Review on Contagious Bovine Pleuropneumonia

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Abstract: Contagious bovine pleuropneumonia is a highly contagious chronic respiratory disease of cattle caused by *Mycoplasma mycoides subspecies. mycoides small colonies*. The disease is characterized by a relatively long incubation period and a highly variable clinical course. Recovered animals may harbor the infection in lung sequestra; necrotic areas of lung tissue separated from the surrounding normal tissue by a fibrous capsule. Contagious bovine Pleuropneumonia is current disease of major concern throughout Sub-Saharan Africa. The principal route of infection is by the inhalation of infective droplets from animals active or carrier cases of the disease. Important pathogenicity factors in *MmmSC* are capsular polysaccharide, hydrogen peroxide and variable surface protein. It is manifested by anorexia, fever and respiratory signs such as dyspnoea, polypnoea, cough and nasal discharges. Diagnosis depends on the isolation of an etiological agent. The common methods used for the diagnosis of the disease are complement fixation test and enzyme linked immune sorbent assays. It is considered to be a disease of economic importance. Commonly used antibiotics include tetracyclines, tylosin, erythromycin, lincomycin, spectinomycin and tilmicosin. The main problems for control or eradication are the uncontrolled movements of animals and the frequent occurrence of sub-acute or subclinical infections and the persistence of chronic carriers after the clinical phase. Therefore, adequate control strategic measures should be implemented for eradication of the disease such as test and slaughter, stamping out, quarantine and vaccination.

Keywords: Cattle, Contagious Bovine Pleuropneumonia, diagnosis, economic importance, epidemiology, control and prevention

1. Introduction

Contagious Bovine Pleuropneumonia (CBPP) is highly contagious respiratory disease of cattle found in most Sub-Saharan African countries, which is caused by *Mycoplasma mycoides subspecies mycoides small colony* (*Mmm*SC) (Amanfu, 2009). Since the global eradication of rinderpest was declared in 2011, CBPP has been considered the most serious disease of cattle (Anon, 2012). On account of its transmissibility and economic impacts, CBPP is now recognized as a priority trans-boundary disease and has thus been categorized as the only bacterial disease in the OIE list "A" diseases (Lefevre, 2000; OIE, 2008).

The disease is endemic to part of Africa, having been eradicated elsewhere during the mid 20th centaury 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). Such policies are difficult to apply in most African countries and it is widespread in pastoral areas of Africa and a major problem for Ethiopian livestock (Masiga *et al.,* 1996). 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; Rovid, 2008). 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).

Factors which are important in the rate of transmission of diseases are closeness of contact, intensity of infection and number of susceptible animals (Thiaucourt, 2004). 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 (OIE, 2002). It has also been postulated that recovered animals with sequestra in their lungs might pose a risk for infection, since viable *MmmSC* has been isolated from sequestra for up to 12 months (FOA, 2004). As long as the bacteria remain encapsulated by fibrous tissue in the intact sequestrum the animal will not be infective, but it is postulated that sequestra might rupture and permit shedding of the bacteria and consequent transmission of the disease (Dedieu-Engelmann, 2008). The main problem in eradication is the frequent occurrence of sub-acute or asymptomatic infections and the persistence of chronic carriers after the clinical phase (OIE, 2008).

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 clinical signs, pathologic lesions, (Pleurisy, lung hepatization), identification and isolation of the agent, immune blotting, serology and PCR techniques (OIE, 2008).

Commonly used antibiotics include tetracyclines, 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. 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 (Radostits *et al*., 2007).

Ethiopia is a tropical African country in which mobile pastoralism is dominant in the arid and semi-arid areas in the eastern, northeastern and southeastern parts of the country (Tegegne *et al*., 2009). The country is one of the top ranking in Africa and among the first ten in the world in terms of livestock resource (OIE, 2002). It possesses the highest number of livestock in Africa with an estimated 56.71 million cattle, 29.33 million sheep and 29.11 million goats (CSA, 2015). However, the productivity of this livestock sector is lower than the potential level of the African production average (Metaferia, 2013). Traditional methods of animal husbandry render the output per unit of domestic breed of livestock to be too low (Behnke, 2010; Metaferia *et al*., 2011). The major biological constraints contributing to low productivity include low genetic potential of the animals, poor nutrition and prevailing diseases (Belay *et al*., 2012a; Belay *et al*., 2012b). Transboundary animal disease such as Contagious bovine pleuropneumonia (CBPP) constrain the livestock sector of the country and affect livelihoods via their impact on animal health, animal food production, availability and quality (Behnke, 2010).

Estimated annual economic cost of contagious bovine pleuropneumonia in Ethiopia due to vaccination is 1,097 Euros and due to treatment is14,987 Euros. 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 (Tambi, 2006). Therefore, the objective of this review paper is: to review on epidemiology, diagnosis, economic importance and control methods of contagious bovine pleuropneumonia.

