

MAKERERE



UNIVERSITY

**OCCURRENCE OF CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN AGAGO
AND OTUKE DISTRICTS, UGANDA**

BY

ATIM STELLA ACAYE

(BVM, Mak)

REGISTRATION NUMBER: 2010/HD17/217U

STUDENT NUMBER: 210003338

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DECLARATION

I **Stella Atim Acaye** acknowledge that this work is original and has not been submitted elsewhere for an academic award. Where it's indebted to the work of others, due acknowledgement has been made.

Signature..... Date:.....

This dissertation has been submitted for examination with approval of the following supervisors:

Dr. Robert Tweyongyere, BVM, MSc, *Ph.D*

College of Veterinary Medicine, Animal Resources and Biosecurity,

Makerere University

Signature..... Date.....

Dr. Chrisostom Ayebazibwe , BVM, MVM, *Ph.D*

National Animal Disease Diagnosis and Epidemiology Center (NADDEC)

Ministry of Agriculture Animal Industry and Fisheries, Entebbe, Uganda

Signature..... Date.....

Dr. Frank Norbert Mwiine, BVM, MVPM, *Ph.D*

College of Veterinary Medicine, Animal Resources and Biosecurity,

Makerere University

Signature..... Date.....

DEDICATION

To my beloved spouse Mr. Geoffrey Edema, children Grace Ayikoru and Jesse Fetaa Edema

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LIST OF ACRONYMS AND SYMBOLS

AFRUS-IDM	Africa-US Integrated Disease Management Network
CCPP	Contagious Caprine Pleuropneumonia
DVO	District Veterinary Officer
FGDs	Focus Group Discussions
HED/CIMTRADZ	Higher Education for Development/ Capacity-Building in Integrated Management of Transboundary Animal Diseases & Zoonoses
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries
<i>Mccp</i>	<i>Mycoplasma capricolum</i> subspecies <i>capripneumoniae</i>
MFPEd	Ministry of Finance Planning and Economic Development
NAADS	National Agricultural Advisory Services
NADDECC	National Animal Disease Diagnostic and Epidemiology Control Centre
OD	Optical Density
PRA	Participatory Rural Appraisal
VACNADA	Vaccination Against Neglected Animal Diseases

ABSTRACT

Contagious Caprine pleuropneumonia (CCPP) is a devastating disease of goats caused by *Mycoplasma capricolum* subsp. *Capripneumoniae* (Mccp). The disease was first confirmed in Uganda in 1995 in Karamoja region. CCPP disease negatively impacts on goat health and production but its extent and magnitude among the local communities remains unknown. A cross sectional study was conducted in the districts of Agago and Otuke neighbouring Karamoja region in Northern Uganda during the months of July and August 2011 to explore the status of CCPP. A semi-structured questionnaire was administered to 162 selected farmers in the study areas to assess their knowledge, attitudes and perceptions on the factors they associated with CCPP occurrence and 8 focus group discussions were also conducted to obtain qualitative information on CCPP. Four hundred and four goats from randomly selected unvaccinated herds and 100 goats from vaccinated herds were examined for antibodies against *Mycoplasma capricolum* subsp. *Capripneumoniae* (Mccp) using ELISA.

The majority of the farmers 121 (74.7%) had knowledge of CCPP and recognised that CCPP was among the diseases affecting goat production in the two districts. There was no association in the occurrence of CCPP among different herd sizes ($p=0.66$), farming practices ($p=0.93$), source of breeding stock ($p=0.28$) and method of acquisition of breeding stock ($p=0.98$). Levels of antibodies against Mccp were higher among the vaccinated goats than unvaccinated ones (mean ODs of 0.905 and 0.776, $p=0.08$). Among the unvaccinated herds seroprevalence of CCPP was 32 (17.7%) and 52 (23.3%) for Agago and Otuke respectively.

This study demonstrated that CCPP was prevalent in Agago and Otuke districts which are outside but close to Karamoja region. Farmers in these districts were generally aware of the disease. Further studies should be undertaken to investigate CCPP in other districts in Uganda to pave way for effective preventive and control measures against CCPP in the country.

CHAPTER ONE: INTRODUCTION

1.1 Background

Contagious caprine pleuropneumonia (CCPP) is a highly infectious and serious respiratory disease of goats clinically characterized by coughing, respiratory distress and very high morbidity and mortality rates (Thiaucourt and Bolske, 1996). CCPP is caused by *Mycoplasma capricolum* subspecies *capripneumoniae* (*Mccp*) (MacOwan and Minette, 1976). The disease is included in the list of notifiable disease by World Organization of Animal Health (OIE) because of very high morbidity and mortality rates causing significant socio-economic impact once declared in a country.

Mycoplasma capricolum subspecies *capripneumoniae* (*Mccp*) has been known to be closely related to three other Mycoplasmas: *Mycoplasma mycoides* subspecies *mycoides Large Colony* (*MmmLC*), *Mycoplasma mycoides* subspecies *capri* (*Mmc*), and *Mycoplasma capricolum* subspecies *capricolum*. All these belonged to the *Mycoplasma mycoides* clusters that have the same origin and their establishment and spread has been reported to have coincided with livestock domestication (Fischer et al., 2012).

Mycoplasma capricolum subspecies *capripneumoniae* (*Mccp*) was originally thought to be a highly host specific pathogen (Thiaucourt et al., 1996), but has also been reported in the Nubian ibex, Wild goat, Gerenuk, Laristan mouflon and other ruminant ungulates (Paling et al., 1978, Hernandez et al., 2006, Arif et al., 2007). *Mccp* is also reported to have been isolated from sheep that were in contact with goats (Litamoi et al., 1990, Bolske et al., 1995).

Globally, Contagious Caprine Pleuropneumonia was documented to threaten the entire world goat population because the disease is very contagious with potential of rapid spread irrespective of national borders (Ayelet et al., 2007). *Mccp* originally known as the F38 biotype has been isolated in a number of African countries including; Kenya, Sudan, Tunisia, Chad, Uganda, Ethiopia, Niger and Tanzania. It has also been isolated in the Middle East countries including Oman, Turkey and the United Arab Emirates. In the past decade the disease spread has been documented to Eritrea (Houshaymi et al., 2002), Thrace in south-eastern Europe and Tajikistan (Ozdemir et al., 2005).

Contagious Caprine Pleuropneumonia (CCPP) has been known to be a disease of major economic importance in Asia and Africa, posing major constraint to goat production because of high mortalities (Rurangirwa et al., 1984). The morbidity and mortality of the disease has been reported by World Organization of Animal Health to be typically very high especially when the disease affects naive flocks, occasionally reaching 100 and 90 percent, respectively. The environment was affirmed to play an important role in its spread when animals congregate at grazing and watering points and this is typical in underdeveloped countries including Uganda.

Mycoplasma capricolum subsp. *capripneumoniae* has been reported to be persistent in chronic and latent carriers which have recovered from active disease without becoming bacteriologically sterile, and it is these latent carriers that have been reported responsible for the perpetuation of the disease in a herd (Thiaucourt and Bolske, 1996, Wesonga et al., 1998).

1.2 Statement of the problem

A survey done in Northwestern Kenya (Wafula, 2006) indicated a high prevalence of CCPP in the areas bordering Uganda suggesting that CCPP may also be particularly highly prevalent in the adjoining areas of Uganda and the Sudan. Indeed CCPP had been previously confirmed in Uganda (Bolske et al., 1995) in the karamoja region, and there have been unconfirmed reports of clinical cases by both the farmers and the veterinarians in a number of goats in the North and North Eastern Districts of Uganda. Further, there are no commercially available diagnostic tests for CCPP. Thus the actual prevalence, risk factors, and impact of the disease in Uganda have not been documented.

1.3 General objective

To explore the associated risk factors for the occurrence and the prevalence of contagious caprine pleuropneumonia in Agago and Otuke districts.

1.4 Specific Objectives of the study

1. To assess the perceived farmers' awareness and attitudes about CCPP in the study area.
2. To describe the factors farmers associated with occurrence of CCPP in the two districts.
3. To determine the seroprevalence of CCPP in selected goats herds in Agago and Otuke districts.

1.5 Hypotheses for the study:

Farmers in Otuke and Agago districts are not aware of the risk factors of CCPP and the losses associated with the disease and yet the disease is prevalent in the two districts.

1.6 Significance and justification of the Study

Livestock contribute greatly to the livelihoods of many farming households in Uganda and important sub sector of agricultural production contributing 9% of the gross domestic product (GDP) and 17% of the agricultural GDP. Out of the 12.4 million national goat population in Uganda as of 2008, a proportion of 2.7 million (21.7%) was reported to found in the northern part of the country (MAAIF and UBOS, 2008). The number of goats in the northern region was ranked second after the western region that constituted the highest (3.5 million, 27.7%). Furthermore, mixed farming small holder farmers and pastoralists own over 90% of the cattle herd and 100% of the small ruminants and yet such practices may be associated with increased risk of spread of infectious diseases including CCPP.

There is therefore urgent need to improve the livestock sector in order to address food safety, security and to meet local and international trade standards. In this regard constraining factors such as infectious diseases need to be addressed. CCPP is a transboundary disease and highly contagious; spread by movement of goats (Nicholas and Churchward, 2012). There is therefore need to determine the prevalence, assess the risk factors, farmers' knowledge and attitudes, and economic impact of CCPP among others diseases affecting goats so that appropriate control measures can be instituted to safeguard trade in livestock as well as improve food security in Uganda. Assessment of the prevalence and associated risk factors of CCPP will help in drawing appropriate intervention measures to control the disease in the country.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction.

