JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

SCHOOL OF VETERINARY MEDICINE

SERO-PREVALENCE AND ASSOCIATED RISK FACTORS OF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN SELECTED DISTRICTS OF SOUTH WEST SHEWA ZONE OF OROMIA, ETHIOPIA

Msc. Thesis

BY

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A Thesis submitted to Jimma University College of Agriculture and Veterinary Medicine, School of Veterinary Medicine in partial fulfillment of Masters of Science (Msc.) in Veterinary Epidemiology

By

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LIST OF ABBREVIATIONS

ADLFRDO	Ameya District Livestock and Fishery Resource Development Office
CBPP	Contagious Bovine Pleuropneumonia
C-ELISA	Competitive Enzyme Linked Immunosorbent Assay
CFT	Complement Fixation test
CSA	Central Statistical Authority
DDLFRDO	Dawo District Livestock and Fishery Resource Development Office
PAs	Peasant Associations
PCR	Polymerase Chain Reaction
PPLO	Pleuropneumonia like Organisms
TADs	Trans-boundary Animal Diseases
TMB	Tetra Methyl Benzidine
WDLFDO	Woliso District Livestock and Fishery Resource Development Office

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ABSTRACT

Contagious bovine pleuropneumonia (CBPP) caused by *Mycoplasma mycoides subspecies mycoides* small colony is a highly contagious disease of cattle which is one of the great plagues which continues to devastate the cattle population in Africa. It is one of the most important threats to cattle-raising and trade in Ethiopia. A cross sectional study was conducted on 384 randomly selected cattle from 96 cattle herds which were selected from herds of cattle managed under the traditional extensive production system in three selected districts (Woliso, Dawo and Ameya) of Southwest Shewa zone of Oromia region with the aim of determining the seroprevalence of contagious bovine pleuropneumonia and to assess the risk factors associated with its occurrence. Competitive Enzyme Linked Immunosorbent Assay test was used to identify 384 cattle sera for *Mycoplasma mycoides subspecies mycoides* small colony specific antibodies. The sero-prevalence of CBPP was calculated as the number of sero-positive samples divided by the total number of samples tested. The association between risk factors and sero-prevalence of contagious bovine pleuropneumonia was computed using multivariable logistic regression analysis. Animal and herd level overall sero-prevalence of contagious bovine pleuropneumonia were 9.4 % (95%CI: 6.5-12.3) and 32.3 %(95%CI: 22.9-41.6) respectively. Among predisposing risk factors assessed (age, sex, body condition score, origin of animal, contact, herd size and introduction of new animal into herd); age (OR= 4.3, 95%CI: 1.8-10.1, P=0.001), body condition score (OR=3.4, 95%CI: 1.3-9.0, P=0.015) and herd size (OR=7.5, 95%CI: 2.7-21.2, p=0.000) were significantly associated with the sero-prevalence of contagious bovine pleuropneumonia. The present study showed that the overall seroprevalence of contagious bovine pleuropneumonia was high in the study area and this indicates a need for intervening and implementing control measures to prevent further spread of the disease in the study area and beyond through the use of better and coordinated vaccination program.

Keywords: *Ameya, Contagious bovine pleuropneumonia, Dawo, Ethiopia, Seroprevalence, Woliso*

1. INTRODUCTION

1.1. Background

Ethiopia is a leading country in the number of livestock population in the African continent with an estimated 59.5 million cattle, 30.7 million sheep, 30.2 million goats and 56.53 million poultry (CSA, 2017). The livestock sector has a significant role in socioeconomic activities of the country and contributes much to the national economy particularly with regard to foreign currency earnings through exportation of live animals, meat, skin, and hides (Abdela and Yune, 2017). However, the output of this livestock sector interms of its contributions to the improvement of the livelihood of animal owners and for the growth of national economy is at alower stage compared to the vast resource on hand (Mamo and Beshah, 2017; Mamo *et al*., 2018). The major constraints contributing to low productivity include low genetic potential of the animals, poor nutrition and prevailing animal diseases (Duguma *et al*., 2012; Eshetu and Abraham, 2016). Among the health constraints, transboundary animal diseases such as Contagious bovine pleuropneumonia (CBPP) cause the major limitation to the livestock sector development in the country and affect livelihood through their impact on animal health, animal food production, availability and quality (Geresu *et al*., 2017).

Contagious bovine pleuropneumonia (CBPP) is highly contagious trans-boundary disease of cattle caused by *Mycoplasma mycoides subspecies mycoides* Small Colony (*Mmm*SC). Under natural conditions, it affects only domestic ruminants of the genus *Bos* (Sori, 2005), mainly *Bos taurus* and *Bos indicus (*Abera *et al*., 2016). It has great potential for rapid spread and causes major impact on cattle production. The disease is manifested by anorexia, fever and respiratory signs such as dyspnea, polypnea, cough and nasal discharges in cattle (OIE, 2014). The principal route of infection is inhalation of infective droplets of diseased animals (Radiostits *et al*., 2007). Factors such as age, stress and concurrent infections may predispose to tissue invasion (Mersha, 2017) and other risk factors for its spread include high-density confinement in night housings and mixing of herds at common grasslands and watering places (Bonnet *et al*., 2005). Due to its high economic impact, OIE declared as one of the most serious contagious animal disease and listed on the group of notifiable animal diseases of high

socio-economic impact and regarded as major trans-boundary animal disease (TADs) (Geresu *et al*., 2017).

Contagious bovine pleuropneumonia (CBPP) has been known to occur in Europe since the 16th century but it gained a world-wide distribution only during the second half of the 19th century because of increased international trade in live cattle (Admassu *et al*., 2015). Although CBPP was once found worldwide, it was eradicated from many countries during the mid 20th century` through the application of restrictions to the movement of cattle, as well as test and slaughter policies combined with compensation for livestock owners (Sacchini *et al*., 2012). Its incidence also began to decline in Africa by the 1970s. However, because of the economic and financial difficulties, the disease came back in the late 1980s and early 1990s (Rovid, 2012). As a result, the disease remains endemic in Africa particularly in tropical and subtropical regions (West, central, east and parts of southern Africa) of the continent (Amanfu, 2009; Neiman *et al*., 2009, Admassu *et al*., 2015) and it is one of the major constraints to cattle-raising and trade in African countries (Alemayehu *et al*., 2014).

Ethiopia is one of east African countries in which CBPP is endemically maintained in various parts of the country (Tambi *et al*., 2006). After rinderpest was eradicated, CBPP has become the most important cattle disease that hinders livestock development in the country. This is mainly caused due to the interruption of the consecutive yearly blanket vaccinations with combined rinderpest (Mersha, 2017). According to update and critical analysis of 20 years (1996-2016) reports by Abdela and Yune (2017), studies undertaken on CBPP so far in different localities of the country both at production area and the quarantine stations revealed the existence of the disease in different parts of the country with seroprevalence which ranges from 0.4% to 96%. It is now re-emerging as one of the most economically important diseases that impede livestock production and remain a threat to livestock export potential in the country (Ebisa *et al*. 2015).

1.2. Statement of the Problem

Contagious bovine pleuropneumonia (CBPP) is a highly contagious disease with serious socio-economic consequences at the farmer and national level hampering the export potential of the country (Gizaw, 2004; Tadesse, 2014). Despite widespread recognition of the prime

importance of livestock for poor communities, this disease continue to be an effective brake on marketing opportunities for sale of live animals and possibly meat and other products (Alemayehu *et al*., 2014). The presence of such diseases makes it difficult for the country to access international livestock markets and impacts both poor and richer livestock producers by marginalizing them from higher price livestock markets and restricting their capacity for value-added trade (Demil, 2017). In addition to the measurable economic impact on a national economy the inability to sell their animal can bring severe hardship to a family with no other income of sources of support (Alemayehu *et al*., 2014).

In order to secure international market, Ethiopian government needs to meet international standards governing trade in animals and animal products by showing their response to CBPP control. The challenge of CBPP control in endemic settings will require active partnerships to overcome the limitations of insufficient epidemiological information from the country on the basis of which to develop control measures, and limited capacity and national resources to apply control measures based on mass vaccination or effective movement control (Alemayehu *et al*., 2014). Previously CBPP was thought to be a problem of lowland pastoral areas with incursion to the adjacent highland but in recent years its incidence in the highland areas has raised (Ebisa *et al*., 2015) and reported by different researchers as one of the major threats to cattle production in mixed farming communities (Daniel *et al*., 2016; Mersha, 2016). In Southwest Shewa zone, where mixed farming is the mainstay of the communities, animal disease is one of the major limiting factors to livestock production and field report showed that various livestock diseases like pasteurellosis and foot and mouth disease (FMD) were considered to be the major diseases affecting cattle production in this areas (Tesfaye *et al*., 2018). These diseases share closely similar clinical signs with contagious bovine pleuropneumonia and it might be confused with this disease when diagnosed tentatively.

Despite of the above facts; no previous investigation has been carried out to determine the status of CBPP in Southwest Shewa zone in general and in Woliso, Dawo and Ameya districts in particular. Therefore, by considering the economic importance of the disease, the absence of systematic study conducted in the study area, limitation of epidemiological information, contagious nature of the disease and challenges for CBPP control; this study was designed with the following objective:

1.3. Objective

- To investigate the sero-prevalence of contagious bovine pleuropneumonia and Its associated risk factors in the study area

2. LITERATURE REVIEW

2.1. The Disease

Contagious bovine pleuropneumonia (CBPP) is an acute, sub acute or chronic respiratory disease of cattle and occasionally water buffalo and it is one of the most serious cattle diseases in Africa (Abera *et al*., 2016). The most common forms of the disease are the sub acute and chronic forms and usually cause sub clinical infections responsible for continuing the spread of the disease. Cattle with acute to sub acute disease develop serofibrinous pleuropneumonia and severe pleural effusion. In animal that develop the chronic form of the disease or recover from the acute disease, persistent pulmonary sequestra occur but their ability to transmit the disease is uncertain (Mbiri, 2017). On account of its transmissibility and economic impacts, CBPP is now recognized as a priority transboundary animal disease and has thus been categorized in the OIE list "A" diseases (March *et al*., 2002; Tambi *et al*., 2006). This respiratory disease is characterized by morbidity rates that could be as high as 75% to 90%. The mortality rate seems to vary from 50% to 90% while the case-fatality rate was found to be 50% (Sori, 2005; Abera *et al*., 2016). The disease is responsible for heavy economic losses due to mortality, loss of weight, reduced working ability and infertility. Additional losses can also be attributed to lost market opportunities due to trade bans (Tambi *et al*., 2006).

