung involvement (Lefevre *et al.*, 1987b, DaMasa *et al.*, 1992).

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

Mycoplasma mycoides sub species mycoides large colony (MmmLC) has one of the widest geographical distributed ruminant mycoplasmas, being found on all continents (Bolske *et al.*, 1988). The gross lesions seen include lung lesions with edema and thickening of the interlobular septa. There may be septicemia accompanied by the enlarged spleen and the lesions present in several organs. Microscopically the alveoli contained mononuclear cells and scattered granulocytes, which fills the bronchioles (Bolske *et al.*, 1988).

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

The natural infection can be characterized by MAKePS syndrome including Mastitis, Arthritis, Kerato conjunctivitis manifestations in various organs appear after an initial septicemia; a stage which can be fatal for kids and adult animals in poor condition (Perreau *et al.*, 1984). The gross pathology is confined to the joints and lungs. The lungs showed interstitial pneumonia and purulent bronchopneumonia. Interlobular septa were broadened due to edema and cellular infiltration mainly by mononuclear cells. The alveoli contained increased number of alveolar macrophages and there was marked peri bronchial lymphoid hyperplasia (Bolske *et al.*, 1988).

Research into the control of CCPP was initially hampered by the uncertainty over the exact cause of the disease. Two mycoplasmas, *Mycoplasma mycoides subsp. mycoides LC* and *M. mycoides subsp. capri*, were for some time implicated in the aetiology of the disease because they caused a pleuropneumonia in small ruminants that resembled CCPP. It was not until 1976 that a mycoplasma, designated F38 but later renamed *Mycoplasma capricolum subsp. capripneumoniae*, was isolated for the first time in vitro by MacOwan and Minette (1976) using a specific medium that they had developed for this highly fastidious organism. Its role as the primary cause of classical CCPP was confirmed following experimental infections.

However, in spite of this evidence, respiratory diseases caused by *M. mycoides subsp. capri* are still referred to erroneously as CCPP particularly in the Middle East and India. According to the World Organization for Animal Health (OIE. 2008), a case should only be defined as CCPP when the following criteria have been satisfied:

1. *Mycoplasma capricolum subsp. capripneumoniae* is isolated or there is strong serological evidence of the mycoplasma.
2. Lesions are restricted to lung and pleura and consist of a pleuropneumonia.
3. There is no enlargement of the interlobular septa of the lung.

The condition is also highly contagious with high levels of morbidity and mortality when immunologically naive goats are infected; however, in endemically infected regions, disease outbreaks may be more sporadic.

2.4. Growth characteristics

The mollicutes grow slowly and generally require 3 to 6 day incubation before colonies are apparent. Growth is best at 37⁰ c in atmosphere of increased CO₂. Sterols are required by all genera except *Acholeplasma* and *Anaplasma*. Most genera are facultative anaerobes except *Anaeroplasma* and *Asteroplasmas*, which are obligate anaerobes. Optimum pH for growth ranges from 6.0 *Ureaplasma* and 7.5 to other Mollicutes. Colony sizes Vary from 0.1 mm to 1.0 mm. When observed with dissecting microscope, many species exhibit “fried egg” morphology (Jones, 1992; Adehana *et al.*, 2004). The umbonate appearance is as a result of the central portion of the colony embedding in to the agar with peripheral zone of surface growth some species produce film and spot which are composed of cholesterol and phospholipids and seen as a wrinkled film on the media surface (Ojo, 1976; Jones, 1992; Adehana *et al.*, 2004).

2.5. Host Specificity

Another characteristic in mycoplasma infection is the high level of host specificity. Usually a mycoplasma species will infect only a single or few closely related animals species. It has been suggested that the degree of host specificity is directly related to the intimate nature of the parasite relationship and dependence of the infecting mycoplasma on

the host cell component to fulfill its fastidious nutritional requirements for successful colonization host may also be related to ability of mycoplasma to successfully invade the cellular host defense mechanism, which differ in various cell types (Levinsohn, 1992).

2.6. Epidemiology

In natural infections, susceptible goats acquire the organisms by inhalation of contaminated droplets from infected goats (MacOwan, 1984). The environment as a whole plays an important role in the appearance, evolution and severity of CCPP. Due to the high sensitivity of mycoplasmas to the external environment, close contact is essential between infected and naive animals for transmission to take place, and, overcrowding and confinement favors close contact and circulation of mycoplasmas, Stress factors due to malnutrition and movement over long distances can predispose the animal to disease, in Africa where extensive and traditional husbandry is practiced, pathogens spread when animals meet at watering points and grazing areas. Breed and sex appear not to affect the epidemiology of CCPP, but age is an important factor. Though all age groups are susceptible, mortality is higher among young animals than adults. Infective *Mccp* may persist in chronic, latent carriers, such as goats, which have recovered from infection without becoming bacteriologically sterile, and are considered responsible for the perpetuation of the disease in a herd (Thiaucourt and Bšlske, 1996; Wesonga *et al.*, 1998). This aspect of the epidemiology was described as early as 1881 in the case of CCPP in South Africa (McMartin *et al.*, 1980, Lefèvre *et al.*, 1987).

2.6.1. Geographic Distribution

While the clinical disease has been reported in nearly 40 countries in Africa and Asia, *M.c. capripneumoniae* has only been isolated in 20 countries because few laboratories have the expertise for isolating and growing mycoplasmas (Nicholas *et al.*, 2008). In 2003, CCPP was diagnosed in Thrace, the region of Turkey on the European mainland bordering Greece and Bulgaria (Ozdemir *et al.*, 2005). Prior to this infection, the only report of CCPP in Europe dates to the 1920s when an outbreak occurred in Greece following the seizure of goats from Turkey although the exact cause was never confirmed.

Interestingly, Greece reported two outbreaks of CCPP in 2006 but it seems likely that this was caused by *M. m. capri* which is endemic in Greece rather than *M. c. capripneumoniae* as the mortality rate was <1% of goats in a herd of above 150 (OIE, 2008). It is also likely that the outbreak of CCPP in Czech Republic in 1902 was similarly misdiagnosed; though, it is clearly impossible to confirm over a century later. There have been no reports of the isolation of *M. c. capripneumoniae* on the American continent although other closely related members have been described there (Nicholas *et al.*, 2008).