2. Literature Review

2.1. The Disease: Contagious Bovine Pleuropneumonia (CBPP) is an acute, sub-acute or chronic respiratory disease of cattle and is one of the most serious cattle diseases in Africa (OIE, 2002). 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, 2006).

2.2. Etiology: The causative agent of contagious bovine pleuropneumonia is *mycoplasma mycoides* subspecies *mycoides* small colony. The causative organism of CBPP was isolated by the French scientists (Nocard and Roux, 1898). It was classified as a mycoplasma (Edward and Freundt, 1956) and given its current name *Mycoplasma mycoides* subspecies *mycoides* small colony type (Cottew and Yeats, 1978).

2.3. Mycoplasma

2.3.1. Taxonomy and morphology: Taxonomically, *Mycoplasma mycoides small colony* belongs to the class *Mollicutes*, order *Mycoplasmatales*, family *Mycoplasmataceae* and genus *Mycoplasma. Mollicutes* (*mollis*; soft, *cutis*; skin, in Latin) (Razin *et al.,* 1998). The mycoplasmas are non-sporulating, Gram negative and non-motile bacteria, which do not possess a determined shape of the cell. There are no internal membrane structure and no cell wall except plasma membrane. Mycoplasmas are not only devoid of cell wall but the genetic capability to produce one, which also renders them completely resistant to β-lactam and other cell wall activity drugs. Due to their small size are able to pass through the usual bacteriological filter (0.1-0.3nm). The cell shapes include spherical, pear, spiral and filamentous form (Quinn *et al.,* 1994). 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, 2004). When observed with dissecting microscope, many species exhibit "fried egg" morphology (Radiostits *et al*., 2007).

2.3.2. Growth characteristics: 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°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).
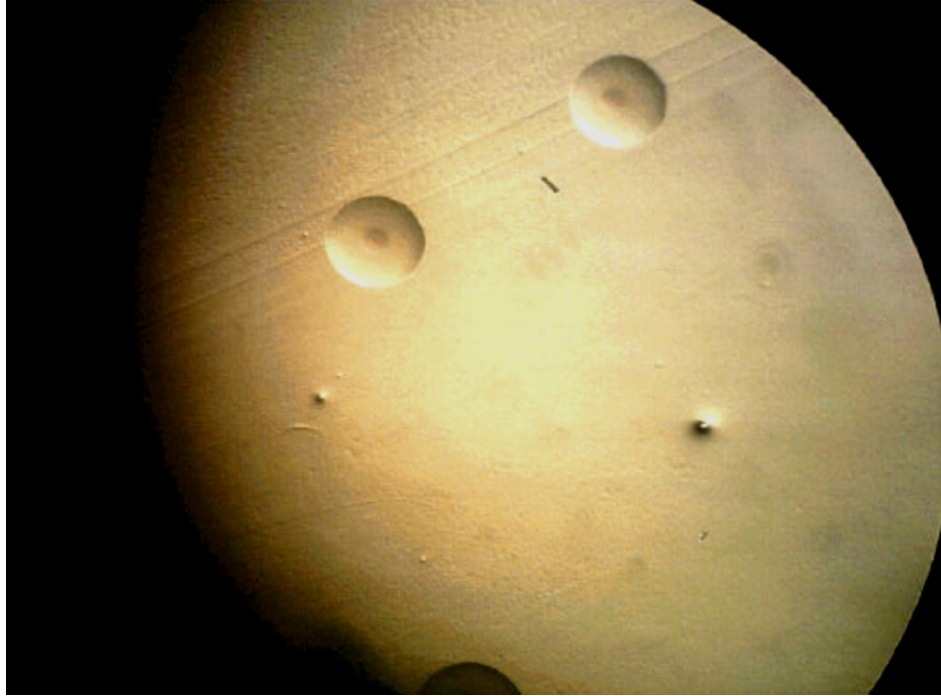
Figure 1: *Mycoplasma mycoides* subspecies *mycoides* colony with the classical appearance of 'fried-egg' with a dense center
Source: (Gedlu, 2004)

2.3.3. Host specificity: Characteristic in mycoplasma infection is the high level of host specificity. Usually mycoplasma species will infect only a single or few closely related animals' species. It has been suggested that the degree of host specificity is directly related to the intimate nature of the parasite relationship and dependence of the infecting mycoplasma on the host cell component to fulfill its fastidious nutritional requirements for successful colonization of host (Levinsohn, 1992).

2.3.4. Resistance to physical and chemical action: *MmmSC* does not survive for long periods in the environment and transmission often involves close contact though, under favorable atmospheric conditions of humidity and wind, aerosols can transport the organism over longer distances. Temperature inactivates in 60 minutes at 56°C or 2 minutes at 60°C. Chemicals or disinfectants inactivates, mercuric chloride (0.01%/1 minute), phenol (1%/3 minutes), and formaldehyde solution (0.5%/30 seconds). The organism survives outside the host for up to 3 days in tropical areas and up to 2 weeks in temperate zones. *MmmSC* may survive more than 10 years frozen (OIE, 2008).