Contagious caprine pleuropneumonia (CCPP) has been known to cause major losses in many goat rearing countries in Middle East, Africa and Asia due to devastating high mortality rates associated with the disease occurrence (Bolske et al., 1996). The disease is included in the List of OIE notifiable diseases list due to its high morbidity and mortality resulting into socio-economic importance within countries as well as its significance in the international trade of animals and animal products.

2.2 Etiology of contagious caprine pleuropneumonia

Contagious caprine pleuropneumonia was first described in the late 19th century (McMartin et al., 1980) and *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) has been reported to be the etiological agent of CCPP (Edward and Fitzgerald, 1953, Jonas and Barber, 1969). The *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) was previously known by the strain name of its type species, F38. *Mccp* has been known to be a member of the *Mycoplasma mycoides* cluster which includes *M. mycoides* subsp. *mycoides* SC (*MmmSC*), *M. mycoides* subsp. *mycoides* LC (*MmmLC*), *M. mycoides* subsp. *capri* (*Mmc*), *M. capricolum* subsp. *capricolum* and *Mycoplasma* species bovine group 7 (Bg 7) a bovine isolate which has been reported to cause other diseases of ruminants (Pettersson et al., 1998). The origin of all these *Mycoplasma mycoides* clusters was reported to be dated way back about 10,000 years ago, and has been suggested that the establishment and spread of the cluster coincided with livestock domestication (Fischer et al., 2012).

2.3 *Clinical signs of contagious caprine pleuropneumonia*

Contagious caprine pleuropneumonia affected goat was previously reported to be reluctance to walk with extreme fever (41°C) though the goat continued to feed and ruminant. Gradually, the respiratory symptoms became prominent, respiration accelerated and painful, and accompanied by violent coughing. In the terminal stages, the goats were reported unable to move and would stand with their legs wide apart, the neck was stiff and extended, saliva continuously dripped from their mouth and their nose reported obstructed by a mucopurulent discharge (Thiaucourt and Bolske, 1996).

Other scholars previously characterized the disease by its contagious nature. Clinically by episodes of fever (40.0–41.4°C) that was reported accompanied by intense coughing and signs of respiratory distress manifested more in the acute cases and less stronger in the sub acute and chronic cases (Thiaucourt et al., 1996), (Wesonga et al., 2004) The associated lesions were documented to cause interstitial, fibrinous pleuropneumonia, interlobular oedema and hepatization of the lung known to cause high mortality rates between 60% and 100% in absence of Antibiotic intervention (Kaliner and MacOwan, 1976), (Jones and Wood, 1988), (Thiaucourt and Bolske, 1996).

The gross pathological lesions were documented to be localized to the lungs and the pleura of the affected goats. It 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). In acute cases, the pleural cavity was reported to contain an excess of straw-coloured fluids with fibrin flocculations (Kaliner and MacOwan, 1976, Wesonga et al., 1998). The

pleural exudates have been reported to solidify forming a gelatinous covering over the whole lungs (Thiaucourt et al., 1996). In chronic cases, there is a black discolouration of the lungs and sequestration of the necrotic lung areas, there is very thick adhesion between the lungs and the pleura (MacOwan and Minette, 1977).

Histological examination of the lung tissues have shown a acute serofibrinous to chronic fibrino-necrotic pleuropneumonia with serofibrinous fluids and inflammatory cells (dominated by neutrophils) in the alveoli, bronchioles, interstitial septae and sub pleural connective tissue (MacOwan and Minette, 1976, Wesonga et al., 1998, Msami et al., 2001). Pulmonary fibrosis peribronchiolar mononuclear cuffing has also been observed (Hussain et al., 2012).

2.4 *Epidemiology of contagious caprine pleuropneumonia*

2.4.1 *Animal species affected by Mccp*

Previously, contagious caprine pleuropneumonia (CCPP) had been reported to affect only domestic goats (Thiaucourt et al., 1994). All age groups of goats have been reported to be susceptible although higher mortalities have been documented among the young animals than adults (Ozdemir et al., 2005, Mekuria and Asmare, 2010). However, *Mccp* have been isolated from healthy sheep that have been in contact with goat herds affected by CCPP in Kenya (Litamoi et al., 1990), Eritrea (Houshaymi et al., 2002) and Ethiopia (Hadush et al., 2009). *Mccp* has also been isolated from sick sheep that had been in contact with goats having CCPP in Uganda (Bolske et al., 1995). Further, *Mccp* have also been isolated from cattle with mastitis (Kumar and Garg, 1991). All these observations suggested sheep and cattle are susceptible to *Mccp* and may play a role in the transmission and spread of CCPP.

CCPP has also been reported in the Nubian ibex, Wild goat, Gerenuk, Laristan mouflon and other ruminant ungulates (Arif et al., 2007) and in Kenya antibodies against CCPP had been detected in some wild herbivores and camels (Paling et al., 1978). These findings have affirmed CCPP as a potential threat to wildlife and the conservation of endangered ruminant species, especially in the Middle East, where it is enzootic (Arif et al., 2007).

2.4.2 Transmission of contagious caprine pleuropneumonia

In natural infections, susceptible goats are infected with the *Mccp* organisms mainly through inhalation of contaminated aerosols from infected goats. The environment has been implicated to play an important role in the spread of CCPP (MacOwan, 1984). Due to the high sensitivity of *Mycoplasma* 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, Mekuria 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 communal grazing areas.

Infective *Mccp* organisms are believed to persist in chronic, latent carrier such as goats or sheep which have recovered from the active clinical disease without becoming bacteriological sterile. These are considered to be responsible for the perpetuation of the disease in endemic areas (Wesonga et al., 1993, Thiaucourt et al., 1996).

2.4.3 Distribution and prevalence of contagious caprine pleuropneumonia

The precise distribution of CCPP is not been well described although the disease has been reported in 30 countries mainly in Africa, the Middle East and parts of Asia (Thiaucourt and Bolske, 1996). The official OIE declaration of CCPP distribution has not reflected the reality in the field as a number of countries might have been infected but fail to confirm or even report the disease occurrence (Thiaucourt personal communication, Regional training course transboundary and zoonotic animal diseases held at MAAIF Entebbe, Uganda 2011).

In Asia and the Mediterranean, *Mccp* have been isolated in Oman (Jones and Wood, 1988), Turkey, the United Arab Emirates and Yemen (Ozdemir et al., 2005, Cetinkaya et al., 2009). CCPP outbreaks have also been confirmed in Pakistan, Tajikistan and Mauritius recently (Nicholas and Churchward, 2012). In Africa, *Mccp* has been isolated in Chad (Lefevre et al., 1987), Eritrea (Houshaymi et al., 2002), Ethiopia (Thiaucourt et al., 1992), Kenya (MacOwan, 1976), Nigeria (Okoh and Kaldas, 1980), Sudan (Harbi et al., 1981), Tanzania (Kusiluka et al., 2000), Tunisia (Perreau et al., 1984), and Uganda (Bolske et al., 1995).

In Uganda, a low sporadic occurrence of CCPP and confined to certain regions had been reported. CCPP was reported to have first appeared in Karamoja province which borders Kenya and Sudan (MacOwan et al., 1975, Harbi et al., 1981). *Mccp* was isolated from goats and sheep in Karamoja (Bolske et al., 1995) but since then, the prevalence, risk factors and socio-economic impact of CCPP in the areas have not been documented to allow effective control measures to be instituted.

2.5 *Diagnosis of contagious caprine pleuropneumonia*

In outbreaks of classical acute CCPP, high mortality and typical early thoracic lesions in goats are known to be highly indicative of *Mccp* infection (Thiaucourt et al., 1996, Hernandez et al., 2006). However, diagnosis of *Mycoplasma pneumonia* may not be fully achieved based on clinical signs and postmortem examination alone. It is recommended that additional laboratory tests be carried out in all cases of caprine mycoplasmosis to establish a presumed diagnosis (Thiaucourt et al., 1996).

The general principles for laboratory diagnosis of *Mycoplasma* had been described to involve mainly; 1) methods for direct microscopic demonstration of *Mycoplasma* cells and antigens in the host tissues or excreta, 2) methods for isolation and growth of *Mycoplasma* from suspected tissues, and 3) techniques for identification and classification of *Mycoplasma* isolates by determination of biochemical characteristics, and/or serological typing (Freundt, 1981). Confirmatory diagnosis of CCPP is achieved by isolation and identification of the *Mccp*, even though specific tests needed to characterize the *Mycoplasma* are quite complicated and time consuming. *Mycoplasma capripneumoniae* is fastidious, grows slowly in broth media, and produces only minute colonies on solid media. Furthermore, it is frequently overgrown by other common mycoplasmas such as *M. ovipneumoniae* (Freundt, 1981, Thiaucourt et al., 1996). Hence, diagnosis of *M. capripneumoniae* infection in animals with CCPP has been reported to be largely hampered by difficulties in isolating the organism from clinical material.

