JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

SCHOOL OF VETERINARY MEDICINE

SERO-PREVALENCE AND ASSOCIATED RISK FACTORS OF CONTAGIOUS BOVINE PLUEROPNEUMONIA IN GUDEYA BILA AND BONEYA BOSHE DISTRICTS OF EAST WOLLEGA, ETHIOPIA

M.Sc. THESIS

BY

TOLESA NEGGASA

SEPTEMBER, 2018

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M.Sc. 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

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SEPTEMBER, 2018

JIMMA, ETHIOPIA

APPROVAL SHEET

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I have completed my thesis research work as per the approved proposal and it has been evaluated and accepted by my advisers. Hence I hereby kindly request the department to allow me to present the finding of my work and summit my thesis.

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DEDICATION

I dedicate this thesis manuscript to my parents for their support in the success of my life.

STATEMENT OF AUTHOR

First, I declare that this thesis was my work and that all sources of materials used for this thesis have been properly acknowledged. This thesis is submitted in partial fulfillment of the requirement for an advanced M.Sc. degree at Jimma University and to be made available at the University's Library under the rules of the Library.

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BIOGRAPHICAL SKETCH

The author was born in 1989G.C in Bodji Chokorsa Woreda, West Wollega Zone of Oromia Regional State of Ethiopia. He attended his elementary education at Chaliya Wera Ilu Elementary School from 1996-2003 and from 2004-2007G.C he attended high school and preparatory school at Bodji Dirmeji School in Bila town. After successful completion of his preparatory school life, he joined Hawassa University in 2008 and graduated with DVM degree in July 2012 G.C. Soon after his graduation, he joined Nedjo woreda livestock and fishery resource development office and served as animal health team leader for four years. After four years of professional service, he joined the School of Veterinary Medicine of Jimma University in October, 2017 to pursue his MSc degree in Veterinary Epidemiology.

ACKNOWLEDGEMENTS

First, I would like to thank God who helped and guided me in all aspects of my life to reach at my today's position.

Second, I convey my sincere gratitude and heartfelt thanks to my major advisor Professor Tadele Tolosa and co-advisor Dr. Feyissa Begna for their noble hearted help and unreserved professional advice and critically reviewing this research and provide constructive comments, and devotion of their time to make valuable contribution to improve it technically. Also I would like to thank Bedele regional veterinary laboratory staffs for their support by materials provision, technical and giving constructive suggestion during my field and laboratory works.

Third, my deepest thanks go to my beloved wife, Tigiste Mekonnen, my son, Labsi and all my family members for nursing me with affection and love, and for their devoted partnership in the success of my life; and also to all my friends for their moral and technical support during working of this research.

LIST OF ABBREVIATIONS

BBLFRDO	Boneya Boshe woreda Livestock and Fishery Resource and Development Office
CBPP	Contagious Bovine Pleuropneumonia
CCPP	Contagious Caprine Pleuropneumonia
C-ELISA	Competitive Enzyme Linked Immunosorbent Assay
CFSPH	Center for Food Security and Public Health
DEFRA	Department for Environment, Food and Rural Affairs
EWLFRDO	East Wollega Zone Livestock and Fishery Resource and Development Office
FAO	Food and Agricultural Organization
GBLSRDO	Gudeya Bila woreda Livestock and Fishery Resource and Development office
MoA	Ministry of Agriculture
OIE	Office of International des Epizootic
PPLO	Pleuropneumonia like Organisms
SC	Small Colony
WTO	World Trade Organization

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ABSTRACT

Contagious bovine pleuropneumonia (CBPP) remains a huge threat to cattle production in sub Saharan African countries in general and hinders livestock development in Ethiopia in particular. To this end, there is limited information about this disease in East Wollega. A cross sectional study was conducted from November, 2017 to June, 2018 in order to determine the seroprevalence and associated risk factors of CBPP in the Gudeya Bila and Boneya Boshe districts of East Wollega Zone, Oromia regional state. The study was conducted in the areas on animal with no history vaccination using simple random sampling to select the study units. A total of 384 blood samples were collected from the jugular vein of each animal and all samples were tested by competitive ELISA (c -ELISA) at Bedele Regional Veterinary Laboratory to detect the specific antibodies to *Mycoplasma mycoides subspecies mycoides* Small Colony (MmmSC). Information on risk factors influencing the occurrence of CBPP using questionnaire survey was collected. Data obtained from both serological and questionnaire survey was analyzed using SPSS software version 20. Multivariable logistic regression was used to analyze the association of exposure variables with MmmSC antibodies circulation. The strength of association between the risk factors and MmmSC antibody prevalence was assessed using Odds Ratio. The results indicated that, the overall seroprevalence of CBPP at individual animal-level and herd-level was 8.6% (95%CI: 5.8% -11.4%) and 26.3% (95%CI: 17.5% -35.2%), respectively. The sero-prevalence of CBPP in Boneya Boshe and Gudeya Bila were 10.8% and 6.6%, respectively. There was a statistically significant association in the prevalence of MmmSC antibody with the body condition score (OR=4.6(1.6-13.2; P<0.01), origin of animals (OR= 12.5(3.1-51.0; P< 0.01) and history of disease (OR=4.9(1.9-12.9; P< 0.01) at individual animal and herd size (OR: 3.6 (1.3-10.3; P<0.01) at herd level. This study showed that the overall prevalence of CBPP in study area was high. This warrants the implementation of appropriate preventive and control practice.

***Keywords**: CBPP, Sero-prevalence, c-ELISA, Risk factors, Boneya Boshe, Gudeya Bila, Ethiopia.*

1. INTRODUCTION

1.1. Background

Agriculture is the backbone of Ethiopian economy and has two sectors; agricultural and livestock sector and the role of livestock is very notable in that it contributes 13-16% of the total gross domestic product (GDP), 30- 35% of agricultural gross domestic product (GDP) and more than 85% of farm cash income (Tsedeke and Endrias, 2011). In addition to their direct role in generating food and income, livestock are valuable assets, serving as a store of wealth, collateral for credit and an essential safety net during times of crisis throughout the developing world and generally generate a livelihood for 1.0 billion poor people in the world (Perry *et al.*, 2003; Abera *et al.*, 2016).

Despite the fact that this magnificent figure is achieved from livestock sector and making the gap of economy very narrow thereby alleviating food insecurity, diseases of animals like contagious bovine pleuropneumonia (CBPP) is playing a principal role and remarkably noticed by many scholars for not to achieve the real asset expected from this sector (Lesnoff *et al.*, 2004; Tegegn, 2017).

Contagious bovine pleuropneumonia is a highly infectious acute, sub-acute and chronic disease of cattle throughout the world; most of sub-Saharan Africa, where it consistently ranks as one of the most serious livestock disease, which caused by *Mycoplasma mycoides subspecies mycoides* small colony (MmmSC). It is one of the diseases recognized by OIE that needs to be controlled or eradicated through a national surveillance protocol (John, 2016; Dereje and Shawul, 2017). It is one of the major constraints to cattle-raising and trade in Africa and is widespread in pastoral areas of Africa. It is also a major problem for Ethiopian livestock (OIE, 2014; Tegegn, 2017).

Although CBPP was once found worldwide, it was eradicated from most continents, by the mid-20th century. Its incidence also began to decline in Africa by the 1970s. However, because of the economic and financial difficulties that affected the ability of governments to adequately fund veterinary services, the disease came back in the late 1980s and early 1990s (Tambi *et al.*, 2006; OIE, 2008). Contagious bovine pleuropneumonia directly impacts

economies through cattle mortality and morbidity. Up to 60% of infected animals are die in naive herds, lactation yields of infected cows are reduced by up to 90%, infected animals grow more slowly and produce less meat and infected draught oxen have a reduced capacity to work (Dereje and Shawul, 2017).

Contagious bovine pleuropneumonia is also a barrier to trade and reduces the value of livestock and the income of value chain stakeholders in many African countries (Joerg, 2014). Major CBPP epidemics have been experienced in Eastern, Southern and West Africa over the last few years. It currently affects 27countries in Africa at an estimated annual cost of 2 billion US dollar loss (Alemayehu *et al.*, 2015). In recent years, CBPP has been found in countries like Botswana from where it was previously eradicated. *Mycoplasma mycoides subspecies mycoides* SC is mainly transmitted from animal to animal in aerosols. This organism also found in saliva, urine, fetal membranes and uterine discharges (Mbithi *et al.*, 2004).

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 (Rovid, 2008). Cattle movements are responsible for the transmission of the CBPP from one herd, region or country to others. Close repeated contact is generally thought to be necessary for transmission; however, *Mycoplasma mycoides subspecies mycoides* SC might be spread over longer distances 50 to 200 meters if the climatic conditions are favorable (Alemayehu *et al.*, 2015).

1.2. Statement of the Problem

Contagious bovine pleuropneumonia is one of the major diseases in Ethiopia that hampering export of livestock and livestock products to the international markets since long time (Farmer, 2010). Pastoral areas output underpins almost all of Ethiopia's live animal and meat exports. However, this area as whole infected since long time, is an endemic area (Afework, 2000). In order to secure international market, Ethiopian government needs to meet WTO requirement by demonstrating their responses towards CBPP control (Afework, 2000; Alemayehu *et al.*, 2015).

The challenge of CBPP control in endemic settings will require active partnerships to overcome the limitations of sufficient epidemiological information from the country on the basis of which to develop targeted measures and limited capacity and national resources to apply control measures based on mass or effective movement control (Alemayehu *et al.*, 2015). Current control measures, like diagnostic tests and vaccines are suboptimal in controlling of CBPP in most of sub-Saharan Africa (Joerg, 2014).