2.3.5. *Mycoplasma mycoides* cluster: The *Mycoplasma mycoides* cluster consists of six pathogenic *Mycoplasma* species, subspecies or strains causing mild to severe disease in ruminant hosts, either bovine or *capri*ne (Johansson and Pettersson *et*

al., 2002). These are *Mycoplasma mycoides* subspecies *mycoides* Large Colony (*Mmm*LC), *Mycoplasma mycoides* subspecies *Mycoides* small colony type (*MmmSC*), *Mycoplasma mycoides* subspecies *Capri* (*Mmc*)*, Mycoplasma capricolum* subspecies *capricolum* (*Mcc*), *Mycoplasma capricolum* subspecies *capripneumoniae* (*Mccp*), and *Mycoplasma* species bovine serogroup seven (*M*BG 7) (Stakenborg *et al.,* 2005).

2.4. Epidemiology: Contagious bovine pleuropeumonia 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 the 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).

Table 1: Member of the *Mycoplasma mycoides* cluster

Species	Disease	Main hosts (other)
MmmSC	CBPP	Cattle (goats, sheep, buffalo)
*Mmm*LC	Caprine pneumonia, contagious agalactiae	Goats (sheep, cattle)
Mmc	Caprine pneumonia	Goats (sheep) but rare
Mcc	Caprine pneumonia, contagious agalactiae	Goats (sheep, cattle)
Mccp	CCPP	Goats (sheep)
*M*Bg 7	Arthritis, mastitis, calf Pneumonia	Cattle

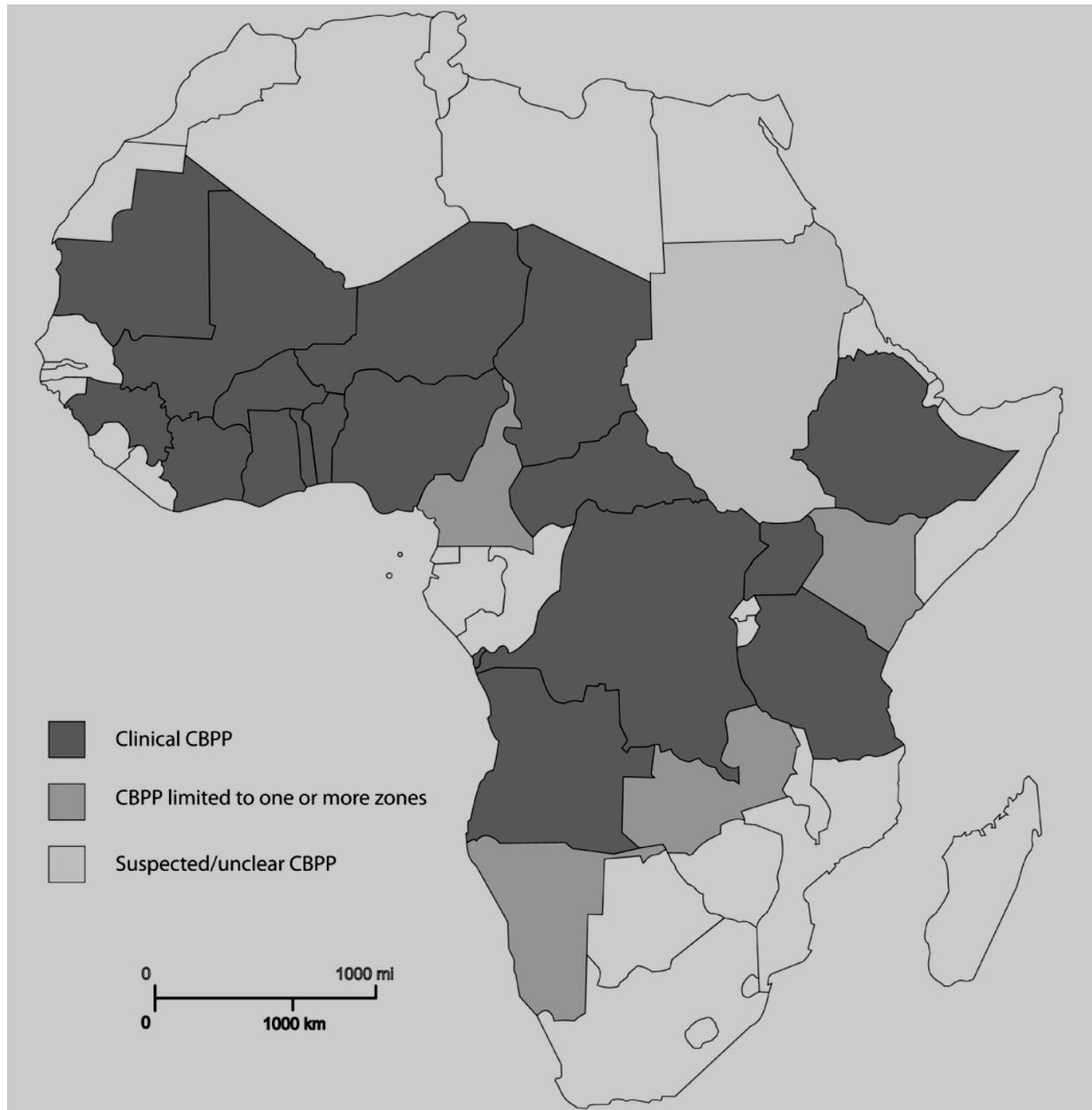**Source:** (Manso-Silvan *et al*., 2009)