Once the mycoplasma has been isolated as the causative agent, it can be identified by immunofluorescent or by growth or metabolic inhibition tests. Several laboratory serological tests can also be used for detection of antibodies to *Mccp* though serological cross reactions

may occur, especially complement fixation (CFT) lack specificity. Other serological test are; passive hemagglutination (PH)(Muthomi and Rurangirwa, 1983), indirect ELISA (Kibe et al., 1985, Wamwayi et al., 1989), the competitive ELISA with specific monoclonal antibodies (Thiaucourt et al., 1994) and the latex agglutination test that has been reported to be very convenient field test for detecting antibodies in whole blood or in serum (Rurangirwa et al., 1987b, March et al., 2000).

The molecular based tests such as Polymerized Chain Reactions (PCR) have been reported to greatly improve detection of *Mccp* (Nicholas and Churchward, 2012). Specific polymerase chain reaction (PCR) assays (Woubit et al., 2004), when applied would address constraints in laboratory diagnosis due to cross reactions that occurred among the *Mycoplasma mycoides* cluster as all members of this cluster share common biochemical and serological properties. Reverse DNA sequence analysis of *Mccp* has been successfully used to study genetic diversity of *Mccp* strains (Taylor et al., 1992b, Taylor et al., 1992a, Heldtander et al., 2001).

2.6. Participatory Rural Appraisal in diagnosis of contagious caprine pleuropneumonia.

Participatory Rural Appraisal (PRA) is defined as approaches and methods used by local people to share, enhance and analyze their knowledge of life and conditions, to plan and to act (Chambers, 1994a). PRA emerged as a participatory tool of learning in the 1980s' and a continuum of rapid rural appraisal (RRA) and other field based and other people oriented approaches (Narayanasamy, 2009). Documented Participatory methods included; mapping and modeling, transect walks, matrix scoring, seasonal calendars, trend and change analysis, well-being and wealth ranking and grouping, and analytical diagramming. Evidence has shown high

validity and reliability of information shared by local people through PRA compared with data from more traditional methods (Chambers, 1994b).

Participatory epidemiological techniques have been based on principles and methods of PRA used for harvesting qualitative epidemiology intelligence contained within community observations, existing knowledge and traditional oral history (Schudel et al., 2006, Bett et al., 2009, Catley et al., 2012). Participatory CCPP investigations were reported to be very usefully in Ethiopia (Mekuria et al., 2008).

2.7. *Differential diagnosis for contagious caprine pleuropneumonia*

The diagnosis of outbreaks of respiratory diseases in goats, and of CCPP in particular, was reported to be complicated, especially where it is endemic and it must be differentiated from other similar clinico-pathological syndromes. These diseases have been known to include peste des petits ruminants, to which sheep are also susceptible; pasteurellosis, which can be differentiated on the basis of distribution of gross lung lesions; and what has been called ‘mastitis, arthritis, keratitis, pneumonia and septicaemia syndrome or more often as contagious agalactia syndrome (Thiaucourt and Bolske, 1996). The disease caused by *Mccp* was argued to be readily differentiated from contagious agalactia syndrome by *Mccp* being contagious and fatal to susceptible goats of all ages and both sexes, rarely affects sheep and does not affect cattle.

2.8 *Economic importance of contagious caprine pleuropneumonia*

Goats are known to be important commodities to a large segment of the worlds’ population as a source of meat, milk and skin. CCPP among infectious diseases of small ruminants have been

documented to pose major constraints to goat production in Africa and Asia (Heldtander et al., 2001, Pettersson et al., 1998, Thiaucourt et al., 1992). There has been direct losses of the disease result from its high mortality, reduced milk and meat yield, cost of treatment, control, disease diagnosis and surveillance in addition to indirect losses associated with the imposition of trade restriction.

2.9 Treatment, prevention and control of contagious caprine pleuropneumonia

The success of treatment of contagious caprine pleuropneumonia 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 lypholised 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 CCPP six months post vaccination (Litamoi et al., 1989, King et al., 1992, Ayelet et al., 2007). Lypholised killed Mycoplasma F-38 vaccine was also reported to confer 100 per cent protection against mortality and 95 per cent protection against clinical disease caused by Mycoplasma species strain F-38 (Rurangirwa et al., 1991). Of note was that multivalent

vaccines had been proposed as a good solution to major upper respiratory disease constraints in small ruminant production systems in lesser developed countries throughout the world (Alexander et al., 1989).

The exact assessment of animal health situation in a country had been documented one of the essential elements in formulating eradication and control programmes, and in regulating international trade in animals and animal products (Lefèvre et al., 1993). Never the less, 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.

CHAPTER THREE: METHODOLOGY

3.1 Study design and area.

A cross sectional study was done in the districts of Agago and Otuke (figure1) during the month of July and August 2011 and involving randomly selected of farmers and their goats. These study areas were selected purposively because they are neighboring Karamoja where CCPP was first confirmed in Uganda (Bolske et al., 1995).

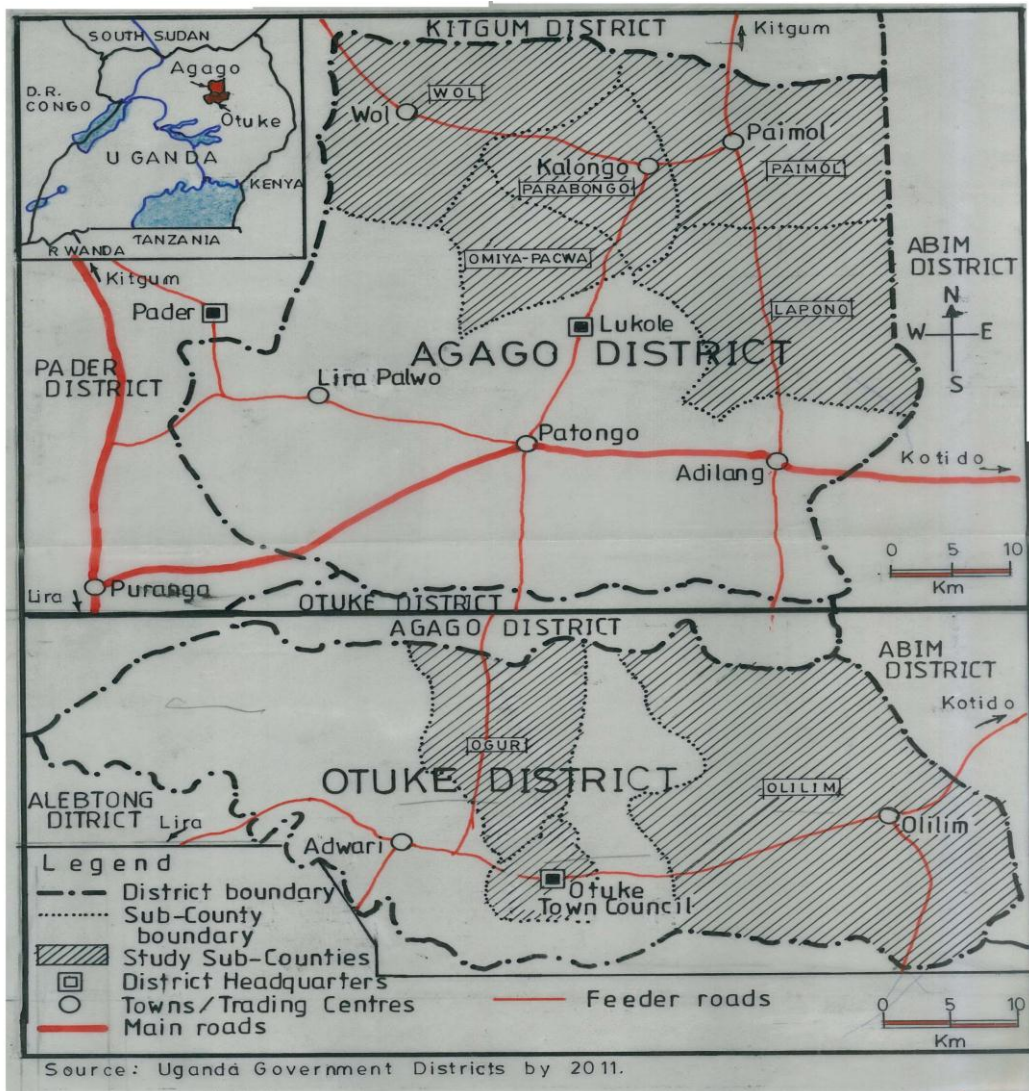


Figure 1: Selected Study Sub Counties in Agago and Otuke Districts

Secondly, multiple vaccinations of CCPP were scheduled to take place in Agago and Otuke districts at the onset of this proposed study. The study covered Omiya Pacwa, Piamol, Lapono, Parabongo and Wol sub counties in Agago, and Olilim, Otuke town councils and Ogor sub counties in Otuke district. These sub counties were chosen because they immediately lie adjacent to Karamoja. Additionally, the sub counties in Otuke were along the main Kotido-Lira highway through which animals were transported since CCPP is known to spread by movement of goats.

To address the stated objectives, semi-structured questionnaires were administered to selected individual farmers and farmer groups in these study areas to gather information on their awareness, attitude and the perceived risk factors for CCPP occurrence in the area. Thereafter, serum samples were collected from both unvaccinated and vaccinated herds and screened against contagious caprine pleuropneumonia antibodies using an in-house indirect ELISA.