Serious problems caused by CCPP exist in Oman where nearly 600 outbreaks were reported between 2008 and 2009 with mortality rates of nearly 10% of 30 000 cases. In Iran, 478 outbreaks were seen affecting more than 16 000 goats between 2006 and 2007 (OIE, 2008/2009). The 38 outbreaks reported in Ethiopia between 2006 and 2007, respectively; almost certainly represent an underestimate as this disease is having a major socio-economic impact there. New outbreaks were reported in Tajikistan in 2009 with four outbreaks affecting 166 goats, most of which died. In Tanzania, 10 outbreaks affected more than 200 goats of which 38 died while another 1128 were culled (OIE, 2009). A dozen new outbreaks occurred in Yemen affecting more than 800 goats of which just above 200 died. Mauritius became infected for the first time in 2009 following the introduction of goats from the African mainland, and within one month, just more than 300 homebred goats had died (Srivastava *et al.*, 2010).

Although the recent confirmation of the presence of CCPP in continental Asia has provided a better estimation of the distribution of CCPP worldwide, some questions still remain. CCPP has only been reported in sixteen countries, while, if we take into consideration reports of clinical disease, over forty countries of Africa and Asia may be affected. The boundaries of the disease in Asia, as well as towards the west and south of the African continent are still uncertain but, taking into consideration the contagiousness of the disease and the movements of nomadic goat herds, CCPP is probably present in central and north-east Africa, the Middle East and all the way through to China.

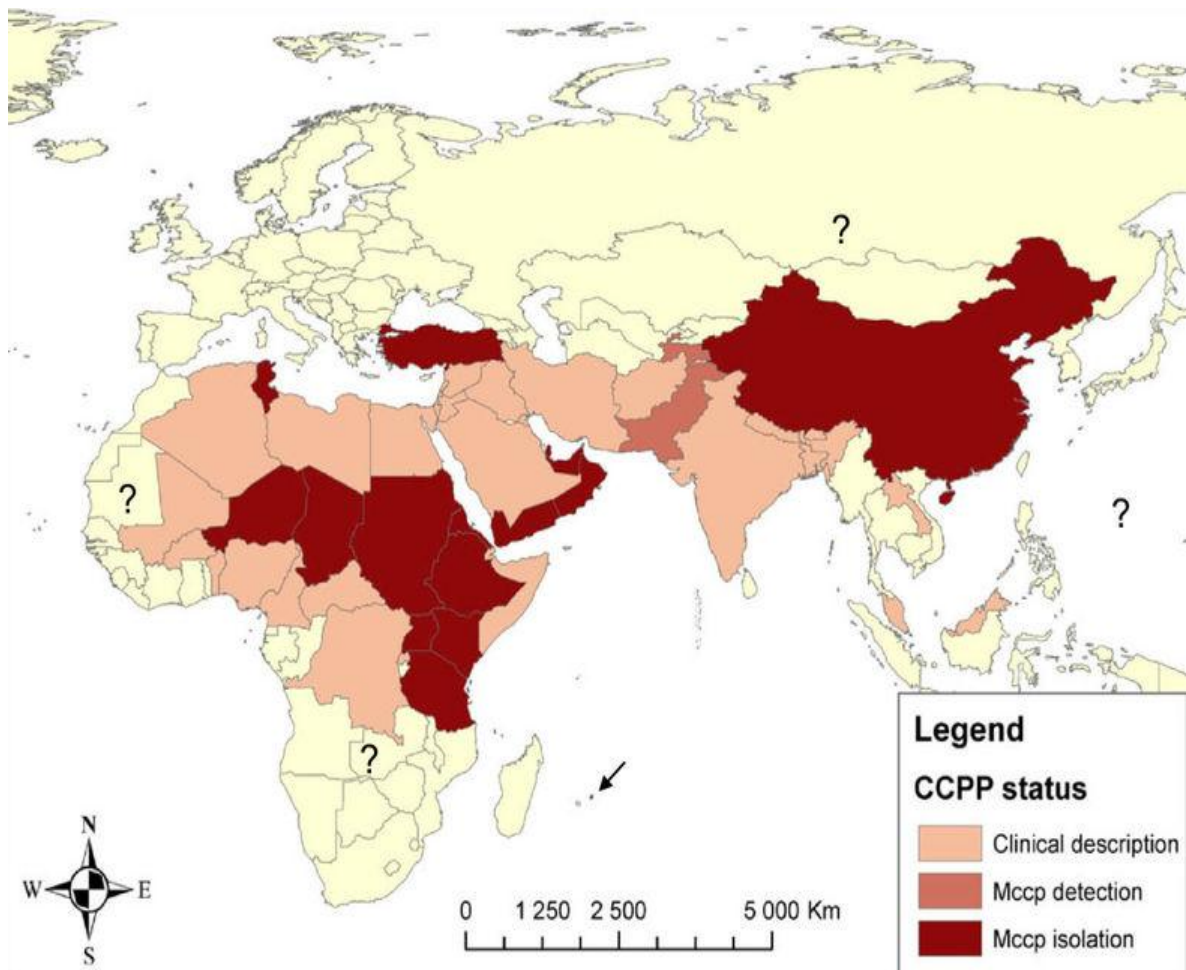


Figure 1. Shows an updated map presenting the probable distribution of CCPP

Probable distribution of CCPP; the countries in which the disease has been described, those in which the etiologic agent has been detected using molecular tests and those in which it has been isolated are indicated. The arrow indicates the presence of the disease in Mauritius, where Mccp was isolated in 2009 (Manso-silvan *et al.*, 2011).

2.6.2. Current status of CCPP in Ethiopia

In Ethiopia CCPP has been suspected to occur for a long period, especially in areas found at the vicinity of endemic areas of Kenya and Sudan. It has been confirmed to be present in Ethiopia since 1980s CCPP has been reported from almost all regions of Ethiopia

including Tigray, Afar, Dire Dawa, SNNP, Oromiya, Benishangul-Gumuz and Amhara regional states (Thiaucourt *et al.*, 1992; Bereket, 1995; Yigezu *et al.*, 2004). It is more prevalent in the arid and semi-arid low land of rift valley; Borena rangelands, South Omo, Afar and other pastoral areas of Ethiopia where about 70% of the national goat population are exist. Various authors as shown in Table 1 reported different sero prevalence rates.