2.2. Etiology

The causative agent of contagious bovine pleuropneumonia is *Mycoplasma mycoides subspecies mycoides* small colony which belongs to the class *Mollicutes*, order *Mycoplasmatales*, family *Mycoplasmataceae and* genus *Mycoplasma* (Mamo and Beshah, 2017). The *Mycoplasmas (Mollicutes)*, formerly called PPLO (Pleuropneumonia-like organisms), are non-sporulating, Gram-negative, non-motile bacteria, which do not possess a determined shape of the cell. The *Mollicutes* are the smallest of the free-living prokaryotes. *Mollicutes* is the correct term to use when collectively referring to members in this order; however, the trivial name *mycoplasma(s)* is also used for this purpose (Andrews *et al.*, 2008).

There are no internal membrane structures and no cell wall external to the plasma membrane; however, many strains possess surface structures equivalent to a capsule. With the exception of *Acholeplasmas, Mycoplasmas* depend on a supply of intact cholesterol, which they incorporate into the membrane, creating sufficient osmotic stability for survival under normal physiological conditions. The *Acholeplasma* synthesize carotenol as a substitute for cholesterol, but will incorporate cholesterol if it is provided. Their polymorphism is the consequence of the missing cell wall. *Mycoplasmas* are devoid of not only cell walls but also lack the genetic capacity to produce one, which also renders the completely resistant to ß-lactam and other cell-wall active drugs (Kasper and Harrison, 2005). Cells sometimes appear as chains and beads, the result of a synchronized genomic replication and cell division. The preferred stains are Giemsa, Castaneda, Dienes and ethylene blue (Andrews *et al*., 2008). When observed with dissecting microscope, many species exhibit "f*ried egg*" morphology (Radiostits *et al*., 2007).

Growth of *mycoplasma* is relatively fastidious and requires special media rich in cholesterol with addition of horse serum. The *mollicutes* grow slowly and generally require 3 to 6 day incubation before colonies are apparent. Growth is best at 37^{o}c in atmosphere of increased Co_2. The optimum PH for growth of *ureaplasma* is 6, whereas 7.5 for other *mollicutes*. Colony sizes vary from 0.1 mm to 1.0 mm (OIE, 2008).

In natural conditions, two types of *Mycoplasma mycoides* are recognized: large colony (LC) and small colony (SC) (Abera *et al*., 2016). They cannot be differentiated serologically but are different morphologically, culturally and in their pathogenicity and can be distinguished through mouse protection tests. *Mycoplasma mycoides subspecies mycoides* Small Colony type (*Mmm*SC) affects only the ruminants of the *Bos* genus (mainly bovine) (Gizaw, 2004). Large colony types occur almost exclusively in goats, rarely in sheep while SC types cause CBPP in cattle (Gorton *et al*., 2005). *Mycoplasma mycoides subspecies mycoides* large colony (LC) type does not result in disease in cattle, but causes septicemia, polyarthritis, mastitis and encephalitis in sheep and goats (Abera *et al*., 2016).

2.3. Epidemiology

Contagious bovine pleuropneumonia epidemiology is characterized by transmission by direct contact, long incubation period and possibility of early excretion of *Mycoplasma* (up to 20 days before apparition of clinical signs), during the course of the disease and after recovery in "lungers" (up to two years). These epidemiological features on one hand and the lack of a reliable screening test to pick up early carriers and lungers on the other hand, make it essential to control cattle movements in order to limit the spread of the disease (OIE, 2008). The epidemiology of CBPP in Africa is dominated by different factors; these are, cattle is the only species affected, transmission is through the direct contact of susceptible animal with clinical cases or chronic carriers and cattle movement play a very important role in the maintenance and extension of the disease (Bessin and Connor, 2000; Radiostits *et al*., 2007).

2.3.1. Host range

Contagious bovine pleuropneumonia is predominantly the disease of cattle and occasionally water buffalo are naturally infected (Andrew *et al.*, 2008). Clinical cases have also been reported in yak (*Poephagus grunniens/ Bos grunniens*) and captive bison (*Bison bison*). Sheep and goats can be infected, although they are not thought to be important in the epidemiology of CBPP (Mitchell, 2007). There are many reported breed differences with respect to susceptibility. In general, European breeds are tends to be more susceptible than indigenous African breeds (Admassu *et al*., 2015). There does seem to be some age resistance, animals less than three years of age are less resistant to experimental challenges (Bashiruddin *et al*., 2005). Experimental work in Australia showed that buffaloes could be infected by artificial means but did not spread CBPP to in contact buffaloes (Mamo and Beshah, 2017). Natural infection has been demonstrated in goats by recovery of the agent from their lungs but experimental inoculation suggested that their susceptibility to the disease is low and the fact that CBPP was eradicated from Botswana by culling only the cattle; although large numbers of goats were present in the affected area, suggests that they do not serve as a reservoir for the disease (March *et al*., 2000). *Mycoplasma mycoides subspecies mycoides* Small Colony (*Mmm*SC) had been isolated from milk of sheep with mastitis and goats with pneumonia (Egwu *et al.*, 2012).

2.3.2. Geographical distribution

Contagious bovine pleuropneumonia is still an endemic disease in Africa, Asia, Eastern Europe, and the Iberian Peninsula (Gizaw, 2004; Tegegn, 2017). With the imminent eradication of rinderpest from Africa, contagious bovine pleuropneumonia (CBPP) has become the disease of prime concern in terms of epizootics that affects cattle (Amanfu, 2009; Ebisa *et al*., 2015) and it is one of the most serious diseases of cattle in African countries (Amanfu, 2009; Mamo *et al*., 2018) particularly in tropical and subtropical regions (West, Central, East and parts of southern Africa) of the continent (Amanfu, 2009; Neiman *et al.*, 2009). This disease was present in many African countries of which Eritrea, Ethiopia, Kenya, Somalia, Sudan, Tanzania, and Uganda are some of the countries quoted (Tegegn, 2017).

2.3.3. Source of infection

The primary source of most of the pathogenic *mollicutes* is the host that is infected with the agent (Hirsh *et al*., 2004; Admassu *et al*., 2015). Cattle in the incubatory phase of the disease may harbor *Mmm*SC in their nasal passages for 40 days prior to developing clinical signs or antibodies and are considered to be a potent source of infection (Mamo and Beshah, 2017). Carrier animals, including sub-clinically infected cattle, can retain viable organisms in encapsulated lung lesions (sequestra) for up to two years. These animals may shed organisms, particularly when stressed. As long as the bacteria remain encapsulated by fibrous tissue in the intact sequestrum the animal will not be infective, but it was thought that condition of stress due to starvation, exhaustions or intercurrent can cause the sequestrum to break down and convert the animal into an active case (Dedieu-Engelmann, 2008; Alemayehu *et al*., 2014). Experimental evidence throws some doubt on this explanation, but droplet infection is usually associated with a donar lesion in the lung (Radostits *et al.,* 2007).

2.3.4. Transmission

Closeness of contact, intensity of infection and number of susceptible animals are important factors in the rate of transmission of the disease (Abera *et al*., 2016). Normally, transmissions are by droplet infection from actively infected animals to susceptible animals in close proximity (Andrews *et al*., 2008). Outbreaks usually occur as the result of movement of

infected animals into a naive herd. Cattle may be exposed to infections for a period of up to 8 months before the disease become established and this necessitates a long period of quarantine before a herd can be declared to be free of the disease. This organism also occurs in saliva, urine, fetal membranes and uterine discharges. Close repeated contact is generally thought to be necessary for transmission; however, *Mmm*SC might be spread over longer distances (up to 200 meters) if the climatic conditions are favorable (Francis *et al*., 2018).

2.3.5. Risk factors

CBPP is typical example of multifactorial diseases, where factors such as intercurrent infections, crowding, inclement climatic conditions, age, genetic constitution, and stress from transportation, handling and experimentation are important determinants of the final outcome of infection (Mamo and Beshah, 2017).

Contagious bovine pleuropneumonia occurs only in cattle; rare natural cases have been observed in buffalo, yak, bison, reindeer and antelopes and the disease has been produced experimentally in captive African buffalo and white tailed deer (Radiostits *et al*., 2007). There are many reported breed differences with respect to susceptibility. In general, European breeds are tends to be more susceptible than indigenous African breeds (Admassu *et al*., 2015). There does seem to be some age resistance, animals less than three years of age are less resistant to experimental challenges (Bashiruddin *et al*., 2005). In addition, Masiga *et al*. (1996) reported that young animals are more susceptible to acute forms of CBPP infection than adult cattle and thus acutely infected young animals may die of CBPP and may not be available for testing. However, chronic stages of the disease are usually seen in adult cattle as the age progresses (Olabode *et al*., 2013).