3.2 Farmers interview by questionnaire and the focus group discussion.

A semi-structured questionnaire (appendix 1) to establish farmers' knowledge of CCPP was administered to selected farmers in the two districts of Agago and Otuke. The questionnaire was pretested in four parishes to ensure its credibility and validity. Thereafter, interviews with the farmers using the semi-structured questionnaire were conducted and information on their perception of CCPP and the underlying risk factors of the disease was obtained. The questionnaires were translated in the local languages (Luo) and administered together with postmortem pictures of the disease (appendix 2).

Focus group discussions were conducted with selected farmer groups in each sub county using open ended focus group guide (appendix 3) to obtain qualitative data on their perceptions of CCPP and the underlying risk factors of the disease.

3.3 Serum Samples Collection.

Whole blood was collected in plain vacutainers and transported in a cool box to the district veterinary laboratory. It was allowed to clot, left overnight to allow the clots to retract and serum separated. Serum was then aliquoted into sterile cryogenic vials and stored in a deep freezer at -20°C. Following the end of the exercise, all the samples were then transported to the National Animal Disease Diagnostic and Epidemiology control centre (NADDECC) laboratory MAAIF in Entebbe and stored at -20°C until analyzed.

3.3.1 Sample Size.

The sample size was determined using the formula,

$$n = (Z^2 \alpha / 2PQ) / e^2 \text{ (Dohoo et al., 2003).}$$

Whereby;

n is the sample size.

Z is a value obtained from statistical tables for the area under a normal curve for a stated level of significance, α .

P is the documented prevalence of CCPP in the area of study but prevalence of the disease in the area has not been documented.

Q is (1-P) and e is the acceptable error.

Since previous studies in Uganda did not document the prevalence of CCPP so far, we assume a prevalence of 50 %, taking a 95% confidence interval with an acceptable error of 5%, then $n = (1.96^2 * 0.5 * 0.5) / 0.0025 = 385$

Therefore the target sample size was approximated to be 400 goats.

Farmers interviewed were selected using formula;

$n = N / [1 + N(e)^2]$ (Yamane, 1967).

n = desired sample size

N = Estimated population size of registered goat farmers in the selected Sub counties

e = level of precision at 95%

$n = 273 / [1 + 273(0.05 \times 0.05)]$

n = 162 farmers

3.4 Laboratory analysis of samples

Laboratory analysis of the serum for antibodies against CCPP was done using an in-house indirect ELISA as described in 3.4.1. The capture antigen was the lyophilised *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp) vaccine (Caprivax Kenya Veterinary Vaccines Production Institute, Nairobi Kenya) suspended in saponin.

3.4.1 In-house indirect ELISA for CCPP

The in-house indirect ELISA for detection of antibodies against Mccp was developed, optimized and performed in the Central Diagnostic Laboratory at the College of Veterinary Medicine, Animal resources and Biosecurity, Makerere University Kampala, Uganda. Briefly:

- ELISA plate, figure 2 (NUNC Immunoplate 439454, Thermo Fisher Scientific, Roskilde, Denmark) were coated with 100µl per well of inactivated Mccp antigen (Caprivax produced by Kenya Veterinary Vaccines Production Institute, Nairobi, Kenya) at 1:100 dilution and incubated overnight in at 4⁰c.
- The plates were washed, 150µl per well of blocking buffer (2% BSA in PBS) added and incubated at room temperature for 1 hour.
- Test serum samples were diluted at 1: 100 diluents (1% BSA + 0.1% Tween 20 in PBS) were added 100ul per well. Known positive sera (strong and weak positive) and negative sera were added on each plate. The plates were incubated at room temperature for 1 hour.
- Conjugate, protein G HRPO at 1:5000 dilutions was added 100ul per well and incubated at room temperature for 1 hour.
- ABTS substrate (1ml of ABST stock solution (4mg/ml) in 11 ml of citrate buffer + 10µl of 30% H₂O₂) was added 100ul per well and incubated at room temperature for 10 minutes.
- The reaction was stopped after 30 minutes incubation by addition of 1% SDS (5g of SDS in 500 mls of dilution water) and the optical densities (ODs) read at 450 nm.

	1	2	3	4	5	6	7	8	9	10	11	12
A	CC	CC	T1	T9	T17	T25	T33	T41	T49	T57	T65	T73
B	CC	CC	T2	T10	T18	T26	T34	T42	T50	T58	T66	T74
C	PS++	PS++	T3	T11	T19	T27	T35	T43	T51	T59	T67	T75
D	PS++	PS++	T4	T12	T20	T28	T36	T44	T52	T60	T68	T76
E	PS+	PS+	T5	T12	T21	T29	T37	T45	T53	T61	T69	T77
F	PS+	PS+	T6	T14	T22	T30	T38	T46	T54	T62	T70	T78
G	NN	NN	T7	T15	T23	T31	T39	T47	T55	T63	T71	T79
H	NN	NN	T8	T16	T24	T32	T40	T48	T56	T64	T72	T80

NN= negative sera, PS+= weak positive sera, PS++= strong positive sera, CC= blank assay diluent, T= test samples (1-80).

Figure 2: Plate layout for the in-house indirect ELISA assay

3.5 Data analysis

The data obtained in the questionnaire was entered using EPIDATA. In- house indirect ELISA data was automatically interpolated into Microsoft excel 2007 and imported into intercooled stata version 9.1 where all analysis was done. Both questionnaire and serological results were analyzed for descriptive parameters; percentages, means, chi-squares and P-values using the intercooled stata version 9.1. Descriptive data was summarized in tables and graphs. Focus group discussion data were summarized into sub themes to establish patterns in the farmers' attitude and their perceived risk factors of CCPP.

CHAPTER FOUR: RESULTS

4.1 Results of the questionnaire survey and the focus group discussions

Questionnaires were administered to a total of 162 farmers of whom 61.7% (n = 100) were from Agago and 38.3% (n = 62) from Otuke. Eight Focus Group Discussions (FGDs) were similarly conducted using Participatory Epidemiology (PE) methods at least 1 group per Sub County consisting of 10-15 farmers was covered to capture relevant qualitative information.

4.1.1 Farmers' perceptions on goat diseases in Agago and Otuke

The common goat diseases in order of importance as listed by farmers were endoparasites (39.7%; n = 91), CCPP (17.9%; n = 41), mange (16.2%; n = 37), orf (13.5%; n = 31), tick infections (12.7%; n = 29) and similarly endoparasites (34.2%; n = 55), CCPP (25.5%; n = 41), tick infections (20.5%; n = 33), mange (13.7%; n = 22), orf (6.2%; n = 10) in Agago and Otuke respectively. Similar patterns were also equally obtained in the proportionate ranking of common goat diseases in the FGDs with the farmer groups (figure 3).



Figure 3: Proportional piling, a participatory epidemiology technique where endoparasites was ranked high in both Agago (A) and Otuke (B) as shown in the focus group discussion ~ konye keni women group in Parabongo Sub county, Agago and Olet farmer group in Ogor Sub county, Otuke district respectively.

4.1.2 Farmers knowledge of CCPP in Agago and Otuke

Through discussion with respondent farmers and applying photographs of typical cases of CCPP, farmers were asked whether they knew CCPP and if their herds had ever been affected by CCPP. Their responses varied as shown in table 1.

Table 1: Perceived farmers' knowledge of CCPP (n= 162)

Parameters	Variables	Number (%)	Number (%)
		Knowledge on CCPP	No knowledge on CCPP
District	Agago (n = 100)	64 (64.0 %)	36 (36.0%)
	Otuke (n = 62)	57 (91.9%)	5 (8.1%)
Sex	Male (n =115)	84 (73.0%)	31 (27.0%)
	Female (n = 47)	37 (78.7%)	10 (21.3%)
Age group	19-28 (n = 22)	18 (81.8%)	4 (18.2 %)
	29-38 (n = 97)	70 (72.2%)	27 (27.8%)
	39 ++ (n=43)	33 (76.7%)	10 (23.3%)
Education levels	No formal education (n=25)	19 (76.0%)	6 (24.0%)
	Primary (n= 83)	58 (69.9%)	25 (30.2%)
	Secondary (n= 41)	31 (75.6%)	10 (24.4%)
	Institutions (n=13)	13 (100.0%)	0 (00.0%)
Herd size reared	1-25 (n=144)	104 (72.2%)	40 (27.8%)
	26-50 (n= 15)	15 (100.0%)	0 (00.0%)
	50++ (n= 3)	2 (66.7 %)	1 (33.3 %)
Farmland size	Small (n = 90)	69 (76.7%)	21 (23.3 %)
	Medium (n= 46)	35 (76.1%)	11 (23.9%)
	Large (n= 26)	17 (65.4%)	9 (34.6%)
Small ruminants reared	Goats (n= 146)	110 (75.3%)	36 (24.7%)
	Goats/sheep (n=16)	11 (68.8%)	5 (31.2 %)
Farmland ownership	Own land (n= 51)	38 (74.5%)	13 (25.5%)
	Communal (n=109)	83 (76.1%)	26 (23.9%)
	Own/communal(n=1)	0 (00.0%)	1 (100.0 %)
	Rent (n=1)	0 (00.0%)	1 (100.0 %)

Of the 162 farmers, most (74.7%) were aware of CCPP and indeed the farmers gave the local Luo name of CCPP as “two-oooy dyel” or “loukoi dyel” (Agago) or “two- iwuku dyel” (Otuke). Of the 121 farmers who had knowledge on CCPP, 86 (82.6%) reported their herd to have suffered from CCPP. Of those (86) who reported to have encountered the disease, associated it with cough (73.3%), fever (54.7%), loss of appetite (54.7%), difficult breathing (38.4%), nasal discharge (36%), difficult movement (33.7%) loss of condition and death (20.9%). Similarly, farmers were also able to identify clinical signs of CCPP in the participatory CCPP investigation FGDs (figure 4).