Figure 2: CBPP occurrences in African countries during 2006-2008
Source: (OIE, 2009)

2.4.1. Host range: Cattle (*Bos taurus* and *Bos indicus*) and Asian buffalo (*Bubalus bubalis*) are the primary hosts for *Mmm*SC. 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. White-tailed deer (*Odocoileus virginianus*) have been infected experimentally (OIE, 2015). Contagious bovine pleuropneumonia is predominantly the disease of cattle and occasionally water buffalo are naturally infected (Andrew *et al*., 2004). There are many reported breed differences with respect to susceptibility. In general, European breeds are tends to be more susceptible than indigenous African breeds (OIE, 2002). 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 (Newton, 1992). 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). *Mmm*SC had been isolated from milk of sheep with mastitis and goats with pneumonia (Egwu *et al.,* 2012).

2.4.2. Geographical distribution: 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). At present, the disease causes vast problems in 21 African countries with severe socio-economical consequences during the time period 2006-2008 (OIE, 2009).

In 2011, CBPP was reported to the AU-IBAR from 18 African countries; spreading across the west, central, east and southern Africa regions. During the reporting period, 304 epidemiological units were affected by CBPP across Africa involving 16,836 cases and 3007 deaths, with an estimated case fatality rate of 17.9%. The highest number of CBPP outbreaks was reported in Ghana (75) followed by Central African Republic (43) and Ethiopia (29) (AU-IBAR, 2011).

Table 2: Countries in Africa reporting CBPP to the AU-IBAR

Country	Outbreaks	Cases	Deaths	Slaughtered	Destroyed
Burkina Faso	4	203	45	0	0
Cameroon	8	384	16	41	0
Central African Republic	43	3674	1270	0	0
Chad	17	342	200	37	18
Congo, DRC	15	8277	458	1361	0
Cote d'Ivoire	18	595	215	13	7
Ethiopia	29	457	112	12	0
Gabon	3				
Ghana	75	127	1	115	0
Mali	4	204	82	119	0
Niger	6	41	10	0	0
Nigeria	22	489	96	221	9
Somalia	12	69	16	0	0
Sudan	2	202	92	108	0
Tanzania	8	399	177	0	0
Togo	9	13	3	1	0
Uganda	22	1330	193	67	0
Zambia	7	30	21	0	1
Total	304	16,836	3007	2095	35

Source: (AU-IBAR, 2011)

2.4.3. Methods of transmission and source of infection: The epidemiology of CBPP is influenced by many factors including the virulence of the *MmmSC* strains, host susceptibility and management systems. Normally transmissions are by droplet infection from actively infected animals to susceptible animals in

close proximity (Andrews *et al*., 2004). 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 occur contact with 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 (Campbell, 2015).

2.4.4. Incubation period: The time from natural exposure to period overt signs of disease is variable but generally quite long. It has been shown that healthy animals placed in CBPP infected herd begin to showing signs of the disease 20 to 123 days. Experimentally subsequent to installation of a large quantity of infective materials at the trachea bifurcation, the incubation period was 2 to 3 weeks (Radiostits *et al*., 2007). When control cattle were placed in contact with naturally affected cattle from a outbreak in Namibia, seroconversion was seen after 6 weeks; rise rapidly in the next two weeks by which time 40% of contacts had died (Nicholas, 2004). However, in the field survey seroconversions continued more than 8 months after the disease onset (Lesnoff *et al.,* 2004).

2.4.5. Mortality and morbidity: The morbidity and mortality rates for CBPP are highly variable. In a naive herd, the outcome varies from complete recovery of all animals to the death of the majority. Morbidity increases with close confinement, due to the increase in transmission, and infection rates can be as high as 50-80% in some situations. The mortality with CBPP is quite varied and ranges from 10 to 70% in various outbreaks (Andrews *et al*., 2004).