Mycoplasma mycoides subspecies mycoides Small Colony is sensitive to all environment influences; do not ordinarily survive outside the animal body for more than a few hours. Restriction enzyme analysis of strains of the organism found that European strains have different patterns than African strains (Radiostits *et al*., 2007). The organism can be grouped into two major, epidemiologically distinct, clusters. One cluster contains strains isolated from different European countries since 1980 and second cluster contains African and Australian strains collected over the last 50 years. The current European strain lack a substantial segment

of genetic information which may have occurred by deletion events. A variety of potential virulence factors have been identified, including genes of encoding putative variables, surface proteins, enzymes and responsible for the production H_2 O_2 and the capsule transport proteins which is thought to have toxic effect on the animal. Molecular epidemiology of CBPP by multilocus sequence analysis of *Mmm*SC strains found a clear distinction between European and African strains. This indicates that the CBPP outbreaks which occurred in European were not introduction from Africa and confirms true re-emergence (OIE, 2008; Mersha, 2016).

The occurrence and incidence of CBPP influenced by management system, disease control policies and regulation of the country, knowledge of the disease by farmers, veterinarians and livestock field officers. The diagnosis capabilities of veterinary laboratory, disease surveillance and monitoring system, adequacy of vaccination programs, government budget allocated to control programs, desires of cattle owner and traders to control the disease are critically important management factors, which influence the effectiveness of controlling disease in a country (Radiostits *et al*., 2007). This affects epidemiology of the disease and crucial factor since CBPP is essentially related to the movement of animals (Mersha, 2016).

In parts of Africa where there are dry climatic conditions, nomadism and transhumant pastoral practice are common making it very difficult not only to control livestock movement, but also to conduct disease surveillance because the farmers and their animals move from one place to another in remote areas with few roads and no means of modern communication. Other risk factors that facilitate the spread of the disease include cattle movement for trading and social activities, keeping many livestock in confinement in night housing and communal grazing where livestock share watering points and grassland are responsible for the spread of the contagion (Mbiri, 2017).

2.4. Pathogenesis

Very little is known about the factors and mechanisms that affect the pathogenicity of *Mmm*SC. No secreted toxins have been identified; neither receptor molecules on the bacterial surface that mediate binding to host epithelium or induce other cellular responses in the host tissues. However, certain factors have been associated with the pathogenesis, but the precise modes of action are still elusive (Mamo and Beshah, 2017).

An essential part of the pathogenesis of the disease is thrombosis in the pulmonary vessels, probably prior to the development of pneumonic lesions. The mechanism of development of the thrombosis is not well understood, but is considered, at least in part, mediated through induction of cytokines (Admassu *et al*., 2015). Contagious bovine pleuropneumonia is lobar variety of pneumonia in which the inter-lobular septa are dilated and prominent due to a great out pouring of plasma and fibrin into them and this dilated septa that give the "*Marbling*" effect to the lung in these areas (Abera *et al*., 2016; Geresu *et al*., 2017).

Bronchitis, bronchiolitis and alveolitis with predominantly neutrophils and mononuclear cellular response constitute the very early inflammation in *Mycoplasma* pneumonia. Contagious bovine pleuropneumonia is characterized by substantial unilateral pulmonary necrosis, sometimes sequestration and marked serosanguinous fluid accumulation in interstitial and pleura (Nicholas *et al*., 2009). Vasculitis appears to be an important component of the pathological changes in this disease, explaining the marked exudation and pleurisy. Thrombosis can explain ischemic necrosis and infarcts of the lung. Death results from anoxia and presumably from toxemia (Mamo and Beshah, 2017). There are various substances produced by the Mollicutes, which are potentially important in disease pathogenesis. Peroxide and super-oxide production may be important in disruption of host cell integrity (Gizaw, 2004).

Mycoplasma phospholipases are potentially important in pneumonia for they may reduce surface tension of the alveolar surfactant, thus resulting in atelectasis. A galactan polymer in *Mycoplasma mycoides subspecies mycoides* has been shown to modulate the immune response and promote dissemination (Admassu *et al*., 2015**)**.

2.5. Clinical Signs

The disease affects the respiratory tract of cattle and characterized by fever, anorexia, dyspnea, polypnea, cough, and nasal discharge. Cattle of all ages are susceptible, but young cattle develop joint swelling rather than lung infections. Many cattle show no disease signs despite being infected and chronically infected animals might act as carriers and sources of infections (Schnee *et al*., 2011; Schubert *et al*., 2011).

Depending on the resistance level of the animal and the intensity of exposure, the disease takes an acute, sub acute to chronic, or the acute course is sometimes followed by a chronic stage which may last for two years (lungers) as a latent phase of the disease (Pilo *et al*., 2007). In an affected herd, CBPP can be seen in hyperacute, acute, sub acute or chronic forms (Masiga *et al.,* 1996; Abera *et al*., 2016). In endemic regions, 13% of cases are of the hyperacute form, 20% of the acute form and 46% of the sub acute form. Approximately 21% of animals are resistant to the disease (Masiga *et al.,* 1996).

2.5.1. Hyperacute forms

Contagious Bovine Pleuropneumonia (CBPP) may be rapidly fatal with no clinical signs observed. This form occurs during the onset of an outbreak and death may be all that is seen. In some cases the animal may die after one to three days with no signs of pneumonia. Death may result from asphyxia, toxaemia or heart failure (Radostits *et al*., 1994; Masiga *et al.,* 1996).

2.5.2. Acute forms

The early stages of CBPP are indistinguishable from any severe pneumonia with pleurisy. Animals show dullness, anorexia, irregular rumination with moderate fever and may show signs of respiratory disease. After a few days, the temperature rises to 40°C or higher, accompanied by a fall in milk yield (in cows), anorexia and cessation of rumination. At this stage, chest pain is evident. Affected animals are reluctant to move, and stand with the elbows abducted and the back arched, the head extended and the nostrils dilated. Breathing becomes short and rapid and a moist cough may be present. In the severe form of the disease, the mouth remains wide open and may contain foam. Mucoid discharge from the nostrils may occur. Exercise will aggravate the respiratory distress (Masiga *et al*., 1996; Admassu *et al*., 2015).

Figure 1: Clinical case of CBPP

Source: FAO (2002)

While classical respiratory signs may be evident in calves, articular localization of the causative agent with attendant arthritis usually predominates calves less than six month of years (Andrews *et al.*, 2008; Radiostits *et al.*, 2007).

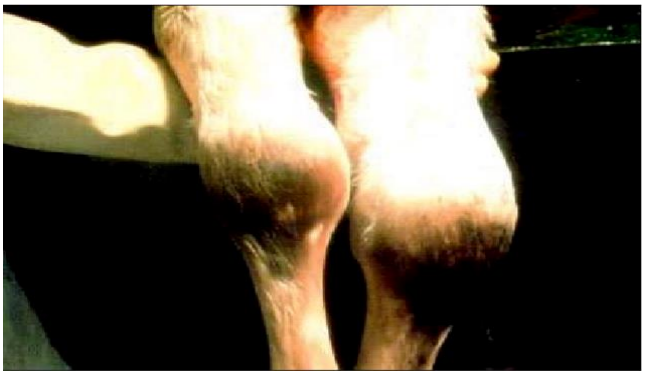

Figure 2: Swollen joints of a calf with acute CBPP

Source: FAO (2002)

In acute CBPP, there is a severe fibrinous pneumonia with copious pleural exudates. The latter is a striking feature and there may be up to 30 liters' of yellow exudates, containing clots, in the chest cavity. One or both lungs may be partially or completely consolidated, giving a characteristic marbled appearance. Affected areas are swollen, vary from pink to dark red, have a moderately firm consistency and exude clear fluid and sometimes blood from cut surfaces (Admassu *et al.*, 2015).

Figure 3: CBPP marbled lung

Source: FAO (2002)

2.5.3. Sub acute forms

Signs may be limited to a slight cough only noticeable when the animal is exercised (Abera *et al*., 2016). Cattle that recover naturally are extremely weak and emaciated. Many infected animals develop chronic or milder forms of the disease, which may be either symptom-less or associated with only a slight temporary rise in body temperature, and some loss of condition. Recovered animals may be clinically normal but in some, an inactive sequestrum forms in the lung, with a necrotic center of sufficient size to produce a toxemia causing unthriftness, a chronic cough, and mild respiratory distress on exercise (Admassu *et al*., 2015). The length of the incubation period depends upon the volume of the infective dose, the virulence of the strain, and the immune state of the animal and it can last from a few days up to several months (in occasional instance up to 6 months) (Cheneau *et al*., 2004).

2.5.4. Chronic form

The chronic form is characterized by an apparently healthy state of the animal even though chronic lung lesions are present. These “silent” carriers of CBPP are infectious and thought to be an important factor in spreading the disease among cattle herds. There is considerable variation in the severity of clinical disease from acute, sub acute to chronic form (Radiostits *et al.*, 2007).

2.6. Differential Diagnosis

In carrying out a CBPP diagnosis, it is necessary to differentiate this disease from other diseases which may present similar clinical signs or lesions. CBPP is hard to differentiate

from other causes of cattle respiratory disease. Pneumonia (particularly unilateral illness) in adults and polyarthritis in calves should be considered warning signs for potential CBPP infection. The way the disease behaves in the herd is as important as the findings in a single animal when carrying out an investigation. Differential diagnosis considered in the diagnosis of CBPP include diseases like Rinderpest, Abscesses, Hemorrhagic septicemia, Foot and mouth disease, Bacterial or viral bronchopneumonia, Theileriosis, Ephemeral Fever, Tuberculosis, Bovine farcy, Actinobacillosis, Foreign body reticulum pericarditis (Radiostits *et al*., 1994; Gizaw, 2004; Abera *et al*., 2016).