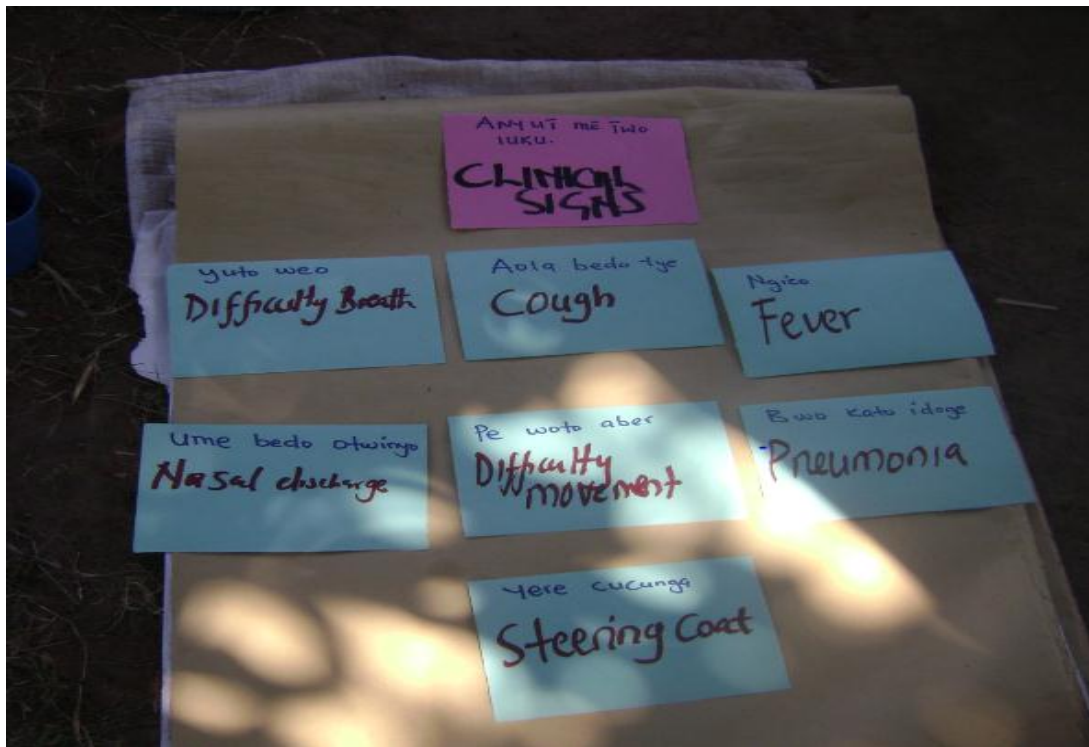


Figure 4: Identification of the clinical signs of CCPP in a FGD conducted with Olet farmers in Ogor Sub County, Otuke district.

Majority of the farmers 86 (82.6%) whose goats suffered from CCPP reported the disease to have occurred in rainy season than dry seasons in both districts. Of interest was that similar

pattern of CCPP occurrence was equally mapped out in FGDs with the farmer groups in both districts (figure 5). Furthermore, a testimony from one of the farmers during FGD within Cancoya group in Ngora parish, Agago district (figure 6) said “after most of the crop harvest in November, we release our goats for three months to feed totally communally starting December and with the onset of rains in April all goats are gathered again under controlled feeding and housing to avoid crop damage. When the goats are housed in April, they start suffering CCPP in July; peaks are observed in July- August and subsequently subsiding until they are released again”.

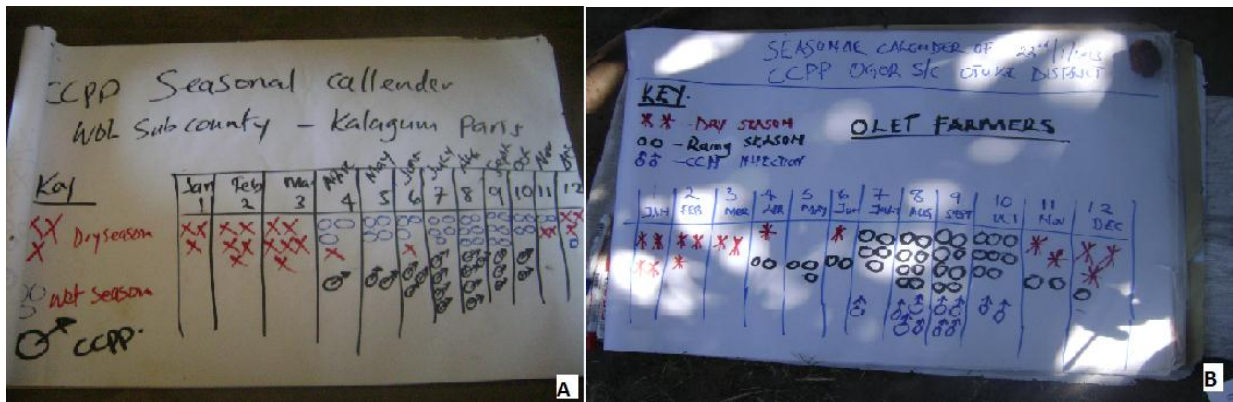


Figure 5: seasonal calendar for CCPP for 2012 as mapped by farmers in Agago (A) and Otuke (B) during FGDs.

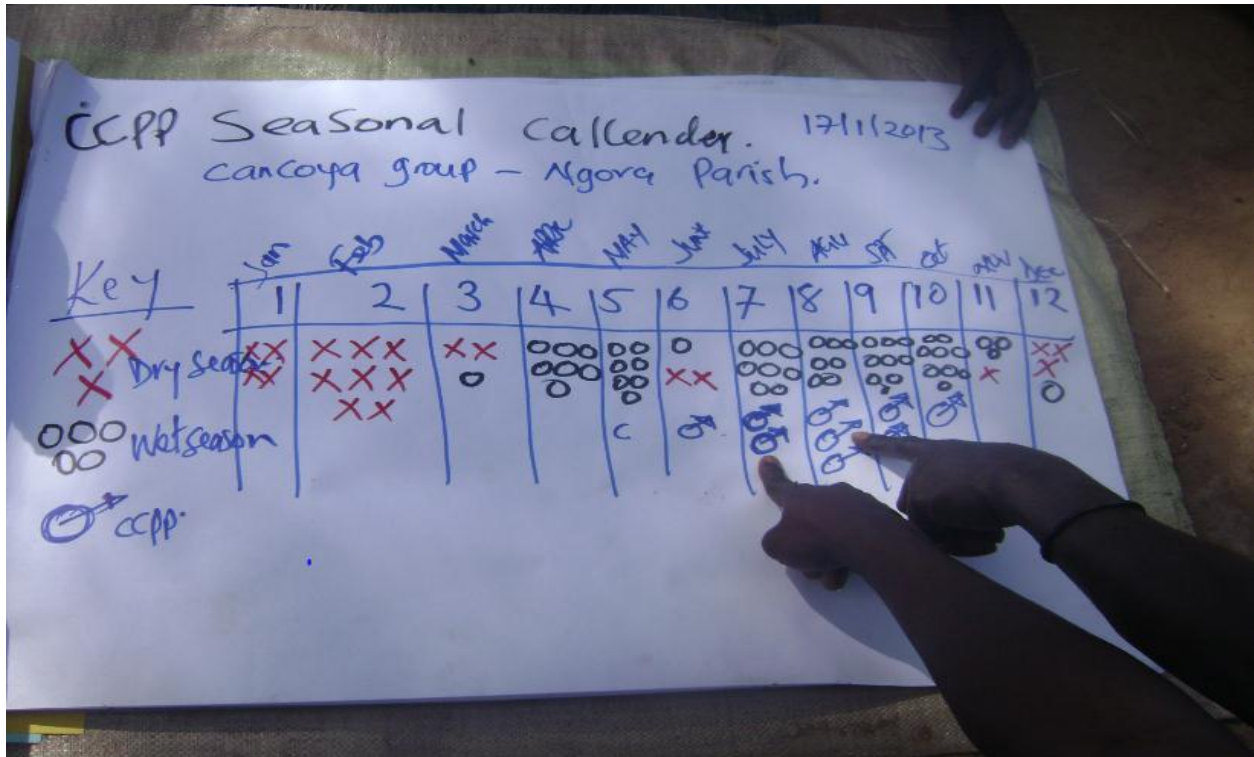


Figure 6: Cancoya farmers in Ngova parish, Agago district associate CCPP with farming practices and seasonal patterns in their area.

4.1.3 Exploring farmers' attitude on CCPP

Responses of the 86 farmers who reported their herds to have suffered CCPP were evaluated with the aim of assessing their varying attitudes towards management of the infection. Sixty one of the farmers (70.9 %) responded to suspected CCPP infection by administering some treatment in form of veterinary medication or local herbs. Table 2 and figure 7 shows a summary of treatment applied in Agago and Otuke to manage CCPP infections. Some of the farmers (n = 25, 29.1%) reported not to have taken any action totally against the disease challenge in their herds.