Among the exacerbating risk factors of contagious bovine pleuropneumonia in Ethiopia are; lack of knowledge of the disease by farmers, veterinary and animal field worker, vaccine shortage, poor diagnostic assays, management system, limitation of epidemiological information about the disease, concentration of livestock at watering points and grazing area and difficulty to control of cattle movements are the principal things which have been cited by many literatures (Ebisa *et al*., 2015).

In western Oromia of the farming communities there are different animal diseases of unknown etiological agent which are often reported, affecting production and productivity of livestock and threatening the livelihood of small scale farmers in the area. East Wollega zone is one of west Oromia zone in which study based on food security was done. Among the main problems reported, feed shortage and massive cattle death attributable to prolonged dry season and diseases of unknown etiologic agent were reported. The disease that caused massive cattle death at that time was tentatively diagnosed as pasteurellosis and it might be other respiratory disease like CBPP (Mersha, 2016).

However there was no systematic study conducted to look into the status of this economically important disease in the Gudeya bila and Boneya boshe districts, challenge for CBPP control, limitations of sufficient epidemiological information, the highly fatal nature of the disease, the ease of spread and the difficulty of detecting carrier animals and limited resources to apply control measures and difficulty to control cattle movement; this study were planned with the following objectives.

1.3. Objectives

- To determine seroprevalence and its associated risk factors for contagious bovine pleuropneumonia in selected districts of East Wollega.

2. LITERATURE REVIEW

2.1. Etiology

Contagious bovine pleuropneumonia is caused by *Mycoplasma mycoides subspecies mycoides* Small Colony type. The *Mycoplasma* (Mollicutes) formerly called Pleuropneumonia like organisms (PPLO) is non-sporulating, gram-negative, non-motile bacteria which do not possess a determined shape of the cell (Admassu *et al.*, 2015). The Mollicutes are members of the order Mycoplasmatales and class Mollicutes (Soft skin) and they are the smallest of the free living prokaryotes (Abera *et al.*, 2016). Mollicutesis the correct term to use when collectively referring to members in this order; however, the trivial name mycoplasma is also used for this purpose (Andrews *et al.*, 2008).

In natural conditions, two types of mycoplasma are recognized: large colony (LC) and small colony (SC) (OIE, 2000). 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 (MmmSC) affects only the ruminants of the *Bos genus* (mainly bovine) and cause CBPP in cattle (Abera *et al.*, 2016). Large Colony types occur almost exclusively in goats, rarely in sheep. *Mycoplasmamycoides subspecies mycoides* Large Colony (LC) type does not result in disease in cattle but causes septicemia, polyarthritis, mastitis, encephalitis, conjunctivitis and hepatitis (Gedlu, 2004).

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. They depend on a supply of intact cholesterol which they incorporate into the membrane, creating sufficient osmotic stability for survival under normal physiological conditions (Quinn *et al.*, 1994). Their polymorphism is the consequence of the missing cell wall. Mycoplasmas are devoid of not only cell walls but also lack the genetic capacity which also renders them completely resistant to ß-lactam and other cell wall active drugs (Kasper *et al.*, 2005).

Due to their small size and their polymorphism, they are able to pass through the usual bacteriological filters. Their shape include spherical, pear shaped, spiral shaped and filamentous forms. Mollicutes stain poorly with gram stain, although they are classified as gram negative (Walker, 1999). Infections range from subclinical to severely debilitating and sometimes fatal disease. Mycoplasmas are unique in microbiology because of their extremely small size and their growth on complex but cell free media (OIE, 2000).

Mycoplasma mycoides subspecies mycoides SC is sensitive to all environment influences, including disinfectants, heat and dry and do not 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 from African strains (OIE, 2008). The current European strains lack a substantial segment of genetic information which may have occurred by deletion events. Variety of potential virulence factors of *Mycoplasma mycoides subspecies mycoides* have been identified, those including genes of encoding, surface proteins, enzymes and transport proteins which are responsible for the production H_2O_2 (hydrogen peroxide) and the capsule which is thought to have toxic effect on the animal (Radostits *et al.*, 2007).

2.2. Epidemiology

Mycoplasma mycoides subspecies mycoides SC type is the etiological agent of contagious bovine Pleuropneumonia (OIE, 2000; Radostits *et al.*, 2007). It can be grouped into two major epidemiological clusters. One cluster contains strains isolated from different European countries since 1980 and a second cluster contains African and Australian strains collected over the last 65 years (FAO, 2003). Molecular epidemiology of CBPP by multilocus sequence analysis of MmmSC 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. The last strains isolated from an epidemic area are usually of lower virulence than the first strains. Generally, strains are most virulent when first isolated and lose their virulence after subculture (Radostits *et al.*, 2007).

Bovines are the main species that are susceptible to CBPP. Infections have also been reported from Asian buffalo. Sheep and goats can also be naturally infected, but with no clear

associated pathology. Wild bovids and camels seem to be resistant and so far, do not appear to be important in the transmission of CBPP (CFSPH, 2006; Brown and Torres, 2008). The African water buffalo is refractory to CBPP. Contagious bovine pleuropneumonia prevalence with respect to age was assessed and cattle over two years were found highly affected as compared to the younger animals with significant variation (Abera *et al.*, 2016).

2.2.1. Geographical distribution

Contagious bovine pleuropneumonia is endemic in parts of Africa, Middle East, Asia and sporadic outbreaks in some European countries (Quinn *et al.*, 2002). It is a problem in parts of Asia especially India and China. Periodically, CBPP occurs in Europe and outbreaks within the last decades have occurred in Spain, Portugal and Italy (Noordhuizen, 2001). Outbreaks usually occur as the result of movement of infected animals into a naive herd. It is widely believed that the recovered animals harboring infectious organisms, within a pulmonary sequestrum, may become active shedders when stressed (Admassu *et al.*, 2015).

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 (Radostits *et al.*, 2007). Some inanimate objects such as placenta and urine can also remain infective for long periods but means of transmission is not general thought to be a problem (Admassu *et al.*, 2015).

2.2.2. Source of infection

Susceptible cattle become infected by inhaling droplets disseminated by coughing in affected cattle. Small ruminants and wildlife are not important in the epidemiology. All ages of cattle 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 (Abdela and Yune, 2017). The organism can also be found in saliva, urine, fetal membranes and uterine discharges. Transplacental infection of the fetus can occur (Abera *et al.*, 2016). Viability of the organism in the environment is poor. The incubation period varies but most cases occur 3–8 weeks after exposure (Andrews *et al.*, 2008). Because carriers may not be detectable clinically they constitute a serious problem in

control programs. Breed susceptibility, management systems and general health of the animal are important factors that influence the infection (OIE, 2000; John, 2016).

Contagious bovine pleuropneumonia is characterized by long incubation period, direct contact transmission, possibility of early mycoplamal excretion about 20 days during course of the disease and after recovery in "lungers" up to 2 years (Admassu *et al.*, 2015). Cattle movement is solely incriminated for maintenance and extension of the disease as there is no wild reservoir to make the transmission route complex (Tegegn, 2017).

2.2.3. Risk factors of the disease

Contagious bovine pleuropneumonia is typical example of multi-factorial 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 (Abera *et al.*, 2016). The lack of a cell wall and endotoxins may enable mycoplasma to colonize the animal without inducing an immune response and the predilection for the mucosal membranes may also limit the humoral response (Admassu *et al.*, 2015).

The occurrence and incidence of CBPP is 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 vaccination programs, government budget allocated to control programs, desires of cattle owners and traders to control the disease are critically important management factors, which influence the effectiveness of controlling disease in a country (Radostits *et al.*, 2007).

2.2.4. Transmission of the disease

Contagious bovine pleuropneumonia is epidemiologically characterized by its ability to transmit through direct contact, long incubation period, possibility of early excretion of mycoplasma up to 20 days before apparition of clinical sings during the course of the disease and after recovery in "lungers" up to two years (Gedlu, 2004). Closeness of contact, intensity of infection and the number of susceptible animals determine the rate of spread of the disease.

It is spread mainly by inhalation of droplets from infected coughing animals, especially if they are in the acute phase of the disease. Because of large numbers of MmmSC are present in bronchial secretions, either cattle with clinical disease or subclinical carriers are actively excreting the organism and transmitted disease to susceptible animals through nasal discharge (Aiello, 1998; CFSPH, 2006).

Closely stabled animals are therefore most prone to infection. However, aerosols containing infected droplets may spread the disease over distances of 50 meters or more. Close proximity is necessary for transmission, occurs primarily through the inhalation of infected droplets from a coughing animal (Abera *et al.*, 2016). Cattle are the only species affected there was no reservoir host in wild animals and cattle movements plays a very important role in the maintenance and extension of the disease. Airborne spread up to 200 meters is thought to be possible. Conditions under which cattle are herded closely together favour rapid spread of the disease (Thiaucourt *et al.*, 2004).

2.3. Pathogenesis

Route of infection determines the pathogenesis of disease. Respiratory lesions result from infection via the respiratory route. This respiratory disease was considered as great economic importance to cattle keepers because of its high mortality rate, production loss, increased production cost due to cost of disease control, loss of weight and working ability, delayed marketing, reduced fertility, loss due to quarantine, loss of cattle trade and reduced investment in livestock production (Tambi *et al.*, 2006). Subcutaneous inoculation of virulent cultures into susceptible cattle causes extensive edema, the so called Willems' reaction, but never the natural disease, intraperitoneal inoculation results peritonitis (Thiaucourt *et al.*, 2004; Abera *et al.*, 2016).