Table 1.Prevalence of CCPP in different part of Ethiopia

Area	Prevalence rate (%)	Animal species	References
East Shoa	51.5	Goat	Gezahegn, 1993
Konso	35	Goat	Bereket, 1995
Arbaminch Zone	36	Goat	Mekonnen, 2006
Gewane	33	Goat	Roger and Bereket, 1996
Fentale East Shoa	16	Goat	Roger and Bereket, 1996
Borena	31.6	Goat	Lakew, 2014
Afar	42.8	Goat	Amare, 2012
Borena (yabelo)	24	Goat	Dawit, 1996
East Shoa	17.5	Goat	Teshome, 1997
Arsi	52.7	Goat	Yigezu <i>et al.</i> , 2004
Dire Dawa	32.36%	Goat	Sherif <i>et al.</i> (2012)

2.6.3. Transmission

CCPP is transmitted directly by an aerogenic route through contaminated droplets (Thiaucourt *et al.*, 1996). The outbreak of the disease follows the introduction of an infected animal into a group of susceptible goats (OIE, 2009). The disease is readily contagious and a short period of contact is enough for successful transmission through coughing (Thiaucourt and Bolske, 1996; OIE, 2009). No evidence of indirect contact has been shown, as the organism is highly fragile in the environment (Thiaucourt and Bolske, 1996). It is quickly inactivated within 2 min at 60 °C but can survive for more than 10 years in frozen infected pleural fluid (OIE, 2009). Disease outbreak may occur after heavy rain, animal transportation over a long distance (OIE, 2009), poor climatic conditions and

primary infections (Thiaucourt and Bolske, 1996). Formaldehyde can inactivate *Mccp* in 30 s at a concentration of 0.05%. A solution of 1.0% phenol can inactivate the organism within 3 min (OIE, 2009).

Incubation period of *Mccp* is normally very short in the lungs (three to five days) but this may be prolonged (three to four weeks) depending on predisposing factors (Thiaucourt and Bolske, 1996). In primary infected goats, CCPP last for about two days with high mortality (McMartin *et al.*, 1980) while in other cases it may last for several days (OIE 2009). However, in the experimental infection model of March *et al.* (2002), *Mccp* was not isolated from the infected lungs of goats (eight-week post infection) due to the development of humoral immunity (IgG, IgM).

2.6.4. Pathology

The gross pathological lesions are localized exclusively to lung and pleura and are often unilateral. Affected lungs can be totally hepatized, and have a port wine colour (Thiaucourt and Bošlske, 1996). A lung section shows a fine granular texture with various colours, but usually without any thickening of the interlobular septa. There is often an abundant pleural exudate and conspicuous pleuritis. The pleural exudates can solidify and form a gelatinous covering sometimes over the whole lung. In acute cases, the pleural cavity contains an excess of straw-colored fluid with fibrin flocculation's (Kaliner and MacOwan, 1976; Wesonga *et al.*, 1993). In chronic cases, there is a black discolouration of the lung tissue and sequestration of the necrotic lung areas. Adhesions between the lung and the pleura are very common and often very thick (MacOwan and Minette, 1977).

Histological examination of the lung tissues may show acute serofibrinous to chronic fibrinonecrotic pleuropneumonia with infiltrates of serofibrinous fluid and inflammatory cells, mainly neutrophils, in the alveoli, bronchioles, interstitial septae and subpleural connective tissue. Intralobular oedema is more prominent but interlobular oedema has also been reported. Peri-bronchial and per-bronchiolar lymphoid hyperplasia with mononuclear cell infiltration is also present (Kibor, 1990; Wesonga *et al.*, 1998; Msami *et al.*, 1998).

2.7. Genetic diversity of Mycoplasma

Mycoplasmas are the smallest free-living fastidious bacteria. They are about 300 nm in diameter, bound by a triple layered membrane and unlike conventional bacteria they don't have a rigid cell wall of murin (Robinson and Bebear, 1997). Their genome size is only one sixth to one third of that of *Escherichia coli* (Bascunana *et al.*, 1994). Mycoplasmas are phylogenetically related to gram-positive bacteria with low G + C content (Razin *et al.*, 1983; Bascunana *et al.*, 1994). The *Mycoplasma mycoides* cluster has two rRNA operons in which intra specific variations have been demonstrated (Heldtander *et al.*, 2001). *Mccp* was once thought to be a homogenous taxon (Abu-Groun *et al.*, 1994; Nicholas 2002; Manso-Silvan *et al.*, 2007), but the discovery of two molecular markers showed some degree of heterogeneity among strains that opened a further channel for studies on the molecular epidemiology of CCPP (Manso-Silvan *et al.*, 2011).

2.8. Immunology

Little is known of the immunology of CCPP despite a number of reported experimental infections. More recently monitored the humoral response of goats infected with a multi-passaged *Mccp* strain 19/2 with several serological tests and PCR. While there was little evidence after infection of the infectious agent or clinical or pathological disease, apart from elevated temperatures and a transient cough in one goat, serological responses were detected by latex agglutination test and competitive ELISA. Immunoglobulin G (IgG) immune-dominant bands of 23, 40 and 44 kDa were seen by immunoblotting in all experimentally infected animals as well as in some sera from a natural outbreak of CCPP in Eritrea which additionally showed bands of 62, 70 and 108 kDa (Muthomi *et al.*, 1983; Perreau *et al.*, 1984; March *et al.*, 2002).

2.9. Diagnosis

2.9.1. Clinical signs

All goats can be affected, whatever their age or sex (Thiaucourt *et al.*, 1996). The incubation period generally lasts 10 days, but may vary between 2 and 28 days. Acute

cases can be observed in regions where CCPP is introduced for the first time to naive populations (OIE, 2008). The first symptom to appear is a reluctance to walk, fever is extreme (41°C) but the animals continue to feed and ruminate, abortions are frequent in pregnant goats, gradually, the respiratory symptoms become prominent, respiration is accelerated and painful, and accompanied by violent coughing, in the terminal stages, the animals are unable to move, they stand with their legs wide apart, the neck is stiff and extended, saliva continuously drips from their mouth and their nose is obstructed by a mucopurulent discharge (Nicholas, 2002; OIE, 2009).