2.7. Diagnosis

Current state of techniques available for the diagnosis of CBPP clearly demonstrates that recent advances in the study of immunology and molecular biology have and will continue to open avenues for improved CBPP diagnosis. The tools currently available for CBPP diagnosis include history of contact with infected animals, clinical signs, pathologic lesions, (Pleurisy, lung hepatization), identification and isolation of the agent, immune blotting, serology and PCR techniques (OIE, 2008; Mamo and Beshah, 2017). CBPP frequently results in disease in only single lung as compared with other types of pneumonia in which both lungs are affected. In a herd with signs of pneumonia in adults and polyarthritis in calves, CBPP should be considered. Post mortem lesions may be more useful in the diagnosis (Abera *et al*., 2016). A definitive diagnosis can be made by recovering *M. mycoides Subspecies mycoides* SC from infected animals (Nicholas *et al*., 2009).

2.7.1. Cultural examination

Samples like nasal swabs, bronchoalveolar washings, pleural fluid obtained by puncture are collected from live animal. Samples taken to necropsy are lung lesions, lymph nodes, pleural and synovial fluid from animals with arthritis. The causal organisms can be isolated culturally from animals during febrile phase or shortly after postmortem from blood, pleural exudates (chest fluid) and/or affected lung tissue & lymph nodes. Because of ‘fastidious’ nature of the agent, samples should be submitted to the laboratory as soon as possible after collection (OIE, 2008; Abera *et al*., 2016).

2.7.2. Biochemical tests

Mycoplasma mycoides subspecies mycoides small colony type is sensitive to digitonin, does not produce '*film spots*', ferment glucose, reduces tetrazolium salts (aerobically and an aerobically), does not hydrolyze arginine, has no phosphatase activity, and has no or weak proteolytic properties. It is where immunological tests give uncertain results that biochemical test is preferred (Tegegn, 2017).

2.7.3. Serological tests

Serological tests for CBPP are valid at the herd level only because false positive or false negative results may occur in individual animals. Tests on single animals can be misleading, either because the animal is in the early stage of disease, which may last for several months, before specific antibodies are produced, or it may be in the chronic stage of the disease when very few animals are seropositive. False-positive results can occur (2%), of which an important cause is serological cross-reactions with other *mycoplasmas*, particularly other members of the *M. mycoides* cluster (Masiga *et al*., 1996, OIE, 2014). The validity of the results has to be confirmed by postmortem and bacteriological examination, and serological tests on blood taken at the time of slaughter. The CFT and ELISA are recommended for screening and eradication programmes. The highly specific immunoblotting test is useful as a confirmatory test but is not fit for mass screening (OIE, 2014). The CFT and c-ELISA are the OIE prescribed herd level tests for CBPP and they are said to have specificity of 98% and 99.9%, respectively and sensitivity for both tests is said to be about 70% (Mtui-Malamsha, 2009).

Complement fixation test: a test suitable for determining freedom from disease and a prescribed test for international trade. The Campbell and Turner complement fixation (CF) test remains the recommended procedure (although the current method is slightly different from the original one), and it is widely used in all countries where infection occurs (OIE, 2008). For antigen titration and harmonization purposes, an international standard positive bovine serum is available from the OIE Reference Laboratory in Teramo, Italy. However, the CFT is still difficult to perform, requiring well trained and experienced personnel (OIE, 2014).

The limitations of the CF test are well known. With a sensitivity of 63.8% and a specificity of 98% (OIE, 2008), the CFT can detect nearly all sick animals with acute lesions, but a rather smaller proportion of animals in the early stages of the disease or of animals with chronic lesions. In addition, therapeutic interventions and improperly conducted prophylactic operations (partial slaughter of the herd) may increase the number of false-negative reactions. However, for groups of animals (herd or epidemiological unit) the CFT is capable of detecting practically 100% of infected groups. The nature of the pathogenesis of the disease is such that the incubation period, during which antibodies are undetectable by the CFT, may last for several months (OIE, 2014)

Competitive Enzyme-linked Immunosorbent Assay(c-ELISA): c-ELISA developed by the OIE collaborating centre for the diagnosis and control of animal diseases in tropical countries (OIE, 2008), has been validated internationally in accordance with OIE standards. Compared with the CFT, the c-ELISA has equal sensitivity and greater specificity (Rurangirwa *et al*., 2000; OIE, 2014). Advice on standard protocols and the availability of reagents can be obtained from the OIE reference laboratories for CBPP or the OIE collaborating centre for ELISA and molecular techniques in animal disease diagnosis (OIE, 2014).

Validation tests that have been carried out in several African and European countries would indicate: the true specificity of the cELISA has been reported to be at least 99.9%; the sensitivity of the c-ELISA and the CFT are similar; and antibodies are detected by the c-ELISA in an infected herd very soon after they can be detected by the CFT, and c-ELISA antibody persists for a longer period of time (Niang *et al*., 2006; OIE, 2014). To enhance its repeatability and the robustness, this cELISA is now provided as a ready-made kit that contains all the necessary reagents, including precoated plates kept in sealed bags. This kit can be obtained commercially and availability can be checked through the OIE reference laboratory in France. Sera are analyzed in single wells. The substrate has been modified and is now TMB (tetra methyl benzidine) in a liquid buffer and the reading is at 450 nm. The substrate colour turns from pale green to blue in the first place and becomes yellow once the stopping solution has been added. Mab controls exhibit a darker colour while strong positive serum controls are very pale. The cutoff point has been set at 50% and should be valid in every country (OIE, 2014).

Immunoblotting test: A field evaluation indicated an immunoblotting test (IBT) is an immuno enzymatic test that is higher sensitivity and specificity than the CF test and has been developed to confirm doubtful CFT or c-ELISA results. A core profile of antigenic bands, present both in experimentally and naturally infected cattle are immuno dominant. The more accurate picture of the immune status of animals given by this test is due to the possibility of a more precise analysis of the host's immune response in relation to the electrophoretic profile of *Mmm*SC antigens; thus the test overcomes problems related to nonspecific binding. It should be used primarily as a confirmatory test, after other tests and should be used in all cases in which the CF test has given a suspected false result (OIE, 2008)

2.7.4. Nucleic acid recognition methods

Polymerase chain reaction is a rapid and sensitive diagnostic method. It allows detection of *Mmm*SC directly in samples of lungs, bronchial lymph nodes, nasal swabs, pleural fluid and blood. Pre-incubation for 24hr of clinical specimens in growth medium may increase test sensitivity. If used for the identification of new isolates it reduces drastically the time required (24-48 hr versus 2-3 weeks). Detection of the causative agent from bovine samples is one way to confirm a suspect CBPP case. However, isolation and serological or biochemical identification tests are time consuming leading to significant delays. To overcome this problem, both single and nested PCR systems have been developed for identification of *Mmm*SC (Admassu *et al*., 2015). Using samples such as lung exudates allows the PCR to be performed directly after differential centrifugations to remove inflammatory cells and pellet *Mycoplasmas*. For fragments, the PCR is applied after DNA extraction. The PCR can also be performed on urine or blood. The main advantage of the PCR technique is that it can be applied to poorly preserved samples (Contaminated or without any viable *Mycoplasmas* as may occur following antibiotic treatment) (Kasper and Harrison, 2005).

The PCR has become the primary tool for identification of *Mmm*SC. If a sample is PCR positive in a CBPP-free zone, the test confirmed by a second and different PCR; infection can be confirmed by the use of only one immunological test. One of the problems with PCR is the possible occurrence of contamination if the necessary precautions and quality management system are not implemented correctly in the diagnostic laboratory. Great care must be taken to respect the strict separation between those parts of the laboratory that may contaminated with

PCR products such as the electrophoresis room and those parts of the laboratory devoted to preparing the reagents (Mamo and Beshah, 2017).

2.8. Control and Prevention

To make the most efficient use of the increasingly scarce resources, disease control programs must be tailored to the needs of particular communities and to high-priority cattle populations to ensure their efficacy, acceptance and sustainability; therefore, economic evaluation should be generalized (Mamo and Beshah, 2017). In most continents, control strategies are based on the early detection of outbreaks, control of animal movements, quarantine, vaccination, test and slaughter policies and a stamping-out policy (March *et al*., 2002; Radostits *et al*., 2007). However, stamping out, test and slaughter policies may not be economically feasible in endemic African countries (Jores *et al*., 2008). This was demonstrated by a stamping-out eradication of CBPP in Botswana during 1996, which led to negative effects on short-term economics and increased malnutrition in children (Boonstra *et al*., 2001). Thus, these methods may not prove realistic and vaccination is the most frequently used control strategy in combination with animal movement control (FAO, 2016; Dereje *et al*., 2018). Extensive vaccination programs and chemotherapy are the remaining options for CBPP control in Africa and of these, vaccination which is major control method practiced in Ethiopia (March, 2004; Admassu *et al*., 2015).

CBPP vaccination was initially (1920"s to early 1970"s) based on broth T1 vaccine which was later replaced by freeze-dried live attenuated *Mmm*SC vaccine T1/44 vaccine (OIE, 2008). A streptomycin resistant variant (T1SR) was developed and used in combination with rinderpest vaccine (Litamoi, 2000; Mtui-Malamsha, 2009). The only commercially available vaccines are live attenuated vaccines using the T1/44 and T1sr strains. The former is more widely used, as it provides coverage for a year, while the duration of immunity of the T1sr vaccine is shorter. The latter has the advantage of inducing fewer adverse reactions and being unlikely to cause clinical disease, as sometimes occurs with T1/44, where especially first time vaccination may induce a Williams reaction that is sufficiently severe to require treatment (Abera *et al*., 2016). To be effective, vaccination must be repeated initially at short intervals and thereafter annually over 3-5 years (FAO, 2002).