Natural infection by inhalation results in lesions that are confined to the lungs in both the acute and chronic forms of the disease and cause bronchitis, alveolitis, bronchiolitis with predominantly neutrophils and mononuclear cellular response constitute the very early inflammation in *Mycoplasma* pneumonia (Nicholas *et al.*, 2009). Infection of cattle by endotracheal intubation resulted in chronic disease, while natural infection by close contact

with infected animals induced severe infection that was more likely to result in mortality (Wanyoike, 1999; Thiaucourt *et al*., 2004).

Actions involved in virulence include evasion of the host's immune system, tight adhesion to the surface of the host's cells, dissemination and persistence in the host, efficient importation of required nutrients and induction of cytotoxicity in the host (Thiaucourt *et al*., 2004). Virulence appears to depend strongly on surface antigens that protect the organism and cause various reactions in the host (Hirsh *et al*., 2004). The capsular polysaccharide galactan has cytopathic and vaso-active effects and contributes to the ability of the organism to spread and persist in the host, probably owing to its ability to protect the organism (Abera *et al.*, 2016). Lipoproteins are also considered to play an important role in immuno pathogenicity (Admassu *et al.*, 2015). The membrane protein L-alpha-glycerophosphate oxidase has been identified as playing a central role in cytotoxicity by catalyzing the oxidation of glycerol-3-phosphate, which results in the release of H_2O_2. The presence of H_2O_2 and/or reactive oxygen in the host cell may cause direct damage as well as triggering an inflammatory reaction (Thiaucourt *et al.*, 2004).

The lesions develop first in the lymphatic system. An essential part of the pathogenesis of the disease is thrombosis in the pulmonary vessels, probably prior to the development of pneumonic lesions (Admassu *et al.*, 2015). Thrombi that develop in the lymphatics cause coagulation of lymph, distension of interlobular septa and focal perivascular round cell infiltration. It is characterized by substantial unilateral pulmonary necrosis, sometimes sequestration and marked serosanguinous fluid accumulation in interstitial and pleura (Abera *et al.*, 2016). Vasculitis is an important component of the pathological changes in this disease, explaining the marked exudation and pleurisy. The formation of cuffs of round cells around the arterioles is the only histological pathognomonic characteristic of CBPP (Nicholas *et al*., 2009).

The secondary lesion is characterized by alveolar involvement due to the accumulation of exudate from the foregoing changes. Necrotic foci surrounded by a band of polymorpho nuclear granulocytes often develop. These foci may develop into sequestra in chronic cases (Walker, 1999; Mariner, 2001). 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 in to them and this dilated septa that give the “Marbling” effect to the lung in these areas (Admassu *et al.*, 2015; Abera *et al.*, 2016).

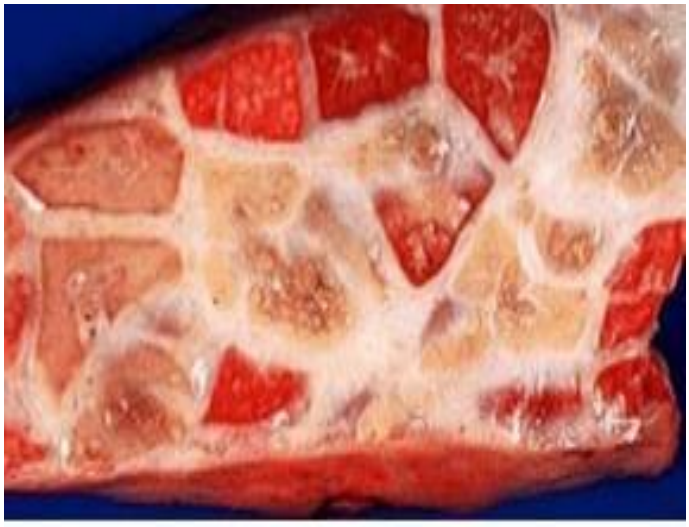


Figure 1: Marbling of lung tissue (Abera *et al.*, 2016).

There are various substances produced by the Mollicutes, which are potentially important in disease pathogenesis. Production of large amounts of hydrogen peroxide (H_2O_2) due to a gene that facilitates active uptake of glycerol, which is phosphorylated and then metabolized further to release H_2O_2, which is then transported into the host cell is important for virulence. Tight adhesion of the pathogen to the host cell is required for the H_2O_2 to be released into the host cell and produce a cytotoxic effect (Thiaucourt *et al.*, 2004). Peroxide and super-oxide production may be important in disruption of host cell integrity (Quinn *et al.*, 2002). Mycoplasma phospholipases are potentially important in pneumonia for they reduce surface tension of the alveolar surfactants thus resulting in atelectasis (Admassu *et al.*, 2015).

2.4. Clinical Signs

There is considerable variation in the severity of clinical disease from hyper acute, acute, sub-acute to chronic form (Radostits *et al*., 2007). Hyper acute form: The clinical signs observed in the hyper acute form are much accelerated. Affected animals may die within a week exhibiting classical respiratory signs. In fatal cases, death occurs after a variable course of from several days to 3 weeks (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. The hyper acute form involving up to10 percent of infected animals and may be observed at the onset of an outbreak; death is sudden and is often not accompanied by any other signs (Andrews *et al.*, 2008).

Acute form: The early stages of CBPP are indistinguishable from any severe pneumonia with pleurisy. Animals show dullness, anorexia and irregular rumination with moderate fever and may show signs of respiratory disease. Coughing is usually persistent and is slight or dry. Sometimes fever goes up to 40 – 42°C and the animal prostrates with difficulty of movement (Radostits *et al.*, 2007). As the typical lung lesions develop, the signs become more pronounced with increased frequency of coughing and the animal becomes prostrate or stands with the back arched, head extended and elbows abducted. While classical respiratory signs may be evident in calves, articular localization of the causative agent with attendant arthritis usually predominates (Andrews *et al.*, 2008). The acute form is observed in approximately 20% of the diseased animals. The course is 5 to 7 days (Wanyoike, 1999).

Sub-acute form may be limited to a slight cough only noticeable when the animal is exercised. Infected cattle many develop chronic or milder form of the disease, which may be either symptomless or associated with only a slight temporary rise in body temperature and some loss of condition (Admassu *et al.*, 2015). Recovered animals may be clinically normal but in some an inactive sequestrum form in the lung with a necrotic centre of sufficient size to produce a toxemia causing unthriftness, a chronic cough and mild respiratory distress on exercise (Abera *et al.*, 2016).

Figure 2: Animal stand with head extended (FAO, 2007).

The earliest signs are a sudden onset of fever up to 40°C or more and in milking cows a drop in milk yield, anorexia and cessation of rumination. There is severe depression and the animals stand apart or lag behind a traveling group and stop eating (Admassu *et al.*, 2015). The clinical symptoms start with the characteristics short, dry cough, which becomes more and more painful; later, the cough usually becomes more severe, the animals shows signs of pain, standing with arched back and extension of the head and neck forwards and downwards, increased grunting respiration, salivation and nasal discharge. In gross pathology, there is a severe fibrinous pneumonia with copious pleural exudates. The pleural exudate is a striking feature and there may be up to 30 liters' of yellow which containing clots in the chest cavity (Masiga *et al*., 1996).

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 clear fluid and sometimes blood from cut surfaces. The interlobular septa are grossly thickened; pleural surfaces over affected areas are thickened and grey (Radostits *et al*., 2007). Local lymph nodes are enlarged, edematous and may contain areas of necrosis. In chronic cases, necrotic lung tissue becomes encapsulated to form sequestrum of 1 to 20 cm

diameter. The tissue within the sequestrum tends to retain much of the architecture of the acute lesion but may eventually become calcified or liquefied. The lesion may either break open to release viable mycoplasmas or be resorbed. Pleural adhesions are commonly found in chronic cases (Andrews *et al.*, 2008).

2.5. Diagnosis

The diagnosis of CBPP is based on a history of contact with infected animals, clinical findings, immuno-diagnosis tests, necropsy findings and cultural examination (Radostits *et al.*, 2007). Contagious bovine pleuropneumonia is difficult to diagnose based on clinical signs alone as there can be many causes of severe pneumonia in cattle. But we can diagnosis CBPP based on a history of contact with infected animals, clinical findings, immuno diagnosis tests, necropsy findings and cultural examination (Admassu *et al.*, 2015).

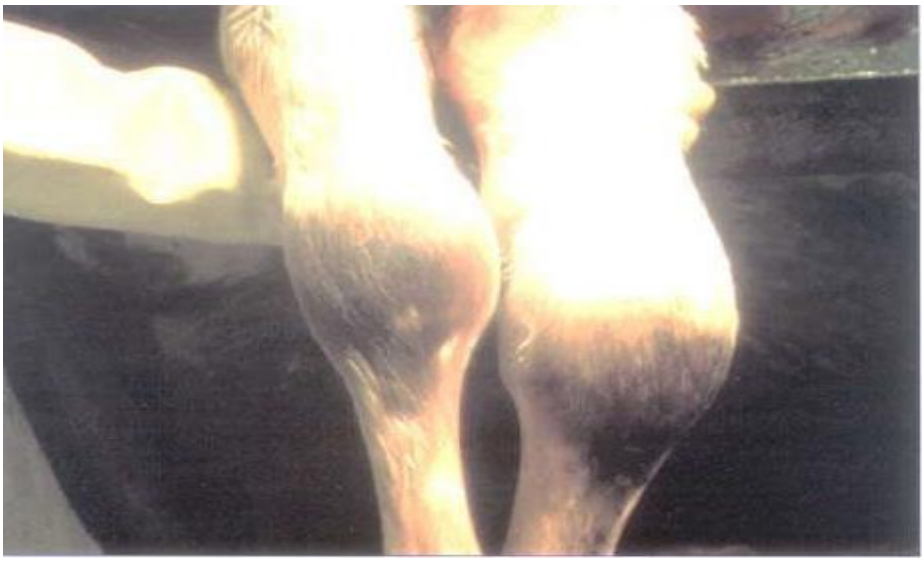


Figure 3: Swollen joints of a calf with CBPP (FAO, 2007).