The duration of the disease varies according to the environmental conditions. This duration may vary from a few days for animals that are subjected to harsh conditions, such as underfeeding, poly-parasitism, the necessity to walk long distances for watering, bad climatic conditions...etc. However, animals can survive more than one month or even recover if they are placed in good condition. Subacute or chronic forms can be observed in regions where CCPP is enzootic. The symptoms are similar to those of the acute cases, but are not as strong. Coughing is irregular and usually follows a physical effort, and nasal discharge may be absent. In the absence of antibiotic treatment, mortality varies between 60% and 100% (Rurangirwa and McGuire, 2012; OIE, 2009).

2.9.2. Post-Mortem Examination

Pathological changes are confined to the chest cavity and consist of pleuropneumonia: unilateral hepatisation, an accumulation of straw-coloured pleural fluid and acute pleuritis of the pleura adjacent to the affected lung (Wesonga *et al.*, 1993).

2.9.3. Isolation of Mycoplasmas

The growth of MccF38 usually takes four to five days, when first isolated, and the diameter of the colonies may be only 0.1 mm, these colonies can only be seen by close observation with a binocular microscope. In a liquid medium, the turbidity is very faint and should be compared with inoculated medium. The composition of the medium is very important and determines the rate of success as well as the size of the colonies. Some authors have recommended using fresh meat infusion to obtain good growth (Kibor, 1990);

though this may not be necessary and commercial components may ensure more regular results.

Horse serum permits good growth of MccF38. The serum should be added at a concentration of 20% to 30% by volume. Once again, the quality of this component must be tested, by comparing different batches from different manufacturers with dilutions of freeze-dried reference mycoplasma strains. Some authors have recommended the use of foetal calf serum or donkey serum (Jones and Wood, 1988). The latter may be interesting for developing countries, as it is very easy and cheap to obtain locally, fresh yeast extract is also an important component of the medium and the yeast extracts that are available commercially are not of the same quality.

The other major difficulty lies in the presence of other bacterial and fungal contaminants in the samples. Usually, the classic bacteria are inhibited by the antibiotics, chiefly ampicillin, that is incorporated into the medium used for the primary culture. However, other mycoplasmas, such as *M. arginini* or *M. ovipneumoniae*, are often isolated. These two mycoplasma species are natural colonizers of the upper respiratory tract of goats and can be regularly isolated from healthy lungs (Bölske *et al.*, 1989), as well as from diseased lungs (Gupta and Verma, 1984; Radwan *et al.*, 1985). There is some indication that *M. ovipneumoniae* may have pathogenic potential but this is probably secondary. *M. ovipneumoniae* may play a role in lung lesions that have a multifactorial origin, particularly in association with viruses or other bacteria, such as the Pasteurella species (Nicholas, 2002).

In the case of CCPP lesions, *M. ovipneumoniae* is often isolated (Jones and wood, 1988). However, where MccF38 and *M. ovipneumoniae* co-exist, the former is present in higher quantities, as has been demonstrated with a quantitative method, such as sodium dodecyl sulfate Polyacrylamide gel electrophoresis (SDS). The isolation of *M. ovipneumoniae* from CCPP lesions should therefore be interpreted with great caution and its aetiological role should be questioned. A highly pathogenic bacterium would presumably disseminate rapidly to numerous animals, hence all isolates from the same herd would be very homogeneous. That is not the case with *M. ovipneumoniae*, as numerous distinguishable

strains of *M. ovipneumoniae* can be isolated from a single herd or even from a single animal (Ionas *et al.*, 1991; Thirkell *et al.*, 1990).

2.9.3.1. Selection of samples

One of the best samples is pleural fluid. Ten milliliters should be harvested aseptically from an animal that is in the acute phase of the disease. Dead animals should be avoided. The best method is to sacrifice at least one animal of the herd that did not receive any antibiotic treatment. Other samples may be gathered from live animals by lower intrathoracic puncture with broad diameter needles. Samples of hepatized lungs are also desirable. Square 3 cm sections should be taken from an area that borders normal lung tissue. There is no need to multiply the number of samples and all efforts should be directed towards obtaining samples of good quality. Samples can be kept at +4°C if transport to the laboratory does not take more than one or two days. Otherwise, they can be deep frozen at -20°C. In the latter case, samples can be stored for months without loss of mycoplasma viability. For storage longer than 10 months, it is recommended to keep the samples at -70°C. In the absence of cold chain, penicillin or ampicillin should be added to the sample to limit contaminant growth. It is important to point out some of the difficulties that can be encountered in isolating MccF38; two main difficulties can be described. First, MccF38 grows very poorly in vitro and, secondly, samples are often contaminated by other mycoplasmas (Thiaucourt *et al.*, 1996; Nicholas and Churchward, 2012).

2.9.3.2. Treatment of samples

Swabs are suspended in 2–3 ml of culture medium. Tissue samples are best minced using scissors, and then shaken vigorously, or pulverized in medium using 1 gm of tissue to 9 ml of medium. Tissues should not be ground. The suspension is usually prepared with a mycoplasma medium, but if parallel bacteriological examination is to be carried out, a high quality bacteriological medium, such as nutrient broth, may be used to provide a suspension suitable for both forms of examination. Pleural fluid, or a tissue suspension or swab, is serially diluted through at least three tenfold steps in the selected mycoplasma

medium and dilutions should also be placed on to solid medium (Thiaucourt and Lefevre., 1992).

2.9.3.3. *Mycoplasma media*

The medium used by MacOwan and Minette to culture Mccp organisms (Mohan *et al.*, 1990) is termed 'viandefoie goat' (VFG), and includes goat-meat, liver broth and goat serum. Alternative suitable media are modified Hayflick's, and modified Newing's tryptose broth (MacOwan and Monett, 1976). Media enriched with 0.2% (or up to 0.8%) sodium pyruvate perform considerably better, both for primary isolation and antigen production of Mccp (Balicki *et al.*, 2008; Cetinkaya *et al.*, 2009).