Antibiotic treatment against CBPP is widely used. It is not part of any official control strategy due to suspicion that its use could facilitate developments of sequestra, increase the number of carrier animals, increase development of resistant strains, and mask the occurrence of clinical disease (Provost *et al*., 1987; Geresu *et al*., 2017). Masking of clinical disease will make diagnosis difficult, which may contribute to unrecognized infections and CBPP transmission. Nevertheless, antibiotics are widely used in pastoralist communities (Mariner *et al*., 2006). Ayling *et al.* (2000) carried out an in vitro trial of the effects of five commonly used antibiotics on a number of strains of *Mmm*SC and concluded that tilmicosin and danofloxacin were effective both in terms of mycoplasmastatic and mycoplasmacidal activity; florfenicol and a tetracycline provide intermediate effectiveness while spectinomycin was ineffective against some strains. Commonly used antibiotics include tetracycline, Tylosin, erythromycin, lincomycin, spectinomycin and tilmicosin. Tylosin and spiramycin are effective in the control of excessive vaccination reactions and should be of value in the treatment of clinical cases resistance to some of these antimicrobials has been noted. Animals that do not respond to treatment often become carriers (Mamo and Beshah, 2017).

2.9. Economic Importance of CBPP

Contagious Bovine Pleuropneumonia is considered to be a disease of great economic importance because of its high mortality rate, production loss, increased production cost due to cost of disease control, loss of weight and working ability, delaying marketing, reduced fertility, trade bans and reduced investment in livestock production (Radiostits *et al.*, 2007). The presence of CBPP in a herd results in direct losses due to its impact on cattle production, through increased mortalities, reduced milk yield, reduced weight gain and reduced fertility rate and therefore it compromises both household and national food security due to loss of protein and draught power (Tambi *et al.*, 2006; Demil, 2017). CBPP also causes indirect losses through additional cost of treatment, preventive vaccination, field diagnostic testing and slaughter of clinical cases, surveillance activities, disruption of trade and the limitation of investment opportunities due to reluctance in adoption of improved breeds (Thornton and Otte, 1999; Demil, 2017). In addition to these, it leads to imposition of rigorous limitations to international trade soon CBPP affected countries in accordance with world organization of Animal Health (OIE) regulations (Bonnet and Lesnoff, 2009).

Contagious Bovine Pleuropneumonia is considered as one of the main stumbling blocks to the growth of the livestock industry in the African continent causing losses estimated to be around two billion US dollars per years (Vilei and Frey, 2010) and the disease has been causing significant economic losses on the livestock sector and the national economy. It accounts for a loss of over 8.96 million US dollars per year (Abdela and Yune, 2017). Thus, over the last decades, the country has lost a substantial market share and foreign exchange earnings due to frequent bans by the Middle East countries (Hurrissa and Eshetu, 2002; Tambi *et al*., 2006).

2.10. Status of CBPP in Ethiopia

Regarding the situation in east Africa in general and Ethiopia in particular, there is a suggestion that CBPP was introduced into East Africa from India by the army of field Marshal Napier when he invaded Ethiopia in 1867–1868 (Masiga *et al*., 1996; Abdela and Yune, 2017). Countries in East Africa reported 66% of the total outbreaks (58% in Ethiopia and Tanzania and 8% in other countries in the region) (Tambi *et al*., 2006). Ethiopia is one of east African countries in which CBPP is endemically maintained in various parts of the country with 25% morbidity and more than 10% mortality (Masiga *et al*., 1996; Tambi *et al*., 2006). A total of 583 outbreaks of CBPP were reported between 1995 and 2002 in Ethiopia in which highest outbreaks (187) were reported in 1998 (Tambi *et al*., 2006; Abdela and Yune, 2017).

Vaccination was the main control strategy practiced in Ethiopia for the last 30 years in combination with Rinderpest vaccine which has rendered protection and restrained the disease to relatively low level until 1992/93. After Rinderpest has been brought under control, CBPP is considered to be among the most important cattle diseases and impediments to livestock development in the country (Mersha, 2016). CBPP is one of the great plagues which continue to devastate cattle herds on which so many people are dependent in the lowlands. In the highlands, the consecutive yearly blanket vaccinations with combined Rinderpest and CBPP have certainly contained the disease to a relatively low level during the past years. But with the adoption of a strategy towards Rinderpest eradication, the vaccinations in the highlands have ceased since 1992/93. Currently, CBPP vaccination in the country is based on targeted

and ring vaccination in the face of outbreaks (Tegegn, 2017). The usual blanket coverage was around 50% and never reached the desired 80-100% level. Generally, the irregularity and low rate of vaccinations since 1993 seems to contribute to the increased incidence of the disease and its further spread (Gizaw, 2004) and now the disease is one of the major threats in the country hindering and challenging the livestock production (Dele *et al*., 2014).

According to reports of various outbreaks, national Serosurveillance and research results from 1997 to 2010, CBPP was found to be present in almost all regional states (Demil, 2017). Studies conducted in Western Ethiopia (Daniel *et al*., 2016), Northern Ethiopia (Teklue *et al*., 2015), Southern Ethiopia (Ebisa *et al*., 2015), Southwest Ethiopia (Mamo *et al*., 2018) and different regions of the country revealed that CBPP is posing a major threat to cattle in many parts of the country thereby causing considerable economic losses through morbidity and mortality(Afework, 2000). Furthermore, CBPP has been reported from different export quarantine centers in the country (Kassaye and Molla, 2013; Dele *et al*., 2014**)** signifying that CBPP remain a threat to livestock export market and may reduce the investment in livestock production.

In general at the country level, CBPP seroprevalence studies have been conducted in different localities of the country. However, there is a great variation of reports from different areas (Ebisa *et al*., 2015; Daniel *et al*., 2016). The seroprevalence in different parts of Ethiopia are summarized in table below.

Table 1: Recently reported seroprevalence of CBPP in different parts of Ethiopia

Parts of the country	Study area	Sample size	Overall sero-prevalence	Reference
Southeastern Ethiopia	Jijiga and Shinille zone of Somali	793	10.3%	Gizaw, 2004
Central Ethiopia	Export quarantine centers in and around Adama	3,111	4%	Kassaye and Molla, 2013
Southern Ethiopia	Amaro special district	400	31.8%.	Ebisa *et al*., 2015
Northern Ethiopia	Southern zone of Tigray (Alamata, Raya Azebo, Ofla, and Endamehoni)	384	11.9%	Teklue *et al*., 2015
Western part of Ethiopia	Western Oromia Zones (Western Shoa, Horro Guduru Wollega, and Eastern Wollega)	386	28.5%	Daniel *et al*., 2016
Southwest Ethiopia	Gimbo district of Keffa zone	384	8.1%	Mamo *et al*., 2018

CBPP diagnostic approaches like clinical examination, post-mortem examination to observe the characteristic lesions in organs of dead and\or slaughtered animals and laboratory examination to confirm the presence of infection, an outbreak investigations to isolate and identify the causative agent of *Mycoplasma* species through postmortem examination and sample collection, sero-prevalence studies, sick animals for autopsy and bacteriological specimen collection, and the clinical and pathological findings as well as the biochemical tests performed so far were the major method that used CBPP diagnosis in Ethiopia (Gizaw, 2004; Sori *et al*., 2005; Mersha, 2017).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted from November 2018 until August 2019 in Woliso, Dawo and Ameya districts which were conveniently selected from Southwest Shewa zone of Oromia regional state, central Ethiopia.

Woliso district is one of the districts of Southwest Shewa zone of Oromia regional state which is located at about 114 km from Addis Ababa (Finfinne) in South-west direction. The area is located at a latitude of 8° 32' 3.01" N and longitude of 37° 57' 54.54" E along Finfinne to Jimma main road with an elevation ranging from 1600 - 2880m above sea level. Agro-ecologically, it is classified into Midland (70%) and Highland (30%) zones. Long rainy season occurs from June to September and short rainy season extended from March to April. The minimum and maximum annual rain fall and daily temperature ranges from 1250 to 1450 mm and 8 to 25℃ respectively. The farming system of the area is mixed crop livestock production system and livestock population of the area is estimated to be about 224,334 heads of cattle, 2,101mules, 16,320 donkeys, 39,543 sheep, 51,042 goats and 127,679 poultry (WDLFRDO, 2018).

Dawo is one of the districts of Southwest Shewa zone of Oromia regional state which is located at about 96 km from Addis Ababa (Finfinne) in South-west direction (80 kilometers paved with asphalt and 16 kilometers gravel). The area is located at a latitude of 8° 44' 59.99" N and longitude of 38° 09' 60.00" E with an elevation ranging from 1650 – 2750m above sea level. Agro-ecologically, it is classified into Midland (69%) and Highland (31%) zones. Long rainy season occurs from June to September and short rainy season extended from March to April. The annual rainfall varies from 900 to 1100 mm; while the annual mean temperature vary from 17℃ to 20℃ with mean value of 18℃. The total land area of the woreda is 64,116.25 ha, of which 48,337 ha (75%) are considered suitable for agriculture. Grazing and forest lands accounts for 6.73% and 7% respectively. The livestock population of the area is estimated to be about 80, 965 heads of cattle, 207 mules, 10,932 donkeys, 39,014 sheep, 10,942 goats and 65,944 poultry (DDLFRDO, 2017).

Ameya is one of the districts of Southwest Shewa zone of Oromia regional state which is located at about 144 km from Addis Ababa (Finfinne) in South-west direction and 30 kilometers far away from the administrative center of Southwest Showa zone -Woliso town. The area is located at a latitude and longitude of 8° 29' 59.99" N and 37° 44' 59.99" E with an elevation ranging from 1600 - 3200m above sea level. Agro-ecologically it is classified into Midland (64%) and Highland (36%) zones. Long rainy season occurs from June to September and short rainy season extended from March to April. The minimum and maximum annual rain fall ranges from 900 to 1800 mm and the mean annual temperature ranges from 12℃ to 32℃. The livestock population of the area is estimated to be about 160,500 heads of cattle, 3,300mules, 15,200 donkeys, 32,600 sheep, 35,400 goats and 101,000 poultry (ADLFRDO, 2018).