Contagious bovine pleuropneumonia frequently results in disease in only one 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 (Cynthia *et al*., 2011). Confirmatory diagnosis is based on the isolation of *Mycoplasma mycoides subspecies mycoides* from clinical samples of lung (Nicholas *et al*., 2009). The causal organism can be isolated from samples taken either from live animals or at necropsy (Admassu *et al.*, 2015). Samples taken

from live animals are nasal swabs or nasal discharges, broncho-alveolar lavage or transtracheal washing and pleural fluid collected aseptically by puncture made in the lower part of the thoracic cavity between the seventh and eighth ribs. Blood may also be cultured (OIE, 2008).

Samples taken at necropsy are lungs with lesions, pleural fluid, lymph nodes of the broncho pulmonary tract and synovial fluid from those animals with arthritis. The samples should be collected from lesions at the interface between diseased and normal tissue. When dispatching samples to the laboratory, it is advisable to use a transport medium that will protect the mycoplasmas and prevent proliferation of other bacteria (heart-infusion broth without peptone and glucose, 10% yeast extract, 20% serum and 0.3% agar). The agent can be detected by culture, serological test, biochemical test and immunological tests (Admassu *et al.*, 2015).

2.5.1. Culture

Mycoplasma mycoides subspecies mycoides Small Colonies needs appropriate media to grow. But it is not intrinsically difficult to grow, unlike other fastidious Mycoplasmas such as one causing CCPP, but requires a fully functioning bacteriological laboratory with access to special *Mycoplasmas* media (OIE, 2008). The media should contain a basic medium Such as heart infusion or peptone, fresh yeast extract and horse serum (Nicholas *et al.*, 2008). To avoid growth of other bacteria, inhibitors, such as penicillin, colistin or thallium acetate are added. The media can be used as broth or solid medium with 1–1.2% agar (Andrews *et al.*, 2008).

All culture media prepared should be subjected to quality and must support growth of Mycoplasma species from small inoculation. The reference strain should be cultured in parallel with the suspicious samples to ensure that the tests are working correctly. After grinding in broth containing antibiotics the lung samples are diluted tenfold to minimize contaminating bacteria and are inoculated into five tubes of broth. Hermetic sealing of the petri dishes or the uses of incubators with controlled humidity are recommended in order to avoid desiccation. To ensure the best conditions for mycoplasma growth, a CO_2 incubator or candle jar should be used. The tubes and petri dishes are inspected at day 5 and at day 10 (Admassu *et al.*, 2015).

In fluid medium, a homogeneous cloudiness usually appears within 2–4 days, frequently with a silky, fragile filament. During the following days a uniform opacity develops with whirls when shaken. On agar media, the colonies are small (1mm in diameter) and have the classical appearance of 'fried eggs' with a dense centre (OIE, 2008).

2.5.2. Serological Tests

Serological tests for CBPP are valid at the herd level. Tests on single animals can be misleading, either because the animal is in the early stage of disease before specific antibodies are produced or it may be in the chronic stage of the disease when very few animals are seropositive (Masiga *et al.*, 1996). Complement fixation test is suitable for determining animal free from disease and a prescribed test for international trade and it widely used in all countries where infection occurs (Admassu *et al.*, 2015). The limitations of the complement fixation test are well known. The complement fixation test can detect nearly all sick animals with acute lesions, but a rather smaller proportion of animals in the early stages of the disease. Despite the high specificity (70%) of the complement fixation test, false-positive results can occur (Sacchini *et al.*, 2012).

A competitive enzyme-linked immunosorbent assay (c-ELISA) developed by the OIE Collaborating Centre for the diagnosis and control of animal diseases in tropical countries has undergone evaluation (Goffe and Thiaucourt, 1998; Admassu *et al.*, 2015). Advice on the availability of reagents can be obtained from the OIE Reference Laboratories for CBPP disease diagnosis validation tests that have been carried out in several African and European countries would indicate that the specificity of the c-ELISA and has been reported to be at least 99.9% (Niang *et al.*, 2006).

The c-ELISA is now provided as a readymade kit that contains all the necessary reagents including precoated plates kept in sealed aluminum foil. The kit has been especially designed to be robust and offer a good repeatability. As a consequence, sera are analyzed in single well (Freundt *et al*., 1999). The substrate has been modified and is now TMB (Tetra methyl Benzedrine) in a liquid buffer and the reading is at 450 nm (Admassu *et al.*, 2015). The substrate color turns from pale green to blue in the first place and becomes yellow once the stopping solution has been added. Monoclonal antibody (MAb) controls exhibit a darker color

while strong positive serum controls are very pale. The cut-off point has been set at 50% and should be valid in every country (OIE, 2008; Muuka *et al.*, 2011; Schubert *et al.*, 2011).

2.5.3. Immunological Test

An immuno enzymatic test designated the immuno blotting test and has diagnostic value. A field evaluation indicated a higher sensitivity and specificity than the complement fixation test (OIE, 2008). 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 complement fixation test has given a suspected false result (FAO, 2004).

2.5.4. Biochemical Tests

These biochemical tests should be carried out by a reference laboratory. For this purpose, after two or three subcultures, antibiotics should be omitted from the medium to check if the isolate is a mycoplasma that will regain its original form in the medium without inhibitors (Freundt *et al*., 1999). Once this test is done and after, the organism can be identified using biochemical tests. *Mycoplasma mycoides subspecies mycoides* Small Colony is sensitive to digitonin, does not produce film and spots, ferments glucose, reduces tetrazolium salts aerobically or anaerobically, does not hydrolyse arginine, has no phosphatase activity and has no or weak proteolytic properties (Admassu *et al.*, 2015).

For these tests, special media have been developed that include the same basic ingredients (Heart infusion broth, horse serum, 25% yeast extract solution, 0.2% DNA solution) (Freundt *et al*., 1999). Once the biochemical characteristics have been checked, immunological tests can be performed to confirm the identification (OIE, 2008). The isolation and identification of the CBPP agent can be difficult and time consuming and depends on careful of the appropriate procedures (Admassu *et al.*, 2015).

2.2.5. Immunohistochemistry (IHC)

Immunohistochemistry (IHC) has proved to be a robust assay in the diagnosis of CBPP, particularly where the causative organism, MmmSC, is not recoverable following long transport distances, where the animal has died of acute disease or where serology cannot be performed or is inconclusive (Alhaji and Babalobi, 2015).

2.2.6. Polymers Chain Reaction (PCR)

PCR is a rapid and sensitive diagnostic method. It allows detection of MmmSC directly in samples of lungs, bronchial lymph nodes, nasal swabs, pleural fluid and blood. Pre-incubation for 24 h of clinical specimens in growth medium may increase test sensitivity (Alhaji and Babalobi, 2015).

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 MmmSC (Schnee *et al*., 2011)

Two PCR detection systems based on the 16S rRNA genes have also been developed for identification of MmmSC with high sensitivity and specificity. Both systems amplify the genes from all members of the *Mycoplasma Mycoides* cluster. Outbreaks of CBPP often require analysis of large numbers of specimens to detect the occurrence of MmmSC. The traditional method for the analysis of PCR products is agarose gel electrophoresis which is useful if a small numbers of samples are involved (Suzuki *et al.*, 2004).

2.6. Differential Diagnosis

Contagious bovine pleuropneumonia is necessary to differentiate from other diseases that may present similar clinical signs or lesions (Admassu *et al*., 2015).

2.6.1. Rinderpest

The confusion with rinderpest results from the fever and discharges observed from the eyes, nose and mouth. However, the characteristic lesions of rinderpest those are essentially erosions in the mouth and throughout the digestive tract, together with the profuse, often bloody diarrhea in advanced cases, should enable easy differentiation from CBPP in which these are not seen. Lung lesions are seen in more chronic cases of rinderpest and these consist of red areas of collapse together with emphysema of lung lobules and the septa separating them. At this stage the erosive lesions of rinderpest may have healed (Cynthia *et al*., 2011).

2.6.2. Haemorrhagic Septicaemia

This is a very acute disease and most affected animals die within 6 to 72 hours after the onset of clinical signs. Edema of the throat and neck to the brisket is often very pronounced. The lung lesions seen in animals that survive the longest can appear very similar to the marbling lesion of CBPP, there may be yellow fluid in the chest and the affected lung may adhere to the inside of the rib cage. Thus, in the individual case distinguishing between haemorrhagic septicaemia and CBPP can be difficult (Admassu *et al.*, 2015).

2.6.3. Theileriosis (East Coast Fever)

Coughing, nasal and ocular discharge and diarrhea are observed. Affected cattle show general enlargement of superficial lymph nodes and especially those of the head. The lungs contain much clear liquid which is also present in the chest cavity; the airways in the lung may be filled with white froth. Cigarette urn-like ulcers (lesion caused east cost fever) are seen in the abomasal folds. Neither pneumonia nor inflammations of the pleura are present (Radostits *et al*., 2007).