2.10. Identification of Mycoplasmas

2.10.1. *Polymerase Chain Reaction (PCR)*

This relatively new diagnostic method has radically improved the detection and identification of microorganisms, which do not grow easily in vitro. PCR is based on the amplification of specific DNA sequences with thermostable enzymes and nucleotidic primers that must be chosen with care (Mcpherson *et al.*, 1992). This novel technique might improve dramatically the laboratory diagnosis of mycoplasma disease in developing countries, as PCR can be performed with dried material, hence removing the need for a constant 'cold chain' for samples. In the case of CCPP, the best sample may be pleural fluid that has been dried onto filter paper. The dried filter paper can then be wrapped in a plastic bag and sent to a reference laboratory (Bölske *et al.*, 1996).

The presence of antibiotics in the sample should not interfere with the reaction. However, it does jeopardize the success of isolation. Drying the sample reduces the size, thus facilitating transport and inactivating many pathogenic organisms. This technique has some limitations. PCR is not a reference technique as the reliability of the process may sometimes be questioned. It is still necessary to isolate MccF38 to confirm without doubt. The isolation of strains is always needed to confirm periodically that the PCR technique used is specific. In addition, the isolation of strains gives access to the whole genome of

the bacteria, whereas PCR amplifies only a portion. Obtaining the whole genome of a strain might be the only way to design improved diagnostic tools, based on different gene locations (Bölske *et al.*, 1996).

Paradoxically, the high sensitivity of the method may sometimes prove to be a limitation, as the manipulation of amplified products may contaminate some reactions and could result in false positive results. The prevention of this risk should be a continual concern for technicians working with PCR. As a result, multiple negative controls must be included in all reaction sets. The PCR method which has been described for the detection of MccF38 (Bascuñana *et al.*, 1994) is based on the amplification of a segment of the gene that codes for the 16S ribosomal RNA (rRNA). This gene is well conserved in bacteria but also possesses regions that are variable enough to ensure a distinction between species or subspecies. These variations are used to construct phylogenetic trees (Weisburg *et al.*, 1989; Weisburg *et al.*, 1991) and diagnostic tools (Johansson, 1993). In the case of CCPP, the primers were chosen specifically to amplify a fragment of this gene for the mycoides 'cluster'. The identification of MccF38 is made in a second step, by digestion of the amplified products with an endonuclease: *pst* I. Three fragments are obtained with MccF38 while only two are seen with other members of the mycoides 'cluster'. This difference indicates a single nucleotide substitution in one of the two genes that code for the 16S rRNA in the mycoplasmas (Bascunana *et al.*, 1994).

2.10.2. Serological tests

Quite a few serological tests are available that are used in the field for the confirmatory diagnosis of CCPP. Indirect Haemagglutination (IHA) and complement fixation tests (CFT) are used to assay the antibody response of goat to Mccp (Massa *et al.*, 1992). The CFT used for the detection of CCPP (Macowan 1976; Macowan and Minette 1977) is more specific, though less sensitive than the IHA (Muthomi and Rurangirwa 1983; Massa *et al.*, 1992). The IHA specificity for the *Mycoplasma mycoides* cluster has been evaluated and the results were found to show cross reactivity between these organisms (Jones and Wood 1988; Litamoi *et al.*, 1989; Massa *et al.*, 1992). The latex agglutination test which detects serum antibodies in CCPP-infected goats is more sensitive than CFT

and can be performed in field conditions using whole blood or undiluted serum with a prompt result (Cho *et al.*, 1976).

An indirect enzyme-linked immunosorbent assay (ELISA) has been developed to screen goat serum at a single dilution of antibody to *Mccp* (Wamwayi *et al.*, 1989). The specificity and suitability of ELISA for large scale testing make it an appropriate tool for epidemiological investigation of CCPP (OIE, 2008). Direct antigen detection and blocking ELISA detects antibodies in the serum of naturally or artificially CCPP-infected goats (Wamwayi *et al.*, 1989). Direct and indirect fluorescent antibody tests are the simple, reliable and rapid serological methods applied to clinical samples for the identification of most *Mycoplasmas* (Thiaucourt *et al.*, 1996). Among many, the indirect fluorescent antibody (IFA) test is the most commonly used and is applied to unfixed *Mycoplasma* colonies on agar (OIE, 2008).

The growth inhibition test (GIT) is the least sensitive and simplest of the tests available for CCPP diagnosis (OIE, 2008). It depends on the direct inhibition of *Mycoplasmas* growth on solid media by specific hyper immune serum, and detects primary surface antigens (Dighero *et al.*, 1970; Rosendal and Black, 1972). 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, 2008).

2.10.2.1. *Competitive enzyme-linked immunosorbent assay (C-ELISA)*

C-ELISA was developed and proved both specific and sensitive (Thiaucourt *et al.*, 1994). This test has recently been reformatted as a kit containing pre-coated plates and ready-made reagents, including MAb 4/52. It is now a strict competition assay instead of a semi-blocking test as in the original publication. The new kit has been re-validated to establish its cut-off value, 55% inhibition (PI), to obtain a strict specificity of 99.9%. It allows the detection of positive sera in CCPP-infected herds, but its true sensitivity at the individual level has not yet been fully evaluated. As it is highly specific, it can be used to evaluate herd status using targeted sampling of recovered animals in the tested herds should greatly enhance the sensitivity without any specificity problem. In the OIE Reference Laboratory,

the uncertainty of measurement for this cELISA has been evaluated at ± 8 PI. This test can be used to evaluate the CCPP vaccine quality as the seroconversion measured one and two months post-vaccination is proportional to the Mccp antigen or saponin content. However, the correlation between c-ELISA titre and protection has not yet been established (Peyraud *et al.*, 2014).

2.11. Treatment, Prevention and Control of CCPP

The success of treatment of CCPP with recommended antibiotics such as erythromycin, tylosin, tetracycline or streptomycin was documented to depend largely on early diagnosis and intervention undertaken against the disease (El Hassan *et al.*, 1984). However, farmers were reported to use various traditional methods to control animal diseases in herds as their first line of response. Iran farmers crushed lung tissues infected with CCPP and obtained extracts that were treated with local herbs. A string dipped in the extract was then passed through the ears of healthy goats (Tadjbakhsh, 1994), thus providing some form of immunity.

An experimental Mycoplasma F-38 vaccine inactivated with lyophilized saponin that protects goats for approximately a year has been produced in Kenya though reported protective but with varied qualities and efficacies (Rurangirwa *et al.*, 1991). The experimental trials of this inactivated Mycoplasma strain F-38 vaccine was reported very effective against natural infection with CCPP six months post vaccination (Litamoi *et al.*, 1989, King *et al.*, 1992, Ayelet *et al.*, 2007).