2.4.6. Risk factor: CBPP 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 (Thiaucourt, 2004).

a)   **Animal related factor:** 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 Africa buffalo and white tailed deer (Radiostits *et al*., 2007). A strong immunity develops after an attack of the natural disease in cattle and vaccination plays important part in control. The lack of a cell wall and endotoxins may enable mycoplasmas to colonize the animal without inducing an immune response and the predilection for the mucosal membranes may also limit the humoral response (OIE, 2008).

b)   **Management related factor**: 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 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).

c)   **Pathogen related factor:** *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. 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. European strain lacks 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 transport proteins responsible for the production H_2O_2 and the capsule which is thought to have toxic effect on the animal. 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 (Radiostits *et al*., 2007; OIE, 2008).

2.5. Pathogenesis: Very little is known about the factors and mechanisms that affect the pathogenicity of *MmmSC*. 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 (Persson *et al*, 2002).

2.5.1. Capsular polysaccharide: An important pathogenicity factor in *MmmSC* is the capsular polysaccharide (CPS), previously known as galactan (Woubit *et al*., 2007). It is made up of the carbohydrate galactose (90%) and to a lesser extent glucose (2-4%) and lipid. Injection of purified CPS to cattle produced severe respiratory collapse and even death. The CPS has been found to play a significant role in the pathogenesis of infection, binding to the host tissue surfaces and inducing resistance to phagocytosis. It has also been associated with the formation of auto reactive antibodies and consequently autoimmune responses. Toxic effects of *MmmSC* have

also been associated with the capsule (Nicholas *et al*., 2000).

2.5.2. Hydrogen peroxide: In a recent investigation reported by Woubit *et al*. (2007), there was indication that glycerol metabolism in *MmmSC* strains release hydrogen peroxide (H_2O_2) as a byproduct, resulting in disruption of host cell integrity. Hydrogen peroxide is produced by a membrane located enzyme L-glycerophosphate oxidase (GlpO) that is involved in glycerol metabolism (Pilo *et al.,* 2005). The initial hypothesis was based on the fact that virulent *MmmSC* African strains possessed an active ATP-binding cassette (ABC) transport system for the utilization of glycerol, which is metabolized to dihydroxyacetone-phosphate (DHAP) releasing H_2O_2, while European strains lacked part of the glycerol uptake genes due to deletion and are less virulent (Vile and Abdo, 2000).

2.5.3. Variable surface protein: *MmmSC* express surface proteins which can undergo reversible changes to alter the antigenic repertoire in a cell population. The gene for these variable surface protein Vmm as they were termed encodes a lipoprotein precursor of 59 amino acids(aa), where the mature protein was predicted to be 36 aa and was anchored to the membrane by only the lipid moiety, as no transmembrane region could be identified (Woubit *et al.,* 2007). The protein was found to undergo reversible phase variation at a frequency of 9×10^{-4} to 5×10^{-5} per cell per generation and this variation enables the *Mycoplasma* organisms to escape the host immune defense mechanism of their host (Pettersson *et al*., 2002; Le Grand *et al.,* 2004; Citti *et al*., 2005 and Wise and Foecking, 2006).

2.6. Clinical Signs: Depending on the resistance level of the animal and the intensity of exposure, the disease takes an acute, subacute to chronic, or the acute course is sometimes followed by a chronic stage which may last for two years (lunger) as a latent phase of the disease (FAO, 2003).

A) 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. 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 (Hirsh *et al*., 2004). 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 calves less than six month of years (Andrews *et al*., 2004; Radiostits *et al*., 2007). Acute CBPP affects approximately 20 percent

of infected animals and the course is 5 to 7 days (Thrusfield, 2007).

B) Sub acute forms: Subacute CBPP is the most common form (40-50%) and is a less severe form of the acute disease with only slight respiratory symptoms and intermittent fever. Signs may be limited to a slight cough only noticeable when the animal is exercised. Cattle that recover naturally are extremely weak and emaciated. Many infected animals develop chronic or milder forms of the disease, which may be either symptomless 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. 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) (FAO, 2002).

C) 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. It is estimated that up to 25% of affected cattle become chronic carriers (Thiaucourt *et al*., 2004). There is considerable variation in the severity of clinical disease from acute, subacute to chronic form (Radiostits *et al*., 2007).

2.7. Diagnosis: The diagnosis of CBPP is based on a history of contact with infected animals, immuno-diagnosis tests, necropsy findings and cultural examination. (OIE, 2008).