2.6.4. Tuberculosis

Tubercular nodules can superficially resemble sequestra but they are degenerative cheese like lesions, sometimes calcified. The lung tissue is destroyed and the same lesions are also seen in lymph nodes in the chest. The capsule of the tubercular nodules is not well defined when compared to that of sequestra (Radostits *et al*., 2007). Contagious bovine pleuropneumonia also differentiated from acute bovine pasteurellosis, bronchopneumonia, Foot and mouth disease, Actinobacillosis and Echinococcal (hydatidcysts) (FAO, 2002).

2.7. Treatment

Under practical field conditions, when the disease outbreaks in a new area, treatment is not applicable and not recommended because of reasons of disease prevention (Admassu *et al.*, 2015). Treatment is recommended only in endemic areas because the organisms may not be eliminated and carriers may develop. Tylosin, 10 mg/kg, IM, bid, for six injections and danofloxacin, 2.5 mg/kg/day for 3 consecutive days have been reported to be effective

(Radostits *et al.*, 2007). Although the *Mycoplasmas* are susceptible to a number of antibiotics treatment failures are common (Walker, 1999).

The use of antimicrobials is controversial because of fears that it could result in more carriers, but cattle owners nevertheless do use antimicrobials to cure clinical disease and save the lives of their cattle. Oxytetracyclines are the antimicrobials most widely used in Africa to treat CBPP. In spite of widespread and probably sub-optimal use over a long period of time resistance has not been detected, but a number of other products have been investigated (Anon, 2012).

2.8. Control and Prevention

The prevention is better than cure is very relevant to dealing with CBPP. Quarantine is the first line of defense against these disease and all countries should devote an appropriate level of resources to ensure that they implement effective border and import quarantine policies and programmes to prevent the introduction of serious livestock diseases (Admassu *et al.*, 2015). Eradication of CBPP has been achieved in North America, Europe, and Australia and apparently in Asia, with only sub-Saharan African continent remaining significantly infected (Nicholas *et al*., 2000). Methods used have generally involved movement control and stamping out of infected herds, although eradication in China, in which infection was widespread finally achieved by the use of an attenuated vaccine involving numerous passages in rabbits that was apparently effective, although it took more than 30 years to achieve eradication (Anon, 2012).

Control of this disease in countries that have achieved eradication relied on the combined use of animal movement controls and slaughter of affected animals, possibly combined with vaccination and surveillance. In Sub- Saharan African countries the difficulty in controlling cattle movement has been identified as one of the major reasons for the spread of CBPP and the same fact has a similarity in Ethiopian condition (Dereje and Shawul, 2017).

The most important resource in the prevention of CBPP is informing animal owner or manager. Cattle owners at all levels of production must be able to recognize CBPP and know what to do when they suspect it. This can only be achieved by intensive farmer training, using

media that are easily understood, highly visual and that will serve as a constant reminder of the disease and its importance (FAO, 2000). Contagious bovine pleuropneumonia control is achieved by eliminating the whole cattle herd population that means stamping out, wherever the disease is detected. However, this may not prove realistic and quarantine coupled with vaccination is the most frequently used CBPP control measure (OIE, 2001).

2.9. Economic Importance of CBPP

Contagious bovine pleuropneumonia is considered to be a disease of 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, loss due to quarantine, loss of cattle trade and reduced investment in livestock production (Tambi *et al.*, 2006; Radostits *et al.*, 2007; Dohoo *et al*., 2009). In the affected countries, enormous losses are experienced each year from the death of animals and the loss of production during convalescence (Catley *et al*., 2002). The highly fatal nature of the disease, the ease of spread and the difficulty of detecting carrier also mean that close restriction must be placed on the movement of animals from enzootic areas (Abera *et al.*, 2015). In addition to these, it leads to in imposition of rigorous limitations to international trades on CBPP affected countries in accordance with world organization of Animal Health (OIE) regulations (Sacchini *et al*., 2012; Admassu *et al*., 2015).

The financial and economic loss caused by the disease in Africa is significant. It has been reported that the continent has lost approximately 2 billion US dollar per year due to death of livestock from the disease (Admassu *et al*., 2015). Contagious bovine Pleuropneumonia has been causing significant economic loss on the agriculture sectors and the national economy in Ethiopia. It accounts for a loss of over 206.5 million Ethiopian birr per year (Laval, 1999). Thus, 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; Admassu *et al*., 2015).

Cattle and cattle products can be lowered by this disease. The loss in draft power was estimated as the product of the number of infected oxen and the number of workdays per year

(Abera *et al.*, 2016). All physical losses in cattle, beef, and milk and draft power were valued using market prices (FAO, 2004). Beef production loss by infected animals was used as a proxy for the absence of weight gain since diseased animals are assumed to not gain weight (Laval, 2001). The losses due to productivity reductions in beef, milk and draft power accounted for 74% of the total value of loss while mortality losses accounted for 26% (Abera *et al.*, 2016).

In Ethiopia the average physical losses from CBPP in terms cattle deaths are 25,115 heads (8,372 in endemic and 16,743 in epidemic areas), 1,852 and 13,396 metric tons of beef meat and liters of milk respectively (Tegegn, 2017). Ethiopia experiences the largest number of cattle deaths and reduction in cattle products under both endemic and epidemic conditions relative to other African countries, due probably to its large cattle population (Tambi and Maina, 2004). It should be noted that the economic evaluation of losses due to CBPP has not been performed systematically throughout Africa. Priority should therefore be given to the cost benefit analysis of control or eradication campaigns (Admassu *et al.*, 2015). Regarding the Ethiopian situation, CBPP has been causing significant economic losses on the livestock sector and the national economy and it accounts for a loss of over 8.96 million US dollars per year (Hurrissa and Eshetu, 2002).

3. STATUS OF CBPP IN ETHIOPIA

Contagious Bovine Pleuropneumonia remains the most important infectious disease of cattle in Ethiopia. It is one of the major threats in Ethiopia hindering and challenging the livestock production system (Malicha *et al.*, 2017). In Ethiopia, various surveys have been carried out to estimate the prevalence of CBPP on livestock in different regions by various investigators (Wondimu, 1996; Takele, 1998; Fikru, 2001; Tesfaye, 2016) 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). The cattle population at risk of CBPP and livestock production systems in CBPP endemic and epidemic zones of Ethiopia is estimated to be a total of 13,325,700 heads of cattle. All of them are considered to be at risk of CBPP, of which 5,510,700 are in endemic ones and 7,815,000 are in epidemic zones (Abera *et al.*, 2016; Dereje and Shawul, 2017).

The irregularity and low rate of vaccinations since 1993 seem to contribute to the increased incidence of the disease and its further spread (MoA, 2003). The usual blanket coverage was around 50% and never reached the desired 80-100% level. Eleven years (1992-2002 G.C.) disease outbreak reports by Federal Ministry of Agriculture indicated that several CBPP epidemics have been recorded from the south, west and north regions of the country (Table1). The passive disease outbreak reports from 1992-2002 shows 587 outbreaks, 16,806 cases and 3,262 deaths. The highest record was in 1998 when 187 outbreaks with 5,652 cases and 1071 deaths were reported (MoA, 2002). Due to the insidious nature of the disease, such official data do not necessarily convey the extent of the problem caused by CBPP in Ethiopia (Gedlu, 2004).

Seroprevalence study from export quarantine centers at Adama-Modjo Livestock Export Industry in five Private beef animals' from November 2013 to May 2014 reported the overall seroprevalence of 8.00% (Birhanu, 2014). Seroprevalence studies from November 2014 to April 2015 in Amaro special district of South Nation Nationality People region were reported 31.8% (Ebisa *et al.*, 2015). In the four districts (Alamata, Raya Azebo, Ofla, and Endamehoni) of southern zone of Tigray region from December 2012 to May 2013 report overall prevalence of 11.9% (Teklu *et al.*, 2015).

Another more recent seroprevalence study conducted in southwest Ethiopia from October 2015 to August 2016 in Gimbo district of Keffa Zone Southern Nation's Nationalities and People's Regional State of Ethiopia were reported 8.1% (Mamo, 2016). In general at the country level, CBPP seroprevalence studies have been conducted in different localities of the country (Abdela and Yune, 2017).

Table 1: Previous reports of CBPP in different part of Ethiopia

Area	**Zone**	**No of examined animals**	**Prevalence**	**Reference**
West Ethiopia	West Wollega	651	48%	Desta,(1997)
	Bodji	506	28%	Fikru,(2001)
Southern Ethiopia	Shashemene	955	6.07%	Asmamaw, (2003)
	Amaro district	400	31.8%	Ebisa *et al.* (2015)
North Ethiopia	Western Gojjam	2073	27.3%	Gashaw,(1998)
	Tigray	384	11.9%	Teklu *et al.(*2015)
Borena	Liben	1014	6.8%	Ahmed, (2004)
	Dire	787	12%	
	Didatuyure	246	9.4%	
Somali	Shinille and Jijiga	793	39%	Gedlu, (2004)