Since the disease occurs in epidemics, antibiotic treatment, as the only control measure would be very uneconomical. Therefore, efforts have been directed towards controlling the disease by vaccination. The first immunization trial using Mccp was carried out by MacOwan and Minnette. They inoculated 20 goats intratracheally with a high-passage culture of *Mccp*. On contact challenge of the inoculated goats one month later, 11 of the 20 vaccinated goats were protected, whereas all 20 control goats contracted CCPP. The results provided an indication that goats could be protected against CCPP by using Mccp (MacOwan and Minnette, 1978).

Study made by Rurangirwa *et al.*, (1991) on lyophilized killed Mycoplasma F-38 vaccine reported 100 per cent protection against mortality and 95 per cent protection against clinical disease caused by Mycoplasma species strain F-38. On the other hand, Food and Agricultural organization (FAO) has recommended trade and movement restrictions, and slaughter of infected goats in newly infected countries with contagious caprine pleuropneumonia since the disease is contagious in nature and transboundary.

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted at Oromia Regional state East Shewa Zone Adami Tullu Agricultural Research Center. It is located in mid rift valley region of Ethiopia 160km from the capital city Addis Ababa to the south. It is found on altitude of 1600m above sea level. This area is characterized by lowland and dry agro climate with annual rainfall of 850-900mm. The temperature is nearly 30⁰c during dry season. The vegetation is characterized by scattered acacia woodland (Adami Tulu Research Center profile, 1998).

The total goat population in the Research Center is estimated to be 500 (Research Center data 2016) which are local breeds. The main husbandry systems includes extensive type where animals were allowed to graze or browse freely during daytime and kept in constructed house at night. Some semi-intensive type of husbandry system was also practiced especially for recently delivered Does and kids. In this case, goats were offered some nutritional supplement and kept usually in-door environment.

3.2. Study Animals

Study animals were local goat breeds managed under extensive production system by the research center. Goats with history of vaccination for CCPP before one year and above 6 months of age and both males and females were included in the study. Age of the animals was determined based on the Research center information and dental eruption. All the sampled goats (Fig 2) were ear tagged for identification and sampling. Animal data were gathered from the Research center recorded information.



Figure 2. Picture of some of the goats used during the research in ATARC.

3.3. Study Design

Experimental study with follow up sampling at pre and post vaccination carried out on Adami Tulu Agricultural Research Center. 358 goats above 6 month of age were selected and tested for sero-positivity of *Mccp* antibody prior to vaccination. Each goat was vaccinated with 1ml of inactivated whole culture CCPP vaccine to evaluate the post-vaccinal antibody response (seroconversion level). Sera samples were collected from 358 goats at day 28 and the seroconversion rate was evaluated by competitive enzyme linked immunosorbent assay (cELISA) according to kit manufacturer's test protocol IDEXX CCPP (Annex 2). Primary data were collected regarding animal vaccination and farm history using questionnaires (Annex 1).

3.4. Sample Size and Sampling Method

Vaccine safety and immunogenicity evaluation was conducted on 358 goats at Adami Tulu Agricultural Research Center. From 500 goats 71.6% (n=358) were used to evaluate the vaccine based on their age group purposively. Those goats above the age of 6 month

(n=358) were selected for the experiment. Primary data were collected from farm supervising veterinarian using questionnaire (Annex 2).

3.5. Blood sample collection

Blood sample was collected from jugular vein of all goats included in this study. Collected blood samples were allowed to stand for 30-45 minutes at room temperature to enhance clotting. Blood samples were identified with their respective goat ear tag identity and transported by putting them in icebox to NVI Research and Development laboratory where sera was separated by centrifugation at 3000 rpm for 3 minutes to extract clear serum (Tuck *et al.*, 2009) and later tested for seroconversion by cELISA.

3.6. Vaccine preparation and Vaccination

3.6.1. Vaccine preparation

Inactivated whole culture CCPP vaccine was prepared from known working seed bank (WSB) also known as Kenyan strain of *Mccp* following the existing standard operating procedure of the manufacturer (Tesgera and Tefera, 2012). The vaccine was evaluated for its quality and identity using OIE guideline for CCPP vaccine quality control tests (OIE, 2014).

3.6.2. Vaccination

Inactivated whole culture CCPP vaccine, the commercially available vaccine produced by NVI (Ethiopia) from Kenyan strain of *Mccp* (Formerly termed as F-38), was used to vaccinate goats in Adami Tulu Agricultural Research Center. The vaccine was homogenized gently and each goat was vaccinated with 1ml of inactivated whole culture CCPP vaccine under the loose skin (subcutaneously) at the thoracic region using sterile treatment syringe with 21Gx1¹/₂"needle to evaluate the post-vaccinal antibody response (seroconversion level).

3.6.3. *Evaluation of safety of the vaccine*

Evaluation of safety of the vaccine was done according to the OIE for vaccine safety parameter (OIE, 2014) vaccinated goats were observed the whole day on the vaccination date and then during the one month of the study by veterinary professionals at the research center and any deviation from normal health (using observation of vital signs) were recorded. Professionals at ATARC performed follow up of study animals. Body temperature of 20 randomly selected goats was taken once per day in the morning for 14 days. Any deviation in the health status of the vaccinated animals was recorded. Minor swelling at vaccination site, which subsided within a week, is expected in vaccinated animals (OIE, 2014).

3.6.4. *Serological test*

Collected sera samples (at day zero and day 28-post vaccination) were examined for the presence of specific antibodies against *Mccp* by using cELISA in serology laboratory at NVI. Sera samples collected (358 pre and 358 post vaccination) were examined according to the test protocol supplied with the kit. Test samples and controls were pre-diluted on the pre-plate (uncoated). Samples to be tested were premixed with a specific monoclonal anti-*Mccp* antibody (Mab 4.52) in a pre-plate (uncoated) and homogenized contents of the pre-plate were transferred into the *Mccp* antigen coated microplate.