2.7.1. Culture

Sample collection for culture

From the living animal: nasal swabs and secretions, tracheal and bronchoalveolar washes and pleural fluid and occasionally blood, urine and synovial fluid should be obtained. From the dead animal: pleural fluid, portions of affected lungs and lung sequestra (scrapings from inside the capsule) and lung-associated lymph nodes, and kidneys should be taken (OIE, 2008). Because of the Mycoplasmas fastidious nature, samples should be submitted to the laboratory as soon as possible after collection. During transportation, it is advisable to use a transport medium that will protect the Mycoplasma and prevent proliferation of other bacteria such as ampicilin and amoxicillin, which are not effective for Mycoplasma but works for contaminants (FAO, 2004). *MmmSC* needs appropriate media to grow. In attempting isolation, 2-3 blind passages may be required. Most isolation attempts fail because the organism is labile,

and is often present in small quantities and is demanding in its growth requirements. Therefore, the media should contain a basic medium such as heart-infusion or peptone, yeast extract (preferably fresh) and horse serum (10%). Besides, several other components can be added such as glucose, glycerol, DNA and fatty acids but the effects vary with the strains. To avoid growth of other bacteria inhibitors such as penicillin, colistin or thallium acetate are necessary.

For demonstration of proteolysis, growth is carried out on casein agar and/or coagulated serum agar (OIE, 2002). The media can be used as broth or solid medium with 1.0–1.2% Pleuropneumonia agar (Andrews *et al*., 2004).

All culture media prepared should be subjected to quality and must support growth of *MmmSC* from small inocula. 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 and on to solid medium. The pleural fluid can be inoculated directly without previous dilution. Hermetic sealing of the Petri dishes or the uses of incubators with controlled humidity are recommended in order to avoid desiccation (OIE, 2008).

2.7.2. Biochemical test

MmmSC 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. For routine field use, the immunological tests are sufficient; but where these give dubious results and in all cases of identification of first isolates, biochemical tests should be confirmed by a reference laboratory (OIE, 2002). For this purpose, after two or three subcultures, antibiotics should be omitted from the medium to check if the isolate is a Mycoplasma or other form of a bacterium that will regain its original shape in the medium without inhibitors. Once this test is done and after cloning (at least three colonies should be selected) the organism can be identified using biochemical tests (OIE, 2008). The peroxidase-antiperoxidase (PAP) test is an excellent confirmatory test (FAO, 2004).

However, differentiation of *Mmm*SC strains by serological and biochemical means has been difficult. This difficulty is caused by immunological cross-reactions and biochemical similarities of *Mmm*SC strains with *Mmm*LC. Therefore, immunological test using growth inhibition test is used as a confirmatory test. The mechanisms by which antibody inhibits the growth of Mycoplasma organisms are not clearly understood. It has been proposed that growth-inhibiting antibodies are directed against exposed surface membrane proteins in which the cells are enclosed in a capsule of carbohydrate which is composed of galactan (Rurangirwa *et al*., 2000).

2.7.3. Immunological tests

The etiological agent or its antigens can be demonstrated by immunochemical tests on infected tissues, tissue fluids and/or cultures of the organism. However, as some of these tests are dependent on a minimum number of organisms being present in the sample; only positive results are taken into account (OIE, 2014).

I) Indirect fluorescent antibody test (IFA): The IFA test can be performed on smears from clinical material using hyperimmune rabbit serum against *Mmm*SC and labelled anti-bovine IgG. Hyperimmune bovine serum has been used, but may have cross-reactive antibodies. The test is satisfactory when applied to pleural fluid smears, but is less satisfactory with lung smears due to considerable nonspecific fluorescence. However, good results can be obtained using lung smears counter stained with Erichrome black (OIE, 2008).

II) Fluorescent antibody test (FAT): The FAT is commonly performed on broth and agar cultures. It is slightly less specific than the IFA test.

1. Broth culture

Place two drops on a microscope slide. Fix for 15 minutes with methyl alcohol, and leave in contact with the labelled hyperimmune serum for 30 minutes at 37°C in a humid chamber. Rinse three times with phosphate buffered saline (PBS), pH 7.2 and examine under an epifluorescence microscope (×80). Colonies grown on solid medium*;* cut a block of agar supporting a number of young colonies and place on a slide with the colonies facing upwards. Place one or two drops of the labelled hyperimmune serum on the block and leave it in a humid chamber for 30 minutes. Place the block into a tube and wash twice for 10 minutes with PBS. Place the block on a slide with the colonies facing upwards and examine as before (OIE, 2008).

2. Petri dish culture

The gel should not be too thick (no more than 3 mm) and should contain as little horse serum as possible. Rinse the gel three times with PBS, flood the surface with 1 ml of labeled serum and incubate for 30 minutes in a humid chamber. Rinse four times with PBS and examine directly under the microscope. The FAT in a Petri dish is used mainly just after isolation and before cloning, as it is very useful in the case of mixed infection with several mycoplasma species. Interpretation of the FAT with broth culture: the mycoplasmas appear bright green on a dark Back ground. However, experience is required for the FAT carried out with colonies on agar, because the background appears dark green (OIE, 2008).