Table 2: Farmers attitude to CCPP management (n=86)

District	Gave some treatment			Did not take any action
	Number (%)			
	Vet drugs	Local herbs	Vet drugs/ local herbs	No action taken
Agago (n =45)	19 (42.2%)	8 (17.8%)	2 (4.4%)	16 (35.6%)
Otuke (n=41)	22 (53.7%)	4 (9.8%)	6 (14.6%)	9 (22.0%)



Figure 7: Local herbs (“*Akeng*” root and “*Iwucuru*” leaves) farmers use in Ogor Sub County, Otuke district to treat CCPP infections in goats. These herbal extracts are mixed with a little salt solution and administered orally to the sick goats.

4.1.4 Relationship of perceived factors associated with CCPP occurrence

The farmers' perceived factors associated with occurrence of CCPP were reported to include; herd sizes, farming practices, source of breeding stock and methods of acquisition of the breeding stock. To assess this Chi square was used to test for the association between the reported CCPP herd status with the respective perceived factors. This test was done at 95% level of confidence interval.

The results showed that there was no association ($p= 0.66$) among farmers who reared small herd sizes 74 (70.5%), medium 10 (71.4%) and large 2(100%) and CCPP herd infection. All the goats irrespective of herd sizes were equally affected by CCPP. It was also established that there were no association between the occurrence of CCPP among different farming practices, source of breeding stock and method of acquisition of breeding stock as shown in table 3.

Table 3: Relationship between the various factors and farmers' perceived CCPP herd status

Factor	Numbers (%) herd infection	P values
Herd sizes		
Small herd size (n=105)	74 (70.5%)	0.659
Medium herd size (n=14)	10 (71.4%)	
Large herd size (n=2)	2 (100.0%)	
Farming practices		
Communal (n=17)	11 (64.7%)	0.934
Tethering (n= 10)	7 (70.0%)	
Tethering/ communal (n=90)	65 (72.2%)	
Fencing/ Paddocks(n=4)	3 (75.0%)	
Source of goats for breeding stock		
Local breeding stock (n=82)	55 (67.1%)	0.276
From other districts (n=12)	10 (83.3%)	
Both locally and other districts (n=17)	14 (82.4%)	
Method of acquisition of breeding stock		
Purchase (n=90)	64 (71.1%)	0.967
Restocking programs (n=5)	4 (80.0%)	
Dowry (n=7)	5 (71.4%)	
Purchase/ restocking (n=19)	13 (68.4%)	

4.2 Examining the seroprevalance of CCPP in Agago and Otuke districts

Samples were obtained from 404 unvaccinated and 100 vaccinated goats. Of the unvaccinated goats 181 were from Agago district and 223 from Otuke district. Table 4 gives a summary description of the samples analyzed in this study.

Table 4 : Description of the goats sampled

	AGAGO				OTUKE		
	Vaccinated	Unvaccinated	Total		Vaccinated	Unvaccinated	Total
Male	22	46	68	Male	16	51	67
Female	28	135	163	Female	34	172	206
Total	50	181	231	Totals	50	223	273

4.2.1 The in-house indirect ELISA for detection of CCPP

It was important that the optimized in-house indirect ELISA gave consistent and reliable results. To monitor for any plate-to-plate variations each indirect ELISA plate assay had a set of 3 controls, namely a strong positive control, a weak positive control and a negative control. Figure 8 shows the results of the controls applied and show that there were no significant plate-to-plate variations. The negative control was serum taken from an experimental goat naïve to *Mccp* while the weak control was serum taken from the same experimental goat after vaccination with Caprivax vaccine. The strong positive was identified from the archived 190 samples initially screened with the rapid latex agglutination test (test results in table 5).

Table 5: Archived sample results with rapid agglutination test

Archived samples	Number (%) Negative	Number (%) Positive
Unvaccinated (n=178)	173 (97.2%)	5 (2.8%)
Vaccinated (n=12)	11 (91.7%)	1 (8.3%)
Total (n=190)	184 (96.8%)	6 (3.2%)

The negative controls were much more consistent on all the assay plates and thus it served as a better cut off to determine seroprevalence in the study. OD concentrations above 3 standard deviations above the mean OD value of the negative sample were considered sero-positive for CCP. The mean OD for the negative sample was 0.1133 and SD=0.036; hence the cutoff was 0.241. Our cuff off at 3 standard deviations above the mean was to enhance the confidence to pick positives.

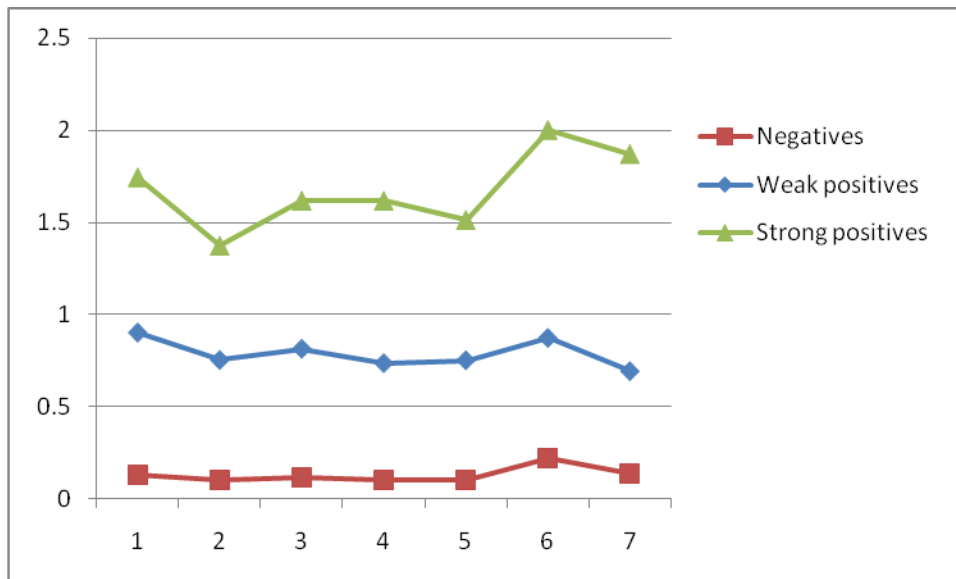


Figure 8: The titre levels for the control samples

4.2.2 Levels of antibodies against Mccp among the goats tested.

Antibody titers (in terms of ODs) were compared between vaccinated and unvaccinated goats. Of note was that although the antibodies among the vaccinated goats generally tended to be higher than unvaccinated goats this difference was of marginal statistical significance, $p= 0.08$ (Figure 9).

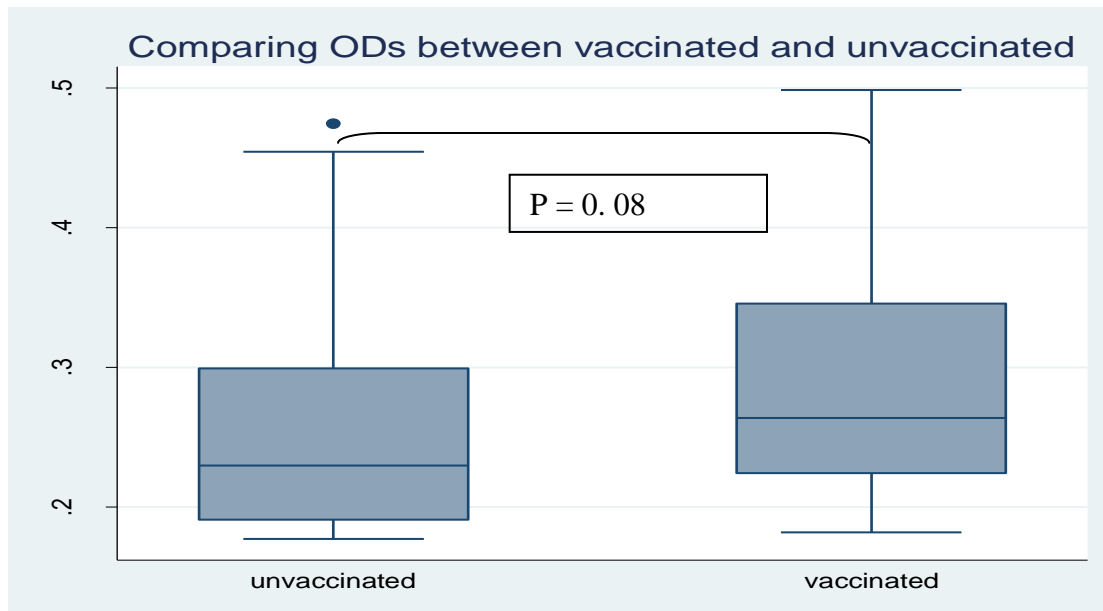


Figure 9: Comparison of titres between vaccinated and unvaccinated sera

Furthermore, the antibodies were compared between sexes and still there were no significant differences observed, $p= 0.2993$ (figure 10).