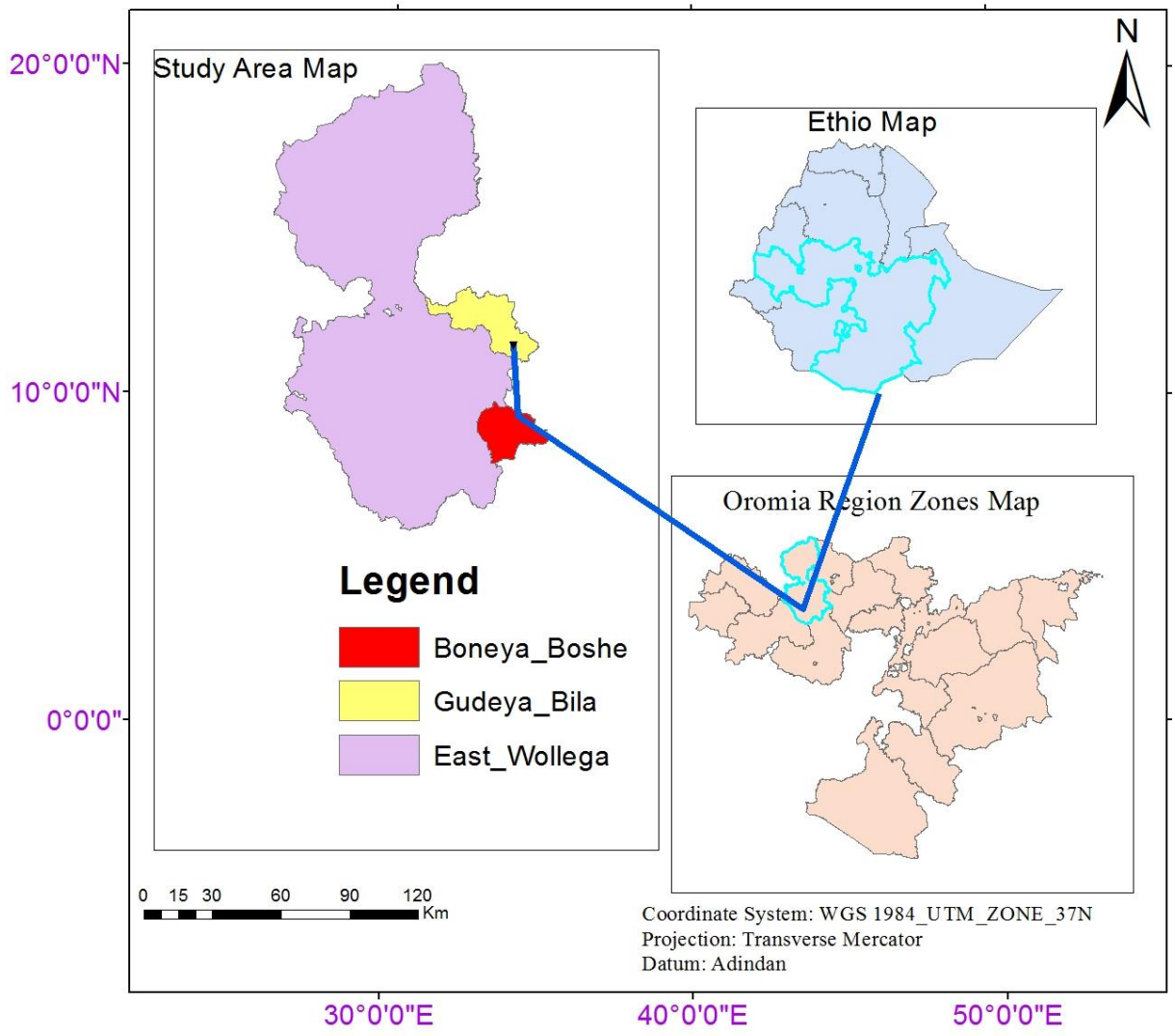
Furthermore, CBPP has been reported from different export quarantine centers in the country signifying that CBPP remain a threat to livestock export market and may reduce the investment in livestock production. Studies undertaken so far in Ethiopia reported seroprevalence that range from 0.4% to 96% (Abdela and Yune, 2017). Generally, based on the available information, the epidemiological situation of CBPP is found in various parts of Ethiopia (Afework, 2000).

4. MATERIALS AND METHODS

4.1. Description of the study area

The study was conducted from November, 2017 to June, 2018 in Gudeya Bila and Boneya Boshe districts of East Wollega zone. Eastern Wollega have broadleaf forest, grasslands and wetland (marshes and swamps) and most common type of vegetation, maize, sorghum, teff, wheat and coffees are the most highly cultivated crops in the area (EWLFRDO, 2017). Gudeya Bila district is found in the East Wollega zone of Oromia Regional State, Western Ethiopia which is located at 274 km West of Addis Ababa, the Ethiopian capital city, at 09º 17'363''N latitude and 037º 01'460'' E longitudes with an altitude ranging from 1876 -2092 meters above sea level. The area is characterized by a humid tropical climate with annual rainfall that ranges from 1000-2200 millimeter per annum. The minimum and maximum temperature ranges from 13ºC and 27ºC respectively, with an overall average of 20ºC (CSA, 2017). Mixed crop-livestock production system was the main form of agriculture in the area. The district has livestock population of 121, 081 cattle, 16,816 sheep, 13,617 goats, 6,834 horses, 4,834 mules, 5,890 donkeys and 48,483 poultry (GBLFRDO, 2017). The district has fifteen kebeles and from this kebeles four kebeles were selected purposively based on cattle population and access to road.

Boneya Boshe district is found in the East Wollega zone of Oromia Regional State, Western Ethiopia which is located at 307 km West of Addis Ababa, at 08º 54'045''N latitude and 037º 00'136'' E longitudes with an altitude ranging from 1613 -1641 meters above sea level. The area is characterized by a humid tropical climate with annual rainfall that ranges from 1000-1200 millimeter per annum. The minimum and maximum temperature ranges from 16.4ºC and 25.3ºC respectively, with an overall average of 20.9ºC (CSA, 2017). Mixed crop-livestock production system was the main form of agriculture in the area. The district has livestock population of 102, 917 cattle, 13,860 sheep, 9,816 goats and 7, 334 equine (BBLFRDO, 2017). The district has ten kebeles and from this kebeles four kebeles were selected purposively based on cattle population and access to road.


Study Area Map
Ethio Map
Oromia Region Zones Map
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20°0'0"N
10°0'0"N
0°0'0"
30°0'0"E
40°0'0"E
50°0'0"E
Legend
Boneya_Boshe
Gudeya_Bila
East_Wollega
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Projection: Transverse Mercator
Datum: Adindan

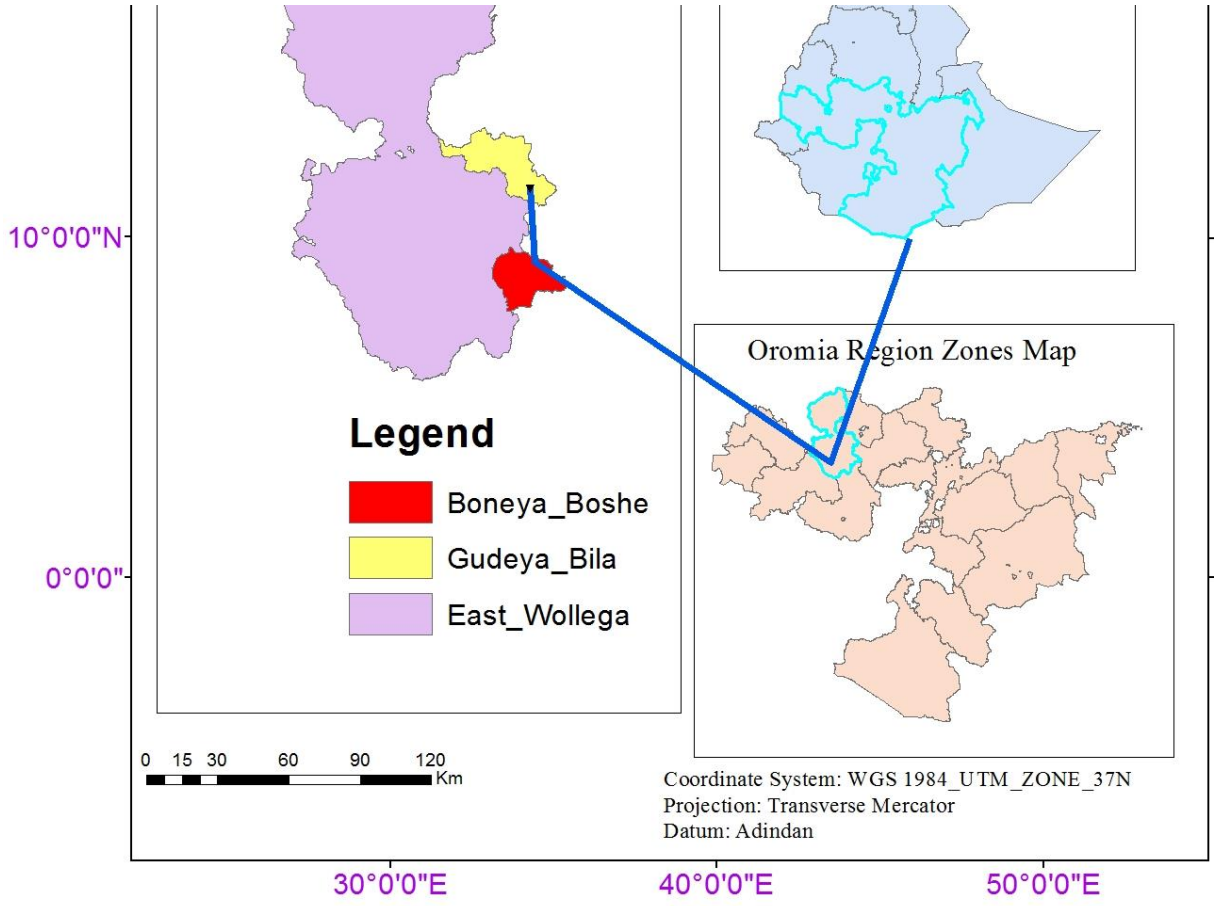

Figure 4: Study area map (CSA, 2017).