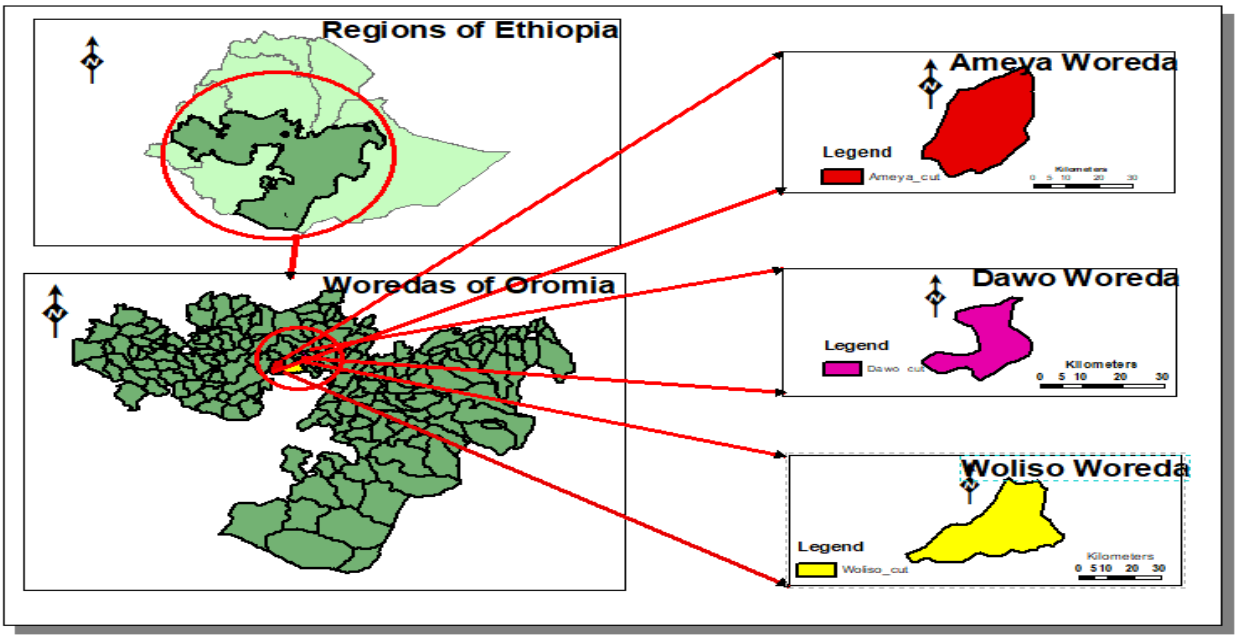

Figure 4: Map showing the location of the study area

3.2. Study Herds and Animals

The target population comprises all cattle above six months of ages with no records of CBPP vaccination in Woliso, Dawo and Ameya districts of Southwest Shewa zone of Oromia region. Herds of cattle managed under the traditional extensive production system were included in this study.

3.3. Study Design

A cross sectional study was conducted in Woliso, Dawo and Ameya districts of Southwest Shewa zone of Oromia region and proportional sample size were considered from these districts according to their cattle population. Body condition scores of animal was scored according to Garnsworthy (2006) and body condition scoring 1 and 2 were recorded as poor body condition and body condition scoring 3, 4 and 5 were recorded as Good body condition (Annex 3).

3.4. Sample Size Determination and Sampling Technique

3.4.1. Sample size determination

Since there was no previous report of sero-prevalence of CBPP in the study area, an expected prevalence of 50% was considered to determine the sample size required for blood sample collection according to Thrusfield (2007) formula and a total of 384 animals were selected.

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

Where n = required sample size, P_{exp} = expected prevalence, and d = desired absolute precision (0.05).

An estimated sample size for households from which animals were selected for blood sample collection was calculated by dividing the total sample size (n=384) to the number of animals sampled from each herd (6) given an estimated of 64 households for inclusion. However, due to the inclusions of households that having less than six cattle, the total sample size of herds was inflated to 96. Therefore, in this study a total of 96 herds (45 from Woliso, 20 from Dawo and 31 from Ameya districts) were selected.

3.4.2. Sampling methods

Southwest Shewa zone was selected because the status of CBPP has not been known yet in the area. Three districts were selected conveniently from the zone based on cattle population and accessibility. By assuming the population is homogeneous, equal numbers of peasant associations were considered from these three districts and from each selected district, three

peasant associations were selected conveniently based on cattle population, accessibility and availability of infrastructure. Accordingly Badesa Koricha, Gurura and Obbi were selected from Woliso district; Karsa Adai, Karsa Bombi and Nano Gabriel were selected from Dawo district; Udad 2, Kota and Gomboro Aliye were selected from Ameya district.

Two stage sampling methods were used to select households (herds) and individual animal. Household that have at least two cattle in each selected PA and defined as herd was considered as primary sampling unit. Lists of households were identified from Peasant association agricultural office. Then households were selected using systematic random sampling and the selected households were informed by animal health workers to provide their cattle for sampling purpose. From Woliso, Dawo and Ameya districts 150, 102 and 132 animals were selected respectively according to their cattle population. Equal numbers of animals were considered from three selected peasant association within each district. Animals from each household were tethered separately by object. The number was assigned to animal by counting tethered animals either from right to left or from left to right. The maximum sample size of six cattle (i.e. the average number of cattle per household of the area) was sampled from each selected cattle herd (household that having animal) for serum sample collection. Only six cattle were sampled randomly from households those having greater than six cattle. However, in case of households those having ≤ 6 cattle, all animals were sampled.

3.5. Data Collection

3.5.1. Blood sample

About 10 milliliters of blood samples were collected from the jugular vein of each cattle using sterile vacutainer tubes and needles by following aseptic procedure after animal was restrained by owner, and sample was properly labeled. The samples were kept protected from sun light in a slanting position for 6-8 hours. Then the serum samples were separated and transferred to a sterile tube (cyrovial) and stored at -20^{o}C at Woliso district veterinary clinic until required for analysis using Competitive ELISA at Bedelle Veterinary Regional Laboratory.

Inclusion criteria

Cattle above six months of ages without records of vaccination for CBPP in both sexes were included.

Exclusion criteria

Cattle of less than six months of age and those with records of CBPP vaccination were excluded.

3.5.2. Herd and animal information

Herd and animal level data/information were collected from selected herds and animals to see the risk factors associated with occurrence of CBPP. Accordingly data on location (both districts and peasant associations), age, sex, body condition score, origin of the animal, history of respiratory related clinical signs, herd size, contact with other herds and introduction of new animal into herds were collected using data collecting format as shown in Annex 1&2.

3.6. Diagnostic Methods

3.6.1. Competitive ELISA

Micro-plates were coated with *Mmm*SC purified lysate. Samples to be tested were premixed with a specific monoclonal Mab 117/5 in a separate plate (preplate) and content of the pre-plate was transferred into the coated micro plate. Any *Mmm*SC specific antibodies present in the sample form an immune-complex with *Mmm*SC antigen coated on the micro plate, competing with Mab 117/5 for the specific epitopes. After washing away unbound material, an anti-mouse antibody enzyme conjugate was added. In presence of immune-complex between *Mmm*SC antigen and antibodies from the sample, Mab 117/5 cannot bind to its specific epitopes and the conjugate was blocked from binding to Mab 117/5. Conversely in the absence of *Mmm*SC specific antibodies in the test sample, Mab 117/5 can bind to its specific epitopes and the conjugate is free to bind to Mab 117/5. After washing away unbound conjugates, enzyme substrate (TMB) was added. In presence of the enzyme, the substrate was oxidized and developed a blue compound becoming yellow after blocking. Subsequent color

development is inversely proportional to the amount of anti-*Mmm*SC antibodies in the test sample. The result was expressed in "percentage of inhibition" by comparing the optical density in the test well with the optical densities in the Mab control wells. The reading was performed at 450 nm (Annex 4).

The percentage of inhibition is given by the formula:

$$\text{PI} = \frac{\text{OD Cm} - \text{OD test serum}}{\text{OD Cm} - \text{OD Cc}} * 100\% \quad (2)$$

Where OD Cm is optical density for the monoclonal antibody; OD test serum is optical density for the test serum; OD Cc is optical density for the conjugate (OIE, 2014).

3.7. Data Storage and Analysis

Data generated from laboratory investigations with herds and animals level information were recorded and coded using Microsoft Excel spreadsheet (Microsoft Corporation). It was transferred to Stata version 13 and analysis was performed. The sero-prevalence of CBPP was calculated as the number of sero-positive samples divided by the total number of samples tested. The statistical significance difference of seroprevalence of CBPP across study districts and among peasant associations was tested by chi-square (X^2) test. At the beginning, association of sero-positivity with risk factors (age, sex, body condition, origin of animal, herd size, history of herd mixing with other herd and introduction of new animal into herd) were screened using univariable logistic regression analysis and variables that found with p-value of less than or equal to 0.25 ($p \leq 0.25$) were subjected to multivariable logistic regression analysis with Generalized linear model logit link. The presence multicollinearity was checked using tolerance and variance inflation factor. Spearman's rho was used to see the correlation between the CBPP occurrence and history of previous respiratory related clinical signs. In all the analysis significance level 0.05 ($p<0.05$) was considered to be statistically significant.