The contents were incubated for 1 hr. at 37°C with a gentle agitation, washed two times, dried and then an anti-mouse IgG enzyme conjugate was added and incubated for 30 min. Then, after three times washing enzyme substrate was added and incubated for 20 min. Finally, stop solution was added and color development was observed and read at 450 nm by ELISA reader to determine the optical density and finally percentage of inhibition was calculated by formula provided with the kit. For the assay to be valid, percentage inhibition of negative control, weak and strong positive controls, and mean values of conjugate control and monoclonal antibody needs to be within the validity criteria set by the manufacturer. Those test samples with percentage of inhibition greater than 55% were considered positive for presence of *Mccp* antibodies. The animals with antibody titer

percentage of inhibition more than 55% were said to seroconvert in response to the administration of the antigen, hence indicating immunogenicity of the vaccine antigen.

3.7. Data Analysis

All collected data were entered into Microsoft Office Excel 2007 computer program and then summarized first by using a descriptive statistics. All statistical analyses were performed using Statistical Package for Social Science (SPSS)-Version 23. The sero conversion rate of study animals (Goats) prior and post-vaccination was calculated as the proportion of the number of cELISA positive animals to the total number of tested animals expressed in percentage.

Paired-sample t-test was used to analyze if there is difference between pre and post vaccination percent inhibition of test sera (paired scores). A P-value less than or equal to 0.05 at 95% confidence interval was considered for significance (Thrusfield, 2007).

3.8. Ethical approval

Sampling from Animals were carried out according to the experimental practice and standard approved by the Animal welfare and Research and Ethics committee at Addis Ababa University Veterinary Medicine and Agriculture , Bishoftu Campus that is in accordance with the International guidelines for Animal welfare, the verification number is **VM/ERC/30/06/09/2017**.

4. RESULTS

4.1. ATARC Farm description

From a total of 500 goats 358 (71.6%) were above the age 6 months and were included in the study as tabulated below (Table 2).

Table 2. Summary of questionnaire survey

Sample area	Production type	Goat Population	Age		Vaccination history
			>6 month	<6 month	
ATARC	Extensive	500	358 (71.6%)	142(28.4%)	Not vaccinated before 1 year

As described in the above table 358 (71.6%) goats were selected and sampled for *Mccp* antibody and the rest 142(28.4%) were under the age of 6 months and excluded from the research. The goats were reared in an extensive type of production system and all were vaccinated for a period of one year.

Safety and immunogenicity of whole culture CCPP vaccine was tested on 358 *Mccp* antibody negative goats at Oromia regional state ATARC.

4.2. Post vaccination immunogenicity test result

Pre and 28-day post vaccination sera was compared. Accordingly, from the total animals 246 (68.7%) were found positive for *Mccp* specific antibody on day 28 (Table 3).

Table 3. Proportion of *Mccp* specific antibody positives on 28 days post vaccination

Study animals	Total vaccinated	Number of <i>Mccp</i> antibody positives	Percentage positives	95% confidence interval
<i>Mccp</i> antibody free goats	358	246	68.7	63.6-73.5

4.3. Comparison of pre and post vaccination percent inhibition of test sera

Paired sample comparison between sera collected before vaccination and sera collected after vaccination on day 28 post vaccination, showed that the day 28 post vaccination sera percent inhibition had strongly higher than that of day zero (P=0.0001) and the difference was strongly significant (table 3).

Table 4. Paired sample comparison of percent inhibition of sera collected before and after vaccination

Item	n	mean	Standard deviation	Stand ard error	95% confidence interval of the difference	t- calculat ed	Df	P value
Day 28 sera	358	49.75	20.32	1.07	12.72-16.92	13.89	357	0.000
Day zero sera PI	358	34.92	10.41	0.55				

NB: n—number of samples, df—degree of freedom

4.4. Safety test result

Safety test was conducted to detect any abnormal local or systemic adverse reactions during the observation periods. The observation made during the vaccination and post

vaccination time indicated that the vaccinated goats showed no abnormal signs except minor swellings at the injection site which subsided within a week, which indicated that the vaccine was safe for farm animal use. The absence of abnormal rise in body temperature in animals, which were monitored during the study, evidenced this issue.

5. DISCUSSION

In the current study a total of 358 goats from ATARC were tested for M CCP antibody at prevaccination and post vaccination after vaccinating with inactivated whole culture CCPP vaccine and the result revealed the rise in *Mccp* specific antibody (seroconversion) was observed in 246 (68.7%) of the vaccinated goats. Comparison of prevaccination percent inhibition of test sera with day 28 post vaccination sera percent inhibition showed that the latter (day 28 post vaccination sera percent inhibition) had significantly higher PI than that of prevaccination sera PI ($p=0.000$).

Thus, the inactivated whole culture *Mccp* vaccine induced seropositivity in majority of the vaccinated goats. The result of present study is comparable to that of Tesgera *et al.* (2015). The study made by Tesgera *et al.*, 2015 by comparing two types of vaccine produced by NVI revealed that the sero-positivity observed after vaccination with inactivated whole culture CCPP vaccine was 60.71% which had no significant difference ($P>0.05\%$) with the current finding as sero-positivity had no significant difference in animals managed intensively at NVI report by Tesgera *et al.*, and animals managed extensively, current research output.

Study made by Lakew *et al.*, 2014 reported rise in *Mccp* specific antibodies to 61.1% after vaccination of goats with inactivated CCPP vaccine which also agrees with the current finding. On the other hand, sero-positivity after vaccination has been known to be affected by several factors like level of nutrition, animals age, physiologic statement (Tizard, 2009), and the reason why the sero-conversion did not rise more than what was observed by this study needs further investigation. The goats used for this study were kept extensively and the level of nutrition obtained by these research animals was not under controlled provision as the result, nutritional shortage is evident as the area is one of the hotter areas with drought.

According to OIE, 2014 the protection rate after vaccinating goats with CCPP vaccine and making in contact challenge with sick animals has been known to be 80% or more. In current study, it was impossible to compare the percent positivity with the OIE, 2014 protection rate as current study was not supported by challenge study, and it only evaluated

safety and immunogenicity ability after vaccination. The current vaccine under evaluation is whole culture based which could elicit antibody against several antigens than the one the cELISA could only detect and therefore it could elicit more protection than the percent positivity showed by this study, though this needs further study.