4.2. Study Design

A cross-sectional study was conducted using a simple random sampling technique to select the study cattle. The size of the households' and complete list of herd distribution were identified from kebele and then both blood sample collection and questionnaire survey was conducted. Pre-tested semi-structured questionnaires were used to collect information on factors influencing the occurrence of CBPP within or between herds using face-to face interview. Data on sex, age, origin of animal, herd size, previous infection history (like clinical sign of pneumonia in adults and polyarthritis in calves), body condition scores of

animal, animal management, introduction of new animal, herd contact and herd contact area were recorded. The body condition scores of animal were scored according DEFRA (2001) and body condition scoring 1 and 2 were recorded as poor body condition score and body condition scoring 3, 4 and 5 were recorded as good body condition score (Annex 4).

4.3. Study animal

4.3.1. Target and study population

The target populations in this study were all local breed cattle above six month of age of both sexes with no history of vaccination in selected kebeles of Gudeya Bila and Boneya Boshe districts of Oromia regional state. The study populations in this study were cattle selected for purpose of this study from selected kebeles of Gudeya Bila and Boneya Boshe districts of Oromia regional state.

4.4. Sample size determination

The sample size was determined using the formula described by Thrusfield (2005) by considering an expected prevalence of 50% with an absolute precision of 5% with 95% confidence level.

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

Where N = sample size of the study population

d = Absolute desired precision

p = expected prevalence in the study area

$$N = \frac{1.96^2 \text{X } 0.5(1-0.5)}{0.05^2} = 384$$

Therefore, a required sample size was 384. A total of 384 blood samples were collected for this study from randomly selected cattle.

4.5. Questionnaire survey

Data were collected by questionnaire surveys from selected 95 households during sampling of study cattle. The questionnaire was covering information on the name of the owner, location, sex, age, origin of animal, herd size, previous infection history based on clinical sign of CBPP like polyarthritis in young animals and pneumonia in adult animals, animal management, contact of herds with one or more animals or herds of different peasant associations at grazing areas or watering points, introduction of new animals, livestock market activity and communal grazing land (Annex1 and 2). This questionnaire was administered by face-to-face interview with the farmers using the local language.

4.6. Sample collection and Laboratory test (Competitive ELISA (c- ELISA)

About 10 milliliters of blood sample was collected from the jugular vein of each cattle using sterile vacutainer tubes and needles by following aseptic procedure after cattle strained by owner and each sample was properly labeled. The sample were kept protect from sun light in a slanting position for 6-8 hours. The serum was separated from it and transfer to a sterile tube and store at -20ºC and analysis with Competitive ELISA at Bedele Veterinary Regional Laboratory.

The serum samples were tested by CBPP competitive enzyme-linked immunosorbent assay (c-ELISA) kit to detect the specific antibodies to *Mycoplasma mycoides subspecies mycoides* Small Colony (MmmSC). Competitive ELISA was an OIE prescribed test and can be used for official CBPP testing. This test was based on a monoclonal anti MmmSC antibody, named Mab117/5 (OIE, 2014) (Annex 3).

$$S\ PI\ \% = \frac{100 \times (MabCx - S\ A_{450})}{(MabCx - CCx)}$$

Where S PI= Samples percentage of inhibition

MabCx = Mab control mean absorbance

S A= Sample absorbance

CCx =conjugate control mean absorbance

Sample with percentage of inhibition greater than or equal to 50% are considered as positive for presence of MmmSC antibodies and sample with percentage of inhibition less than 50% are considered as negative for presence of MmmSC antibodies.

4.8. Data analysis

Data obtained from both serological tests and questionnaire surveys were entered and stored in Microsoft (MS) Excel spreadsheet program and analyzed using SPSS software programs version 20. Individual animal risk factors like age, sex, body condition scores, history of disease and origin of animal and herd level risk factors like herd size, management, herd contact with other herds, contact area and introduction of new animal were analyzed. The total seroprevalence of individual animals was calculated by dividing the number of c-ELISA positive animals by the total number of animals tested and herd prevalence was calculated by number of herd positive to total number of herd tested. A herd was considered seropositive, if at least one animal in the herd was found seropositive. Herds in this study were two or more animals kept by single individual farmer.

Univariable logistic regression was used to select the exposure variables forward for multivariable analysis. Factors were selected for final multivariable logistic regression analysis if the p-value was ≤ 0.25. The strength of association between the risk factors and the occurrence of the disease was assessed using Odds Ratio (OR). Pearson correlation coefficients were used to check the variables for co-linearity. Then, multivariable analysis was conducted and non-significant variables were removed sequentially using backward elimination at $p < 0.05$.

5. RESULTS

5.1. Prevalence

From the total animals of 384 examined 33 of them were found to be positive for *Mycoplasma mycoides subspecies mycoides* SC (MmmSC) antibodies. The overall sero-prevalence of contagious bovine pleuropneumonia at individual animal-level was 8.6% (n=33/384) (95%CI: 5.8% -11.4%). From total of 95 herds examined 25 of them are positive to MmmSC antibodies. The seroprevalence of contagious bovine pleuropneumonia in herd-level was estimated to be 26.3% (n=25/95) (95%CI: 17.5%-35.2%). The positive animal for MmmSC antibodies in the Boneya Boshe and Gudeya Bila were 10.8% (20/186) and 6.6% (13/198), respectively. Among the kebeles sampled, 2% (1/50) in Haro gudisa, 9.6% (5/52) in Hena jawaja, 8.3% (4/48) in Jare, 6.3% (3/48) in Agalo gidami, 23.5% (12/51) in Ejersa gute, 11.6% (5/43) in Gala gore, 4.4% (2/45) in Bilo and 2.1% (1/47) in Jawis kebeles animals are positive for MmmSC antibodies (Table 2).

Table 2: Sero-prevalence of contagious bovine pleuropneumonia in study area

Factor	Categories	No of examined	Prevalence (%)	**95%CI**	
				Lower	**Upper**
Districts	Gudeya bila	198	13(6.6)	3.1	10.0
	Boneya boshe	186	20(10.8)	6.3	15.2
Gudeya bila	Haro gudisa	50	1(2.0)	1.9	5.9
	Hena jawaja	52	5(9.6)	1.6	17.6
	Jare	48	4(8.3)	0.5	16.1
	Agalo gidami	48	3(6.3)	0.6	13.1
Boneya boshe	Ejersa gute	51	12(23.5)	11.9	35.2
	Gala gore	43	5(11.6)	2.1	21.2
	Bilo	45	2(4.4)	1.6	10.5
	Jawis	47	1(2.1)	2.0	6.3

5.2. Risk factors

5.2.1. Animal –level risk factors

Male animals have high sero-prevalence 11.3% (17/151) than female 6.9% (16/233) and sex was not statistical association with MmmSC antibodies circulation. Animals with age > 5 years 10.6% (19/180) have high sero-prevalence than animals with age ≤ 5 years 6.9% (14/204) and there was no statistical significant association with MmmSC antibodies

circulation. Animal with poor body condition score 12.1% (28/ 231) has high sero-prevalence than good body condition score animals 3.3% (5/153) and there was statistical significant association (P<0.05) between poor body condition score and *Mycoplasma mycoides subspecies mycoides* small colony antibodies circulation. Animal with history of disease 13.3% (23/173) has high sero-prevalence than cattle with no disease history 4.5% (10/211) and there was statistical significant association (P<0.05) between animal with history of disease and MmmSC antibodies circulation. Animal replacement from outside the herd was statistically associated with MmmSC antibody circulation where the herds with animals replaced was found to be higher 17.1% (9/52) sero prevalent than animal with own source origin 7.2% (24/332) (Table 3 and 4).

Table 3: Sero-prevalence of CBPP antibody with risk factors at individual animal level

Factor	Categories	No of examined	Prevalence (%)	95%CI	Univariable analysis Crude OR(95%	p-value
Sex	Male	151	17(11.3)	6.2-16.3	0.4(0.2-1.0)	0.06
	Female	233	16(6.9)	3.6-10.1		
Age	≤5years	204	14(6.9)	3.4-10.3		
	>5 years	180	19(10.6)	6.0-15.0	1.7(0.7-4.1)	0.20
Bcs	Poor	231	28(12.1)	7.9-16.3	5(1.6-15.8)	0.09
	Good	153	5(3.3)	0.5-6.1		
Disease history	Yes	173	23(13.3)	8.2-18.3	5.6(2.0-15.9)	0.001
	No	211	10(4.5)	1.9-7.6.0		
Origin	Own source	332	24(7.2)	4.4-10.0		
	Outside	52	9(17.1)	7.0-27.6	12.4(3.0-51.9)	0.001

Table 4: Results of multivariable analysis of potential risk factors at individual animal level

Factors	Categories	No of examined	Prevalence (%)	Multivariable analysis	
				Adjusted OR(95% CI)	P-value
Bcs	Poor	231	28(12.1)	4.6(1.6-13.2)	0.01
	Good	153	5(3.3)	*	
Disease history	Yes	173	23(13.3)	4.9(1.9-12.9)	0.01
	No	211	10(4.7)	*	
Origin	Outside	52	9(17.3)	12.5(3.1-51.0)	0.01
	Own herd	332	24(7.2)	*	

*= reference

5.2.2. Herd level risk factors

Among the potential risk factors assessed at herd level large herd size 41.9% (13/31) has high sero prevalence than lower herd size 18.8% (12/64) and there was statistical significant association between large herd size with MmmSC antibodies (OR: 3.6 (1.3-10.3; P<0.01). From management, herds from extensive management system 28.4% (19/67) have high sero prevalence than herds found in semi extensive management system 21.4% (6/28) and management is not statistically associated with MmmSC antibody. Herd with history of contact with other herds 26.1% (24/92) has lower sero prevalence than herds with no history contact with other herds 33.3% (1/3) and there were no statistical significant association with MmmSC antibody. Herds with history of new animal introduction 21.7% (10/42) has lower sero prevalence than herds with no history of new animal introduction 30.6% (15/49) and there were no statistical significant association with MmmSC antibody. Herds which had contact with other herds at watering point has 32.1% (9/28) high sero prevalence than herds contact with other herds both at watering and grazing point has 24.6% (15/61) and there were no statistical significant association with MmmSC antibody (Table 5).