4. RESULTS

4.1. Overall Sero-prevalence of CBPP

Out of 384 selected animals, 36 were sero-positive for *Mycoplasma mycoides subspecies mycoides* Small Colony (*Mmm*SC) specific antibody. The overall animal level sero-prevalence of CBPP in the study area was 9.4 % (95%CI: 6.5-12.3). From 96 cattle herds, 31 were seropositive for CBPP specific antibody. The overall herd level sero-prevalence of CBPP was 32.3 %(95%CI: 22.9-41.6). In this study different sero-prevalence was recorded across the study locations in which the highest sero-prevalence (12%, 95%CI: 6.8 - 17.2) was observed in Woliso districts while the lowest sero-prevalence (7.6 %, 95%CI: 3.1 - 12.1) was recorded in Ameya district. Similarly, the highest sero-prevalence (20%, 95%CI: 8.9 - 31.1) was observed in Gurura peasant association while the lowest sero-prevalence (2%, 95%CI: 0.1- 5.9) was recorded in Obbi peasant association. However, chi-square(X^2) test showed that there was no statistically significance difference in seroprevalence of CBPP among study districts and peasant associations(Table 2).

Table 2: Sero-prevalence of CBPP across study districts and peasant associations

Variables	Categories	No of cattle examined	No tested positive	Prevalence % (95%CI)	X^2 (p value)
Districts	Woliso	150	18	12(6.8 - 17.2)	
	Dawo	102	8	7.8(2.6 - 13.1)	2.00(0.368)
	Ameya	132	10	7.6(3.1 - 12.1)	
PAs	Badesa Koricha	50	7	14(4.4 - 23.6)	
	Gurura	50	10	20(8.9 - 31.1)	
	Obbi	50	1	2(0.1 - 5.9)	14.75(0.064)
	Karsa Adai	34	3	8.8(0.7 - 18.0)	
	Karsa Bombi	34	1	2.9(0.1 - 8.6)	
	Nano Gabriel	34	4	11.8(0.9 - 22.6)	
	Udad2	44	2	4.5(0.1 - 10.7)	
	Kota	44	5	11.4(2.0 - 20.7)	
	Gomboro Aliye	44	3	6.8(0.6 - 14.3)	
Total		384	36	9.4(6.5-12.3)	

CI= Confidence Interval; PAs = Peasant Associations

4.2. Risk Factors Analysis

4.2.1. Animal level risk factors analysis

In this study various animal level sero-prevalence were recorded across different potential risk factors like age, sex, body condition and origin of the animal. The findings of the present

study showed that the sero-prevalence observed in adult age group (>5years)(14.1%, 95%CI: 9.3-18.8) was higher than sero-prevalence observed in young age group(≤5years) (3.9%, 95%CI: 1.1- 6.8), the sero-prevalence observed in male cattle (11.2%, 95%CI: 6.8-15.6) was higher than sero-prevalence observed in female cattle (7.4%,9 5%CI: 3.7-11.2), the sero-prevalence observed in cattle with poor body condition (<3) (11.7%, 95%CI: 8.4-16.1) was higher than sero-prevalence observed in cattle with good body condition (≥3) (4.2%, 95CI: 0.6-7.8) and the sero-prevalence observed in cattle bought from outside of the herd (16.5%, 95%CI: 9.5-23.5) was higher than sero-prevalence observed in cattle born within the herd (6.5%, 95%CI: 3.6-9.5) (Table 3).

Initially, these risk factors were screened at ($p \leq 0.25$) using univariable logistic regression analysis and this analysis revealed that age, sex, body conditions and origin of animal were entered into multivariable logistic regression analysis (Table 3).

Table 3: Univariable logistic regression analysis of risk factors with animal level seroprevalence of CBPP (n=384) Southwest Shewa zone, Ethiopia

Variables	Categories	No of cattle examined	No tested positive	Prevalence %(95%CI)	OR(95%CI)	p-value
Age	Young(≤5yrs)	178	7	3.9(1.1-6.8)	*	*
	Adult(>5yrs)	206	29	14.1(9.3-18.8)	4.0(1.7-9.4)	0.001
Sex	Female	188	14	7.4(3.7-11.2)	*	*
	Male	196	22	11.2(6.8-15.6)	1.5(0.8-3.2)	0.207
Body condition score	Good(≥3bcs)	119	5	4.2(0.6-7.8)	*	*
	Poor(<3bcs)	265	31	11.7(8.4-16.1)	3.0(1.1-8.0)	0.026
Origin of animal	Born	275	18	6.5(3.6-9.5)	*	*
	Bought	109	18	16.5(9.5-23.5)	2.8(1.4-5.7)	0.003

*=Reference group; CI= Confidence Interval; OR=Odd ratio

Finally, further analysis of risk factors was performed using multivariable logistic regression analysis. Multivariable logistic regression analysis of potential risk factors with sero-prevalence of CBPP found that age and body condition had statistically significant ($p<0.05$) association with sero-prevalence of CBPP. Cattle in adult age group (>5years) (OR= 4.3, 95%CI: 1.8-10.1, P=0.001) were more than four times more likely to be sero-positive of CBPP than cattle with young age group (≤5years). Cattle with poor body condition (OR=3.4,

95%CI: 1.3-9, P=0.015) were more than three times more likely to be affected by CBPP than cattle with good body condition score (Table 4).

Table 4: Multivariable logistic regression analysis of risk factors with animal level sero-prevalence of CBPP (n=384) Southwest Shewa zone, Ethiopia

Risk factors	Categories	Prevalence %(95%CI)	OR (95% CI)	P-value
Age	Young(≤5yrs)	3.9(1.1-6.8)	*	*
	Adult(>5yrs)	14.1(9.3-18.8)	4.3(1.8-10.1)	0.001
Body condition	Good(≥3score)	4.2(0.6-7.8)	*	*
	Poor(<3score)	11.7(8.4-16.1)	3.4(1.3-9)	0.015

*= Reference group; CI= Confidence Interval; OR=Odd ratio; yrs=years

4.2.2. Herd level risk factors analysis

In this study the risk factors that considered at herd level seroprevalence were herd size, contact (herd mix), and introduction of new animal into the herd. Results of risk factors analysis with herd level sero-prevalence showed that among herd level risk factors that considered, only herd size had statistically significant effect on seropositivity of CBPP ($p<0.05$). Larger herds (OR=7.5, 95%CI: 2.7-21.2, p=0.000) were more than seven times more likely to be affected by CBPP than smaller herds (Table 5).

Table 5: Final herd level risk factors analysis to CBPP sero-prevalence (n=96) Southwest Shewa zone, Ethiopia

Variables	Categories	No of cattle herds examined	No tested positive	Prevalence %(95%CI)	OR(95%CI)	p-value
Herd size	Small size(<5cattle)	48	6	12.5(3.1-21.9)	*	*
	Large size(≥5cattle)	48	25	52.1(37.9-66.2)	7.5(2.7-21.2)	0.000

*=Reference group; CI= Confidence Interval; OR=Odd ratio

4.3. Spearman correlation analysis

Correlation analysis using Spearman's rho was conducted to test correlation between history of previous respiratory problems and sero-prevalence of CBPP which revealed that there was statistically significant association between history of respiratory problems and sero-prevalence of CBPP (rs=0.2016, P=0.001).

5. DISCUSSION

Based on the serological results of this study, CBPP was a major cattle health problem in the study areas. In this study, a total of 384 serum samples were tested from Woliso, Dawo and Ameya districts of Southwest Shewa zone of Oromia region and an overall seroprevalence of 9.4% (95%CI: 6.5-12.3) was recorded by using c-ELISA. This finding is closely in agreement with the results of various researchers as reported by Mamo *et al*. (2018) from Gimbo District, Southwest Ethiopia (8.1%), Biruhtesfa *et al*.(2015) from Bishoftu and Export Oriented Feedlots around Adama using c-ELISA (8.7%), Schnier *et al*.(2006) from Southwestern Kenya (9.7%), Kassaye and Molla (2013) from Export quarantine center of Adama (9.5%), Molla and Delil (2015) from Dassenech district of South Omo zone (10%) and Teklue *et al*. (2015) from Southern part of Tigray testing using CFT test (11.9%). However, the overall seroprevalence is by far much lower than the previous findings reported by Ebisa *et al*. (2015) from Amaro special districts, southern part of Ethiopia (31.8%) and Daniel *et al*. (2016) from three districts of Western Oromia (28.5%). On the other hand, the findings of this study was higher than the results reported by Alemayehu *et al*. (2014) from bulls originated from Borena pastoral area of Southern Ethiopia (0.4 %) and Dele *et al. (*2014) from Export quarantine center of Adama (0.4%).

The overall herd level sero-prevalence of CBPP was 32.3% (95%CI: 22.9-41.6) which is closely similar with the finding of Gizaw (2004) who reported seroprevalence of 30.4% in Somali regional state of Ethiopia. However, this result was higher than the previous report of Bonnet *et al*. (2005) with 4.6% in the Ethiopian highlands. On the other hand the finding was lower than the finding reported by Suleiman *et al*. (2015) from agro-pastoral areas of Nigeria (54.7%) and Mersha (2017) from selected districts of East Wollega and West Shewa zone of Oromia region (54%).

This variation in seroprevalence reported from various researchers might be due to variation in temporal and spatial distribution of the disease, differences in agro ecological systems, cattle management and production systems, population density, number of examined animals, and the types of tests used to determine seroprevalence of the disease (Ebisa *et al*., 2015).

Out of predisposing risk factors that were assessed with sero-prevalence of CBPP; sex, origin of animal, herd mix and introduction of new animal into herds were not significantly ($p>0.05$) associated with sero-prevalence of CBPP. The absence of significance difference in sero-prevalence of CBPP between sex (male and female) was similar with study reported by Daniel *et al.* (2016) in west Oromia but contradicts with study reported by Schnier *et al.* (2006) in Kenya who reported a significantly higher prevalence in female animals. The absence of significance difference in this finding may be due to similar exposure of male and female to the disease. Origin of animal had no statistically significant ($p>0.05$) association with the sero-prevalence of CBPP. The findings of this study was in agreement with study reported from Bishoftu abattoir and Export Oriented Feedlots Around Adama (Biruhtesfa *et al*., 2015) in which statistically significant association was absent in the occurrence of CBPP between origins. The reason for absence of significant difference in the present study may be due to similar agroclimatic zones as bought and born animals may be originated from the same agroclimatic condition.