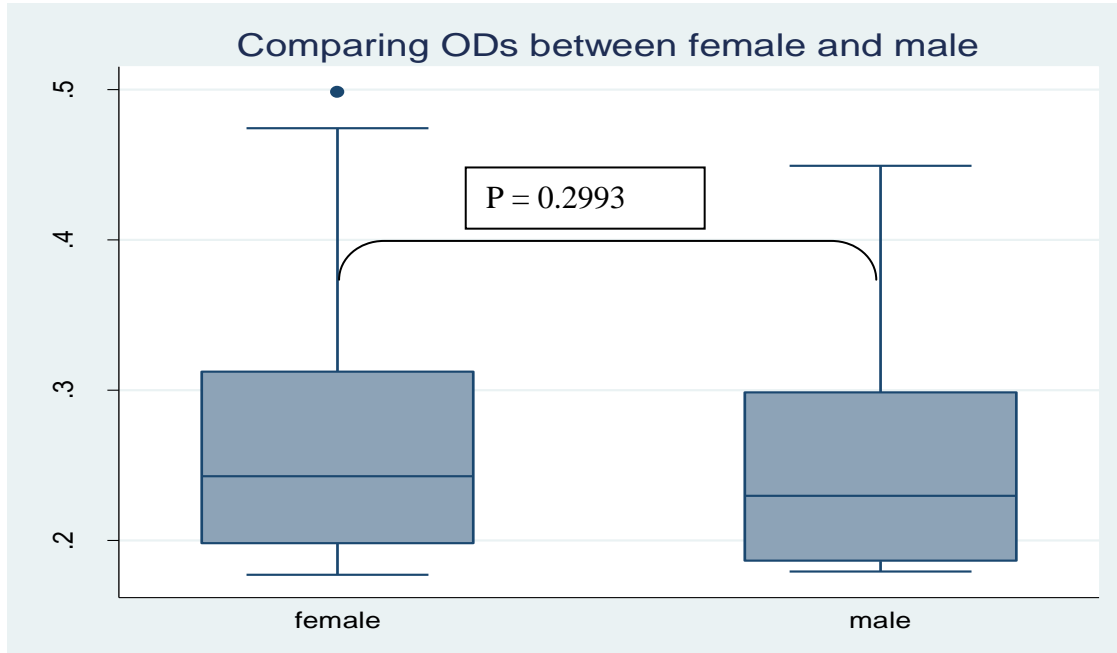


Figure 10: Comparison of titres between female and male goats

4.3 Seroprevalence of CCP in Agago and Otuke districts

Using the cutoff of 0.241 OD value as described above (4.2.1) and considering the distribution of the OD values, an arbitrary categorization of the results was made as negative ($OD < 0.25$), trace ($0.25 < OD < 0.5$), weak positive ($0.5 < OD < 1$) or strong positive ($OD > 1$). Table 6 gives the summary of the categorized results. To compute the seroprevalence, weak and strong positives were considered as positive while those with trace were considered to be negative. The seroprevalence was not significantly different between Agago (17.7%) and Otuke (23.3%) districts (Chi square $P=0.165$), and the combined overall seroprevalence was 20.8%.

Table 6: Seroprevalence of CCPP by district

Districts	Number (%) Negative	Number (%) Trace	Number (%) Weak positives	Number (%) Strong positives
Agago (n=181)	64 (35.4%)	85 (47.0%)	28 (15.5%)	4 (2.2%)
Otuke (n=223)	73 (32.7%)	98 (44.0%)	36 (16.0%)	16 (7.2%)
Total (n=404)	137 (33.9%)	183 (45.3%)	64 (15.8%)	20 (5.0%)

CHAPTER FIVE: DISCUSSION OF RESULTS

The main aim of this survey was to explore the associated risk factors for the occurrence of and the seroprevalence of contagious caprine pleuropneumonia in Agago and Otuke districts, which are outside the Karamoja sub region where CCPP had been previously confirmed (Bolske et al., 1995). Of note was that no previous documentation of CCPP status in the study area had been reported. Therefore a simple semi structured questionnaire administered to selected goat farmers and the FGDs with selected farmer groups was thought prudent to at least obtain some preliminary information on the disease and its associated risk in these areas.

Majority of the goat farmers 144 (88.9%) in Agago and Otuke reared not more than 25 goats, a trend that has not probably changed much in this region for over ten years from the previously documented typical goat herd sizes in Uganda (Kabagambe et al., 2001). Furthermore, designated grazing grounds were mostly owned communally 109 (67.3%) and such kind of husbandry practices are constrained in terms of instituting herd health and management programs even though farmers may be aware of their herd health challenges as was observed in this study.

Contagious caprine pleuropneumonia was ranked second to endoparasites among diseases constraining goat production in both Agago and Otuke. This finding was at variance with Southern Ethiopia participatory investigation of CCPP where farmers ranked the disease as a major constraint in goat production (Mekuria et al., 2008). And similarly also minimal variance in Agago (17.9%) with Turkana southern districts in Kenya (Bett et al., 2009) where relative

incidence of CCPP was ranked highest (25%) relative to PPR (23.5%) and mange (20%) though this agreed with the relative incidence of CCPP ranked by farmers in Otuke (25.5%).

The present study has shown that farmers have indigenous knowledge of diseases affecting their goats and they were able to name the diseases and their clinical signs in local language, and further map the seasonal calendar for such diseases. Farmers described the local name of CCPP, clinical sign and postmortem lesions in the local languages that were closely related to previous clinical signs of the disease documented (Thiaucourt et al., 1996), (Wesonga et al., 2004). The seasonal variation of the CCPP as noted by farmers during both interviews and the FGDs revealed that most cases occurred during long period of rains (July to September). Thus farmers in both districts associated the disease with rainy season and were in agreement with previous reports from Southern Ethiopia (Mekuria et al., 2008). This specific period could be targeted as the best time for control of CCPP in these areas. This also affirmed previous reports that seasonal calendars were important tool in designing disease control programs (Catley et al., 2002, Catley et al., 2012).

The farmers' attitude towards treatment of CCPP in Agago and Otuke was largely related to use of modern veterinary drugs than use of local herbs even though a few used both. This observation was different from purely pastoral communities who were documented to depend highly on local materials mainly plants to manage livestock health problems as first line of treatment (Githiori et al., 2005, Giday and Teklehaymanot, 2013). Of note was that up to 29.1% of the farmers did not take any action against CCPP infections in their herds and this could probably signify the low value some farmers still attached to small ruminant.

The farmers' perceived factors associated with CCPP herd infections among the different herd sizes, farming practices, sources of breeding stock and method of acquisition of the breeding stock were not significant. Of note was that irrespective of the different factors all goat husbandries were still traditional. Such kind of traditional husbandry practices were previously reported to favor spread of CCPP when animals meet at watering points and grazing areas because of increased contact rates between infected goats and naïve ones essential for effective transmission of Mccp (Lefevre et al., 1987, Thiaucourt et al., 1996, Mekuria and Asmare, 2010).

Considering that there are no commercially available serological screening tests for CCPP, an in-house indirect ELISA was optimized and used to screen the sera samples in the present study. Other tests like the latex agglutination test for CCPP, a rapid screening pen-side test developed by VLA (Weighbridge) and the competitive ELISA developed by CIRAD have been developed but not been rolled out for commercial use in field application. The Office International des Epizootics (OIE) indicated such tests having little demand yet constrained by issues of stability of the reagents that hinders them from being commercially available on the market. On the other hand CCPP as a disease is limited to the developing countries reported to lack precise knowledge on disease occurrences (Lefèvre et al., 1993). Thus CCPP probably has not generated enough concern for commercial investment by pharmaceutical companies. Nevertheless, in this study we show that we can locally develop and optimize diagnostic tests in addressing our local challenges.

The present study estimated the overall seroprevalence of CCPP at 20.8%. A closer value to our finding was reported in a cross sectional study done in southern Ethiopia by (Mekuria and Asmare, 2010) recorded overall seroprevalence of 18.61%. The seroprevalence of 20.8% was at variance with (Eshetu et al., 2007) where a higher seroprevalence of 31% in an export abattoir was obtained. Similarly, a higher overall seroprevalence of 32.68% in a study conducted in Kefta Humera, Alamata (Tigray) and Aba-‘ala (Afar), northern Ethiopia was also reported (Hadush et al., 2009). A higher seroprevalence of 32.5% in CCPP outbreak investigation in Beetal goats in Pakistan (Hussain et al., 2012), as well as 37.5% CCPP prevalence in East Turkey were documented (Cetinkaya et al., 2009). On the contrary, (Mekuria et al., 2008) reported a lower overall seroprevalence of 15.5% in a participatory investigation of CCPP in the Hammer and Benna- Tsemay districts of southern Ethiopia. The differences observed may be due differences in situation of the disease during the time of sampling or temporal and partial factors, probable variation in the sensitivity and specificity of the serological tests employed as earlier discussed by (Hadush et al., 2009).

The seroprevalence of the disease was evaluated between the districts, Agago (17.7%) and Otuke (23.3%) using chi square test and the results showed no significant difference. This is because Agago and Otuke lie within the same agro ecological zone. Similar explanation was also reported (Hadush et al., 2009) in seroprevalence study of CCPP in Kefta Humera, Alamata (Tigray) and Aba-'ala (Afar) for no significance obtained.