Table 5: Sero-prevalence of bovine mycoplasma antibody with risk factors at herd level

Factor	Categories	No of examined	Prevalence (%)	95%CI		P-value
				lower	**Upper**	
Herd size	≤36 herd size	64	12(18.8)	9.2	28.3	
	>36 herd size	31	13(41.9)	24.6	59.3	0.02
Management	Extensive	67	19(28.4)	17.6	39.2	0.20
	Semi extensive	28	6(21.4)	6.2	36.6	
Herd contact	Yes	92	24(26.1)	17.1	35.1	0.6
	No	3	1(33.3)	20.0	86.7	
New animal	Yes	46	10(21.7)	9.8	33.7	0.90
introduction	No	49	15(30.6)	17.7	43.5	
Contact area	Watering	28	9(32.1)	14.8	49.4	0.90
	Water and grazing	61	15(24.6)	13.8	35.4	

6. DISCUSSION

This study shows that CBPP is one of major cattle health problem and threatening the livelihood of farmers in the eastern Wollega zones of the Oromia regional state. The overall seroprevalence of MmmSC antibodies was estimated to be 8.6%. Nearly similar result was reported by other investigators, 9.4% in Borena (Ahmed, 2004) and 9.7% in south western Kenya (Schnier *et al*., 2006).

The higher seroprevalence was previously reported from different regions of the country and outside of the country by other investigators, 39% in Somali Regional State (Gedlu, 2004), 28.5% in western Oromia (Daniel *et al.*, 2016), 28% in the Bodji district of Western Wollega (Fikiru, 2001), 25.3% in Sidama Zone (Malicha *et al.*, 2017) and 16% in Kajiado district of Kenya (Matua-Alumira *et al.*, 2006) and on the other hand the lower seroprevalence also previously reported by different investigator, 4% in and around Adama (Kassaye and Molla, 2012), 6.14% in Southern Ethiopia (Asmamaw, 2003), 1.4% in Bale zone (Dereje and Shawul, 2017). The variation in prevalence of CBPP reported from different parts of Ethiopia and other countries might be due to differences in agro ecological systems, cattle management and production systems, population density, sample size and the types of tests used to determine the seroprevalence.

Poor body condition was found significantly ($P<0.05$) associated with the occurrence of MmmSC antibodies circulation which agrees with finding of Biruhtesfa *et al*. (2015) in Bishoftu abattoir. This might be due to the fact that animals with poor body conditions are more susceptible to the disease due to low immunity to resist disease.

Animals with history of disease was found significantly ($P<0.05$) associated with the presence of MmmSC antibodies. This might be due to the fact that previous diseased animals are carrier of *Mycoplasma mycoides subspecies mycoides* in lung sequestra. Furthermore, the disease is mainly transmitted from animal to animal in aerosols and the organism is usually found in saliva, urine, fetal membranes and uterine discharges and easily transmitted from affected animal to healthy animals (Radostits *et al.*, 2007).

Statistically significant (P< 0.05) association between introducing new animals to the herds from outside origin replacement and seropositivity might be due to the fact that animal replaced from diseased herds and this finding is in agreement with the study reported by Tadese, (2014) at Adama-Modjo Quarantine in which there was significant association in the prevalence of the CBPP antibodies and origins of animals.

The statistically significant association between the large herd sizes and sero-prevalence of MmmSC antibodies was in agreement with report of Alemayehu *et al.* (2015) in which high sero-positive recorded in large herd size in Borena pastoral area of Southern Ethiopia. This might be related to the health management of large herd size and risks of an individual animal become infected with disease increases as herd size increase due to overcrowding of animals (Radostits *et al.*, 2007).

Among the potential risk factors assessed at individual animal-level, age of the animals were not associated significantly (P>0.05) with the presence of the CBPP antibodies. This finding is in agreement with that of Teshale *et al*., (2015) and Biruhtesfa *et al.,* (2015) who found no significant association between age groups. This might be due to similar exposure of animals to the disease since the disease is contagious that all animal in the herd have equal chance of exposure and a single diseased animal can serve as continuous source of infection to the herd. Furthermore, the disease is mainly transmitted from animal to animal in aerosols and the organism is usually found in saliva, urine, fetal membranes and uterine discharges. Hence, there could be uniformity of infection in all age groups (Radostits *et al.*, 2007).

There was no statistically significant association (P > 0.05) in the presence of the MmmSC antibodies based on sex. This result was agreement with finding of Daniel *et al.*, (2016) in west Oromia but does not agree with report of Schnier *et al.*, (2006), who reported a significantly higher prevalence in female animals. This might be due to similar exposure of male and female animals to disease.

From potential risk factors assessed at herd-level, there was no statistical significant association between management system and presence of MmmSC antibodies. This might be due to the fact that great majority of cattle management and the production fashion was not

technically and scientifically supported and both semi-intensive and extensive management system enhance close or repeated contact of cattle and the propagation of CBPP make simple (Tegegn, 2017).

There were no statistically significant association between history of new animal introduction and presence of MmmSC antibodies. The absence of statistically significant association between histories of new animal introduction with presence of MmmSC antibody might be due to the fact that there was no restriction on movements of animals and replacement of animal from outside.

There was no statistically significant association between MmmSC antibodies and herd contact history. The absence of statistical significant association between herd contacts with other herds and MmmSC antibody might be due to the fact that both semi-intensive and extensive management system enhances close or repeated contact of cattle.

There was no statistically significant association between presence of MmmSC antibodies and contact areas. The absence of statistical significant association between herd contact area and MmmSC antibodies might be due to the fact that both semi-intensive and extensive management system enhance close or repeated contact of cattle at all contact area and all area of contacts are equally risk for animals.

7. CONCLUSIONS AND RECOMMENDATIONS

Contagious bovine pleuropneumonia had been recognized in Ethiopia for many years. The sero-prevalence of MmmSC antibodies in the study areas at individual animal and herd level was 8.6% and 26.3%, respectively. The result of this study also shows that the disease is prevalent in the study area. Animals kept in these study areas are always at the risk of contracting CBPP because of their uncontrolled replacement of animals from outside origin.

The results of these study shows that animals with poor body condition score, large herd size, replacement of animal from outside herd and previously diseased animal are showing high sero-prevalence of the disease and statistically associated with MmmSC antibodies. The presence of statistically significant association in the sero-prevalence of MmmSC antibodies with the above risk factors suggests that favors the occurrence and spread of the disease. Based on the above conclusion the following recommendations are forwarded.

- Animals should be kept in high plane of nutrition especially cattle in poor body condition in order to develop resistance against the disease.
- Diseased animals should be separated from the herd and treated;
- Care should be taken during replacement of animal from other herds
- Control measures directing at preventing further spread and lowering the prevalence of the disease in the zone through the use of better and coordinated vaccination program should be recommended to control disease in large herd size.

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9. ANNEXES

Annex 1: A questionnaire survey used to collect information on CBPP

Date ...../...../.....

Questionnaire Number.....

Name ---------- kebele----------

Answer the following questionnaire corresponding to your cattle

1. What is herd composition of your cattle on age basis?

A ……..D ……..

B ……..E ……

C ……..F ……

2. What is herd composition of your cattle on sex basis?

A ……..D ……..

B ……..E …….

C ……..F…….

3. What is breed composition of your herds?
    A) Local breed
    B) Cross-breed
    C) Exotic breed

4. Is there cattle morbity in your herds (disease history)?

A) Yes

B) No

5. Is there is contact of your herd with other herd?

A) Yes

B) No

6. If your answer yes for question 5, where they contact?

A) Watering point

B) Grazing area

C) Both at grazing and watering point

7. Is there cattle disease after contact in your herds?

A) Yes

B) No

8. Is there is introduction of new animals from outside source?

A) Yes

B) No

9. Is there livestock market activity around your kebele?

A) Yes

B) No

9. How your animal is managed?

A) Extensive

B) Semi extensive

10. Is there is contact of your herds of different households?

A) Yes

B) No

Annex2: Serological sample collection sheet (Woreda_____, Kebele_____).

Sample code	Site		Owner name	Breed	Age	Sex	BSC	Origin of animal		Serology result
	Woreda	Kebele						Born	Bought	

Annex 3: C-ELISA procedure

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/deionized 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:
3. Sample distribution	Transfer 100µl from each well of the preplate to the appropriate well of coated microplate.
4. Sample incubation	Cover the microplate (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 microplate (with lid, aluminium 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 three times.
9. Substrate distribution	Add 100µl of TMB Substrate N.13 in each wells
10. Substrate incubation	Incubate 20 minutes (±3 min.) at + 37^{o}c (±3 ^{o}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 MmmSC Antibodies.

Source: OIE, 2014

Annex 4: Body condition scoring format

Score	Condition	Detailed Description
1	Poor	Tail head – 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 – end of horizontal process 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: DEFRA, 2001.