In addition, the management situation at the time of vaccination (before and after vaccination) was important for a very good immune response (antibody production). Vaccines should be administered at times of low stress and several weeks prior to expected changes in management, that may increase stress or exposure to infectious agents. Good nutrition, both in protein and energy as well as trace minerals and vitamins is required for an adequate immune response (Rashid *et al.*, 2009). With the present field trial, study animals were owned and managed by the research Center extensively with no additional supplement. Thus, several factors like poor nutrition, high environmental temperatures and immunosuppressive concurrent diseases may have interfered with ability of the animal to mount a good immune response following field vaccination.

Observations were made on field performance (safety and immunogenicity) of the inactivated whole culture CCPP vaccine after vaccination of goats in Adami Tulu Agricultural Research Center. Safety test of the vaccine was evaluated by measuring rectal temperature and general clinical condition, and 100% of vaccinated goats were found none febrile with healthy profile during safety test observation period implying that the vaccine did cause any systemic disturbance to the vaccinated animals and hence proved safe for use in field condition. During the observation period, the body temperature fell between 38.6 and 40⁰c, which was within the goat normal body temperature (Radostitis *et al.*, 2010).

6. CONCLUSION AND RECOMMENDATION

The study showed that the vaccine antigen induced sero-positivity (sero conversion) in majority of the vaccinated goats. Even though the protection level of this vaccine was not evaluated by this study by contact challenge to make a comparison with set standard of OIE 80% protection level of CCPP vaccine, at the prevailing field condition, the seroconversion attained by study can be assumed promising level of immunogenicity. It also showed that the inactivated whole culture CCPP vaccine was safe in all of the vaccinated goats. So the vaccine was safe and immunogenic at field though some of the goats vaccinated with this vaccine did not show sero-positivity after vaccination within twenty-eight days which could be attributed to several factors like level of nutrition, weather condition during which the vaccine was given, presence of any other stressing factors, etc. The inactivated whole culture CCPP vaccine is also believed to possess several antigens that could aid protection against the disease than the one that the current test for evaluation could detect.

Therefore, based on the above conclusion the following recommendations were forwarded:

- Further detailed study should be conducted and monitored routinely on field effectiveness of the vaccine in different parts of the country.
- Controlled challenge study needs to be conducted to correlate the protection against the disease by seropositive and non-seropositive vaccinated animals.

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8. ANNEXES

ANNEX 1 . CCPP Whole culture vaccine field assessment questionnaires form

Region: _____

Zone: _____

Farm site _____

Date: _____

Farm history

Production type: _____

Breed _____

Sex _____

Age _____

Total population _____

Vaccination history

Conclusion

ANNEX 2. C-ELISA protocol

Test procedure

All reagent must be allowed to come to 18-25⁰c before use. Reagents should be mixed by gentle inverting or swirling. Controls may be dispensed anywhere on the microplate.

1. Obtain the required number of coated microplates and uncoated microplates for sample preparation and record the position of each sample.
2. Dispense the dilution buffer N24, Controls, samples and detection solution in the uncoated microplate(s),
 - a. Dispense 100µl of Dilution Buffer N.24 into each well of the uncoated microplate(s).
 - 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 two 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 preplate(s).
 - g. Dispense 110µl of UNDILUTED Detection Solution into each well of the preplate except in CC.
3. Homogenize the contents of the wells of the uncoated microplate and transfer 100µl from each well to the appropriate wells of the coated microplate(s).
4. Cover the microplate and incubate 1 hour (± 5 min.) at +37⁰c (± 3 ⁰c) under gentle agitation, avoiding desiccation of the plates.
5. Remove the solution and wash each well with approximately 300 µl of wash solution 2 times. Avoid plate drying between plate washings and prior to the addition of the next reagent. Tab each plate onto absorbent material after the final wash to remove any residual wash fluid.
6. Add 100µl of DILUTED Conjugate in each well.

7. Cover the microplate and incubate 30 minutes (± 3 min.) at $+37^{\circ}\text{C}$ ($\pm 3^{\circ}\text{C}$) under gentle agitation avoiding desiccation of the plates.
8. Repeat step 5 but this time washing three times.
9. Add 100 μl of TMB Substrate N.9 in each well.
10. Incubate 20 minutes (± 3 min.) at $+37^{\circ}\text{C}$ ($\pm 3^{\circ}\text{C}$) away from direct light.
11. Dispense 100 μl of stop solution N.3 per well.

12. Measure and record the absorbance values of samples and Controls at 350 nm.
 Note: When using robotics, incubation of microplates in an incubation chamber allows working without covers. Use of robots is also not compatible with gentle microplate tapping or wiping. Plates can be held up to 1 hour in the dark prior to reading. The duration of substrate incubation can be adjusted to yield an OD of 1.000 in the MabC wells.

13. Calculation:

Controls

Calculate Conjugate Control Mean Absorbance (CCx) and Mab Control Mean Absorbance (MabCx).

$$\text{CCX} = (\text{CC1A (450)} + \text{CC2A (450)}) / 2$$

$$\text{MabCx} = (\text{MabC1A (450)} + \text{MabC2A (450)} + \text{MabC3A (450)} + \text{MabC4A (450)}) / 4$$

Samples and controls

Calculate the Percentage of Inhibition (SPI) for each sample and control.

$$\text{SPI}\% = 100 \times (\text{Mab Cx} - \text{S A (450)}) / (\text{Mab Cx} - \text{CCX})$$

Validity criteria

$$0.500 \leq \text{Mab Cx} \leq 2.000$$

$$\text{CCx} < 0.300$$

$$\text{Mean NC PI} \leq 35\%$$

$$50\% \leq \text{Mean PC PI} \leq 80\%$$

$$60\% \leq \text{Mean SPC}$$

$$\text{PI} \leq 90\%$$

For invalid assays, technique may be suspect and the assay should be repeated following a thorough review of the package insert.

14. Interpretation

Negative	Positive
S PI% < 55%	S PI% \geq 55%

Note: for this test positivity threshold is set at 55% of inhibition. However, every measurement has a certain uncertainty which depends on the kit itself and on the testing laboratory. Sera with PI values within the range $55\% \pm$ uncertainty of measurement should be considered with care and distinguished from the others that are positive or negative with certainty. It is advisable to perform this ELISA testing under quality assurance and, whenever possible, with an accreditation (i.e.: ISO 17025).

Note: IDEXX has instrument and software systems available which calculate results and provide data summaries.