III) Disk growth inhibition test (DGIT): The DGIT is based on the direct inhibition of the growth of the agent on a solid medium by a specific hyperimmune serum. However, cross reactions within the mycoides cluster are common and great care should be taken to differentiate *Mmm*SC (bovine biotype) from *Mmm*LC (caprine biotype; large colonies). It is a simple test to perform, but some results require experience to be interpreted. Small inhibition zones (less than 2 mm wide) partial inhibition with break through colonies false-negative and false-positive reactions (very rare). The quality of the hyperimmune serum used in this test is critical for good results (OIE, 2014).

IV) Agar gel immunodiffusion test: The agar gel immunodiffusion (AGID) test can detect the specific antigen present at the surface of *Mmm*SC and the circulating galactan invading the haemolymph system of sick animals. Pleural fluid ground lung fragments or even sequestrae can be tested against a hyperimmune serum in two wells cut 5 mm apart in the gel. The gel is composed of noble agar (12 g) and thallium acetate (0.2 g/liter) in PBS pH 7.2 (1000 ml). The test is considered to lack sensitivity and little is known about its specificity, but it has served as a screening test and only positive reactions should be taken in to account. The results are better when the plate is incubated at 37°C and can be read within 24 hours. A simpler field test has been developed using impregnated paper discs instead of wells (OIE, 2014).

V) Dot immunobinding on membrane filtration: The use of special 96-well micro plate whose well bottoms are made of a specific membrane filter offers further advantages over other tests, such as practicality, rapidity, ready standardization and the possibility of treating many samples against several sera simultaneously. These plates allow the removal of well fluids by vacuum filtration. In this way, mycoplasmas are separated from broth media by trapping them on the filtration membranes and the broth proteins that do not bind to the membrane are easily removed. The membranes are then incubated with specific hyperimmune sera. The unbound immunoglobulin is removed by filtration as above and the bound antibodies (IgG) are detected by means of an enzyme-conjugated anti-IgG (OIE, 2008).

VI) Immunohistochemistry (IHC): Immunohistochemistry (IHC) has proved to be a robust assay in the diagnosis of CBPP, particularly where the causative organism *Mmm*SC is not recoverable (e.g. following long transport distances) where the animal has died of acute disease or where serology cannot be performed or is inconclusive. *Mmm*SC immunoreactive sites can be detected in lung lesions using the peroxidase-antiperoxidase method on sections of paraffin-embedded material. Because the isolation of the agent is not always achieved from chronic cases and after treatment with antimicrobial drugs, this test is only supplementary to the diagnosis of CBPP; a negative result is not conclusive (OIE, 2014).

2.7.4. Serological tests: To detect latently or chronically infected animals, almost all serological tests are suitable. Galactan, the major antigenic component of *MmmSC* can be found in all body fluids during and for some time after the acute stage of the disease. It has been found that not all *Mycoplasma mycoides* subspecies *mycoides* strains, which have been isolated so far present immunological differences. Moreover, the level of antibodies against *Mycoplasma mycoides* subspecies *mycoides* that can be detected serologically does not necessarily also gives information about the level of protective antibodies being present (Rurangirwa *et al*., 2000).

I) Complement fixation test: At present, the most reliable test for detecting serum antibodies that is currently prescribed test for international trade by the OIE, which applies at herd level for interpretation. Great care is needed in collecting and storing sera to be used for this test which is complex to perform. False negative results can be found both early and late in the disease course (Martel *et al*., 2004). It is recommended that any fixation of complement, even partial (25, 50 or 75%) at a serum dilution of 1/10 should be followed by additional investigations. The limitation of the CF test is well known with a sensitivity of 70% and a specificity of 98% and it can detect nearly all sick animals with acute cases (Sacchini *et al*., 2012). In addition, therapeutic interventions and improperly conducted prophylactic operations may increase the number of false-negative reactions. The nature of the pathogenesis of the disease is such that the incubation period, during which antibodies are undetectable by the CF test, may last for several months. Despite the high specificity of the CF test, false-positive results can occur, of which an important cause is serological cross-reactions with other mycoplasmas, particularly other members of the *Mycoplasma mycoides* cluster. The validity of the results has to be confirmed by post-mortem and bacteriological examination, and serological tests on blood taken at the time of slaughter (Martel *et al*., 2004; Regassa *et al*., 2005).