From risk factors that were assessed with sero-prevalence of CBPP; Age (P=0.001), body condition score (P=0.015), and herd size (P=0.000) were significantly associated with the seroprevalence of contagious bovine pleuro-pneumonia. The sero-prevalence of the disease was higher in animals within adult age group. Cattle within adult age group (>5yrs) (OR= 4.3, 95%CI: 1.8-10.1, P=0.001) were more than four times more likely to be affected by CBPP than cattle within young age group (≤5yrs). Bashiruddin *et al*. (2005) reported that adult cattle were more resistant to CBPP infection than younger animals. In addition, Masiga *et al*. (1996) reported that young animals are more susceptible to acute forms of CBPP infection than adult cattle and thus acutely infected young animals may die of CBPP and may not be available for testing. However, In contrast to Bashiruddin *et al*. (2005), this study revealed that the seroprevalence observed in adult cattle was higher than seroprevalence observed in young cattle which is in line with the finding reported by Kassaye and Molla (2013). This may be associated to the fact that chronic stages of the disease are usually seen in adult cattle as the age progresses (Olabode *et al*., 2013). This might be due to long time exposure and persistence of sequestrum for a long period of time in CBPP recovered animals.

Body condition score was significantly (P<0.05) associated with the sero-prevalence of CBPP in which higher sero-prevalence of 11.7% was recorded in cattle with poor body condition (<3 bcs) as compared to sero-prevalence of 4.2% in cattle with good body condition (≥3). Cattle with poor body condition (OR=3.4, 95%CI: 1.3-9, P=0.015) were more than three times more likely to be affected by CBPP than cattle with good body condition score (≥3bcs). This finding is in agreement with the finding reported by Biruhtesfa *et al.* (2015) from Bishoftu abattoir. This statistically significant association may be due to the fact that animals with poor body conditions are more susceptible to the disease and they may have low immunity to resist the disease. Besides, animals with poor body conditions are more susceptible to the disease than animals with good body condition (Radostatits *et al*., 2007), this findings was also in agreement with an assumption that CBPP sero-positive animals had poor body conditions than sero negative animals; thus, this result may also indirectly describes the impact of the disease associated with loss of productivity.

Herd size had statistically significant association (P<0.05) with seroprevalence of CBPP. The seroprevalence of the disease was higher in large herd size (52.1%, 95%CI: 37.9-66.2) compared to small herd size (12.5%, 95%CI: 3.1-21.9). Large herd groups (OR=7.5, 95%CI: 2.7-21.2, p=0.000) were more than seven times more likely to be affected by CBPP compared to the small herd groups. The significant difference in the sero-prevalence of the disease between the herd sizes was in agreement with the findings of the study conducted in bulls originated from Borena pastoral area of Southern Ethiopia (Alemayehu *et al., 2*014). This significance difference may be due to the fact that probability of getting the disease increase as herd size increase due to crowding of animal and the disease spread within the large herd size easily because of the contagious nature of disease.

Correlation analysis using Spearman's rho revealed that there was statistically significant association between history of previous respiratory problems and sero-prevalence of CBPP (P=0.001). Among the clinical signs of CBPP, the respiratory signs such as dyspnea, polypnea, cough and nasal discharges in cattle are the common to be mentioned (OIE, 2014). The reason for this statistically significant association between history of previous respiratory problems and sero-prevalence of CBPP might be due to the clinical characteristics of the

disease. Hence, based on the present study result, if animals were exhibited any respiratory health problem in the study area; it could be an indication of CBPP infection.

6. CONCLUSION AND RECOMMENDATIONS

The present study revealed that animal and herd level overall sero-prevalence of CBPP in the study area were 9.4 % and 32.3 % respectively. This result showed that the disease is prevalent in cattle in selected (Woliso, Dawo and Ameya) districts of Southwest Shewa zone of Oromia region, suggesting the disease could cause considerable economic losses through morbidity and mortality. The presence of statistically significant differences in the sero-prevalence of CBPP among the predisposing risk factors like age, body condition and herd sizes suggests that variation in certain animal and herd related risk factors favors the occurrence and spread of the disease. Therefore, based on the above conclusion the following recommendations are forwarded:

- Further investigation in wide geographical areas and large sample size using reliable tools like molecular technique and biochemical test are needed in order to know the exact epidemiological scenario of the disease.
- Controlling measures should be implemented to prevent further spread of the disease through the use of better and coordinated vaccination program.

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8. ANNEXES

Annex 1: Herd data collecting questionnaire format

Questionnaire No _____

Date _____

1. Areal information

Region_____

Zone _____

District_____

PA _____

Geo. Ref._____

Altitude _____

2. Respondent information

Name _____

Sex _____

Age _____ _____

Educational background _____

3. Answer the following question regarding your cattle

3.1. What is your herd composition on the following basis?

Oxen _____,

Bull _____,

Lactating cow _____,

Dry cow _____,

Heifer _____,

Calf _____,

3.2. What breed of Cattle do you rear? _____

3.3. What are the main purposes for keeping animals?

Cattle _____

Sheep _____

Goat _____

Donkey _____

3.4. List other activities undertaken by the a farmer _____

_____

3.5. Who takes care of the animals? _____

3.6. What type of management system do you use to keep the animals (Watering and Grazing)?

_____

3.7. Is there contact of your herd with other herd?

A) Yes

B) No

3.8. Is there introduction of new animals?

A) Yes

B) No

3.9. Is there livestock market activity around?

A) Yes

B) No

3.10. Have you encountered disease of cattle in your herds?

A) Yes

B) No

3.11. If your answer for question 3.11 is yes, is there cattle mortality in the herd?

A) Yes

B) No

3.12. If your answer for question No. 3.11 is yes, what are the clinical signs of disease observed?_____ _____ _____

_____ _ _____

Annex 2: Animal information collecting format.

Sample code	Site		Owner name	Breed	Age	Sex	Bscs	Disease history		Origin of animals		Serology results
	Woreda	Kebele						D^+	D^-	Born	Bought	

D^+ = Animal with history of disease; D^-= Animal with no history of previous disease

Annex 3: Body Condition Scoring

Score	Condition	Detailed description
1	Poor	Tail heed – deep cavity with no fatty tissue under skin. Skin fairly supple but coat condition often rough. Loin – spine prominent and horizontal processes sharp.
2	Moderate	Tail head – shallow cavity but pin bones prominent; some fat under skin. Skin supple Loin – horizontal processes can be identified individually with ends rounded.
3	Good	Tail head –fat cover over whole area and skin smooth but pelvis can be felt. Loin – ends of horizontal processes can only be felt with pressure; only slight depression in loin
4	Fat	Tail head – completely filled and folds and patches of fat evident. Loin - cannot feel processes and will have completely rounded appearance.
5	Grossly fat	Tail head buried in fatty tissue, pelvis impalpable even with firm pressure.

Source: Garnsworthy (2006)

Annex 4: Procedure of c-ELISA

Steps	Action
1.Preparation of reagents	Reconstitute detection Solution with 1ml of distilled water before use. Reconstituted detection solution must be diluted 1:120 in dilution Buffer N.24. Wash concentrate must be diluted 1:20 with distilled/de ionized water before use. Concentrated conjugate must diluted 1:100 in the Dilution Buffer N.24.The lyophilized controls must be reconstituted one day in the advance with 1 ml of sterile distilled water.
2. Preparation of samples	Dispense 100 µl of Dilution Buffer N. 24 into each well of the preplate. Dispense 110µl of Dilution Buffer N.24 into two appropriate wells (conjugate control wells: cc). Dispense 11µl of undiluted Strong Positive Control in four appropriate wells (strong positive control wells SPC). Dispense 11µl of undiluted positive control in two or four appropriate wells (positive control wells: pc).Dispense 11µl of undiluted Negative control in two appropriate wells (Negative control wells; NC). Dispense 11µl of undiluted Sample per well into remaining wells preplate. Dispense 110µl of diluted Detection Solution into four appropriate wells (Mab Control wells: MabC).
3. Sample distribution	Transfer 100µl from each well of the preplate to the appropriate well of coated micro plate.
4. Sample incubation	Cover the micro plate (with lid, aluminium foil or adhesive) and incubate 1 hour (± 5 min.) at 37 ^{o}c (± 3^{o}c) with a gentle agitation.
5. Washing the plate	Wash each well with approximately 300µl of wash solution two times
6. Conjugate distribution	Add 100µl of diluted Conjugate in each well
7. Conjugate incubation	Cover the micro plate (with lid, aluminum foil or adhesive) and incubate 30 minutes (± 3 min.) at 37^{o}c (±3^{o}c) with a gentle agitation.

8. Washing the plate	Wash each well with approximately 300µl of wash solution tree times.
9. Substrate distribution	Add 100µl of TMB Substrate N.13 in each wells
10. Substrate incubation	Incubate 20 minutes (±3 min.) at + 37°c (±3 °c) in a dark place.
11. Stopping the reaction	Dispense 100µl of stop solution N.3 per well
12. Measure the plate	Blank micro plate reader on air. Measure and record the absorbance values of samples and controls at 450nm. Calculate the results.
13. Interpretation	Samples with percentage of inhibition less than or equal to 40% are considered Negative for the presence of MmmSC Antibodies. Sample with percentage of inhibition greater than 40% and less than 50% are considered Doubtful and must be retested. Sample with percentage of inhibition greater than or equal to 50% are considered positive for presence of *Mmm*SC Antibodies.

Source: OIE (2014)