Important to note was that comparison of antibody levels (ODs) among the vaccinated goats were slightly higher than unvaccinated but the difference still insignificant ($p=0.08$). We

expected a significant difference in the antibody levels between two. This may suggest that vaccination may not be provoking sufficient sero-conversion thus putting the efficacy of this vaccine into question. Never the less, this findings were in disagreement with high efficacy reported with inactivated Mycoplasma strain F38 saponin vaccine in natural infection with CCPP (Rurangirwa et al., 1987a, Litamoi et al., 1989, King et al., 1992) and experimentally lyophilized killed F38 vaccine that conferred 100% protection against mortality and 95% clinical disease of Mycoplasma species strains F38 (Rurangirwa et al., 1991).

Seroprevalence had also been evaluated between sexes of the goats that agreed with findings of (Kusiluka et al., 2000) in Tanzania ; (Hadush et al., 2009) and (Mekuria and Asmare, 2010) in Ethiopia that similarly suggested no sex difference in CCPP epidemiology.

As much as this study was conducted successfully, there were challenges in developing a diagnostic test used in screening the sera samples against antibodies of Mccp. The main setback was absence of a gold standard against which the in house indirect ELISA could have been compared. Secondly, there was a possibility of cross reaction since capture Mycoplasma F 38 antigen used was not specific and could have cross reacted with other Mycoplasmas in the same cluster. Otherwise better results could have been obtained.

CHAPTER SIX: CONCLUSIONS AND RECCOMENDATIONS

6.1 Conclusion from the findings.

The preliminary questionnaire survey, focus group discussions and the serological findings strongly demonstrated that CCPP is prevalent in Agago and Otuke districts which are outside but close to Karamoja region where the disease was previously confirmed. This study also showed most farmers to be aware of CCPP and the use of participatory disease investigations approaches which could be of relevancy in designing and timing CCPP control programs in these districts.

There were no associations in the occurrence of CCPP among different herd sizes, farming practices, source of breeding stock, and method of acquisition of breeding stock. This study has also shown that we can locally develop and optimize diagnostic tests in addressing our local challenges.

6.2 Recommendations.

The study covered only Agago and Otuke, therefore more detailed and bigger studies should be undertaken to investigate further CCPP in rest of the districts in Uganda to pave way for effective preventive and control measures against CCPP in the country. Secondly, there is need to develop a diagnostic test which will be easy to use and readily available in Uganda.

APPENDICES

Appendix I: Questionnaire

MAKERERE



UNIVERSITY

COLLEGE OF VETERINARY SCIENCES, ANIMAL RESOURCES AND

BIOSECURITY

DEPARTMENT OF BIOSECURITY, ECOSYSTEM AND VETERINARY PUBLIC HEALTH

P.O. BOX 7062 Kampala Uganda

Web address: <http://www.vetmed.ac.ug/>. Tel: 256-41-530065

Fax: 256-41-554685

Questionnaire Number.....

Date.....

Interviewer.....

**QUESTIONNAIRE ON THE PREVALENCE AND RISK FACTORS OF
CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN AGAGO AND OTUKE
DISTRICTS**

INTRODUCTION;

Dear respondent,

Makerere University in conjunction with Ministry of Agriculture, Animal Industry and Fisheries is conducting a research to determine the prevalence and risk factors underlying spread of Contagious Caprine Pleuropneumonia in Agago and Otuke districts. This Questionnaire is intended to generate information that shall be used widely to contribute towards the fight against this disease. Your responses will be treated strictly confidential and it

is for purposes of this study only. Please you are free to ask any questions and give us correct information as much as possible.

Thank you.

Part A: Respondent information.

1. Name of respondent/farmer/farm.....
2. Sex of the respondent.
 - a. Male ()
 - b. Female ()
3. What is your age in complete?.....
4. How many people live in this household? (H/H size).....
5. Number of dependents (categorical)
6. What is your education level?
 - a) No education ()
 - b) Primary school ()
 - c) Secondary school ()
 - d) Institution ()
 - e) University ()
5. Location:
 - a. Village.....
 - b. Sub County.....
 - c. District.....

Part B: Goat/sheep demographics:

1. Farm size: a) small (≤ 5 acre) () b) Medium (5 – 10 acre) () c) Large (> 10 acre) ()
2. Farm Land ownership: a) Own land () b) Rent land () c) communal land ()
3. What species of small ruminants do you rear?
a) Goats only () b) sheep only () c) both goats and sheep ()
4. How many are they?
a) Bucks/Billy..... b) Castrates..... c) Does/ ewes.....d) kids.....
5. What is your main purpose for keeping these small ruminants?
a) Meat production () (b) Dairy production () (c) Meat and milk production ()
6. Other Agricultural activities undertaken by the a farmer
a) Crop cultivation () (b) Bee farming () (C) Aquaculture () and (d) others.....

Part C: The management systems:

1. Who takes care of the animals.....
2. What type of management system do you use to keep the animals?
a) Zero-grazing () (b) Tethering () (c) Fencing/ Paddocking () (d) Communal grazing ()
3. How do you water your animals?.....
4. How did you get the animals?
a) Gift () b) Purchase () c) Restocking programs () d) Dowry ()
e) Other (Specify).....
f) Details of restocking.....
5. Do you purchase small ruminants from other districts?
If yes, please specify.....

6. Apart from small ruminants, what other animal species do you keep?

a).Cattle () b) Pigs () c) Dogs () d) Donkeys ()

e) Others (specify).....

Part D: Awareness of CCPP by the farmers

1. What are the diseases of goats in your herd?.....

.....

2. Rank the diseases in order of importance.....

.....

.....

3. Have you ever heard of CCPP?

a) Yes () b) No ()

4. If yes, did your goats suffer CCPP?

a) Yes () b) No ()

5. If yes, what symptoms of CCPP did you observe?.....

.....

.....

6. When did your herd experience this disease (outbreak)?

Seasonal occurrence of CCPP (seasonal calendar).....

Frequency of occurrence of CCPP (time line).....

7. How many goats were affected?

.....
.....

8. What control measure did you undertake when the goats felt sick of CCPP?

.....
.....

9. In your view, how did your goats get infected?

.....
.....

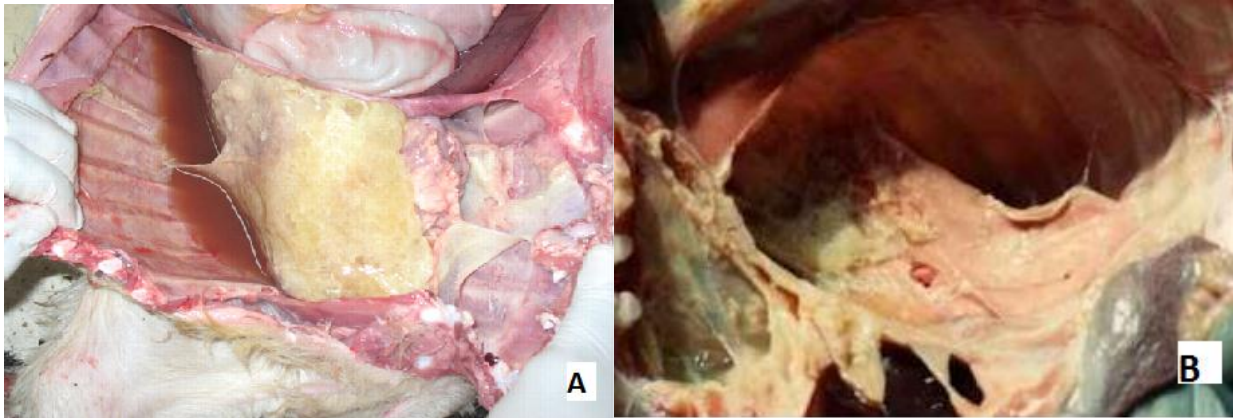
10. What precautions are you undertaking to avoid your herd from getting infected with CCPP?

.....
.....

The end

Appendix 2: Pictures of CCPP postmortem lesions used during questionnaire interview

Lung of a goat affected by CCPP, showing the presence of pleural fluid, fibrin deposition and adhesions



Adopted from Srivastava A K et al. Veterinary Record 2010; 167:304-305

The DVO Agago (C) researcher (D & E) during interview with goat farmers at Kalagum parish in Wol Sub County, Agago and Ogor Sub County in Otake respectively (2011).



Appendix 3: Focus group discussion guide

TOPIC: PREVALENCE AND RISK FACTORS OF CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN AGAGO AND OTUKE DISTRICTS

Purpose: The main purpose of the study is to collect information on the risk factors of Contagious Caprine Pleuropneumonia and gather information on the farmers' probable knowledge, Attitude and Practice of the disease. The information gathered here is expected to provide preliminary findings upon which larger studies can be done so that effective control measures can be instituted against the disease.

- List the diseases that affect goats in your herds
- Rank the diseases listed above using propionate piling method in order of frequency of occurrence
- Has your goats ever suffered from CCPP?
- What are the symptoms/ clinical signs of CCPP?
- What could be the probable causes of CCPP in the herds?
- Could you map the seasonal calendar of CCPP in your area for a period of one year?
- Are there any steps undertaken to control the disease?
- Which steps?.....
- Any challenges in controlling the disease.....
- Any more suggestions to control the disease better.....

The end

Appendix 4: The researcher with Mr. Bahati and Ms Mary during sample collection at Angeta parish in Olilim sub county, Otuke district (2011)



A farmer in Piamol Sub County, Agago district holding a goat suspected to have been suffering from CCPP (2011).



Appendix 5: Temporal night sheds for goats in the study area (2011)



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