II) Competitive enzyme-linked immunosorbent assay (cELISA): Competitive enzyme-linked immunosorbent assay developed by the OIE Collaborating Center for the diagnosis and control of animal diseases in tropical countries (Le Goff & Thiaucourt, 1998), has been validated internationally in accordance with OIE standards (Amanfu *et al*., 1998). In May 2004, this cELISA was designated as an OIE prescribed test for international trade by the OIE International Committee (now the World

Assembly). The performance of this cELISA method has also been validated by the French Committee for Accreditation in 2009 (OIE, 2014). The OIE Collaborating Center for cELISA and Molecular Techniques in Animal Disease Diagnosis Validation tests that have been carried (OIE, 2008). No cross-reactions with other *Mycoplasma* species were reported during its development and a relative sensitivity (SE) of 96% and specificity (SP) of 97% were reported (Le Goff and Thiaucourt, 1998). The cELISA 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 wells. The substrate has been modified and is now TMB (Tetra methyl Benzedrine) in a liquid buffer and the reading is at 450 nm. 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 point (OIE,2000; Niang *et al*., 2006).

III) Immunoblotting Test: An immune enzymatic test designated the immunoblotting test (IB test) has been developed and is of diagnostic value. A field evaluation indicated a higher sensitivity and specificity than the CF test. A core profiles of antigenic bands present both in experimentally and naturally infected cattle's. 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).

2.7.5. Polymerase chain reaction (PCR): 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 *MmmSC* (OIE, 2008*).* 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 *et al*., 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 (FAO, 2004).

2.8. Treatment: Under practical field conditions, when the disease out breaks in a new area, treatment is not applicable and not recommended because of reasons of disease prevention. Treatment is usually undertaken and indicated only in areas where the disease is endemic but in practice farmers are treating their animals when they have no other alternative. Although the Mycoplasmas are susceptible to a number of antibiotics *in vitro*, treatment failures are common (Radostits *et al*., 2007). 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 tetracyclines, 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 (Nicholas *et al*., 2012).

2.9. 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 (OIE, 2002). The major obstacles to the control and eradication of the CBPP is difficulty in

controlling of animal movements (especially in sub-Saharan Africa), complications of applying quarantine and slaughter policies, lack of rapid pen side diagnostic test, ineffective vaccine and insufficient funds to implement control policies (Radiostits *et al*., 2007). A variety of management options exists when local, national or international authorities face decisions on transboundary animal disease, like CBPP (OIE, 2000).

In African countries with endemic CBPP cannot afford eradication by slaughtering of all infected herds (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). Stumping out, testing and slaughtering infected animal may not prove realistic and quarantine coupled with vaccination is the most frequently used CBPP control measure (Quinn *et al*., 2002). Extensive vaccination programs and chemotherapy are the remaining options for CBPP control in Africa and of these, vaccination still is the preferred method (March, 2004).

2.9.1. Stamping Out: The ideal method to control a transandboundary disease like CBPP is the application of the stamping out policy of complete elimination of infected and exposed animals along with attendant zoo-sanitary measures. This strategy is generally design to for slaughtering of animals during the epidemicity of the disease to reduce the risk of transmission (OIE, 2002; Thrusfield, 2007).

This policy will probably be most important for countries with highly developed livestock industries. It involves the eradication of disease by distraction of all infected animals. It should not be contemplated unless there are adequate provisions for compensation. If there is no any compensation for stumping out, then producers, particularly small scale producers are reluctant to participate and, if they participate it may mean that no longer can afford to produce. In order to avoid decapitalization, small scale producer who rely on solely their animals for income may move their animals across the border rather than killing them, further spreading infection (FAO, 2004).

2.9.2. Test and slaughtering of infected Animals: In eradication campaign, infected animals may be slaughtered to remove source of infection. Eradication of a disease from herds after involves a test and removal strategies, in each all animals are tested and only those positive are removed and slaughtered (Thrusfield, 2007).

2.9.3. Quarantine: Uncontrolled animal movements during transhumance, trade and cattle theft have facilitated the spread of the disease throughout the world. Although the quarantine and checkpoints have been in place, weak legislation and a lack of means and resources to enforce control of livestock movements are making the situation worse (Msami *et al*., 2001). Then this is strategy for isolation of animals that are either infected or suspecting of being so, or of non infected animals that are at risk. It is also important to isolate animals suspected of being infected, until infections is either confirmed or discounted by clinical examination or laboratory testing. Within each quarantine areas, clinical cases are separated and confirmed to a hospitalized zone and such animals are slaughtered under strict veterinary supervision (Thrusfield, 2007).

2.9.4. Vaccination: The first crude vaccine against CBPP was published in 1852 by Louis Willems and consisted of pleural exudates from diseased animals (Egwu *et al*., 1996). Since then, different attenuated live *Mmm*SC strain vaccines have been used and the vaccines currently recommended by the OIE are the live strain vaccines T1/44 and its streptomycin resistant derivate T1-SR (Litamoi, 2000; Thiaucourt *et al*., 2000 and Dedieu-Engelmann, 2008). When the application of the stamping out policy of eradication is not feasible, the control of CBPP has relied on preventive immunoprophylaxis using live attenuated cultures of the causative agent along with restriction of cattle movement if possible (Radiostits *et al*., 2007). Both T1-SR and T1/44 are freeze-dried vaccines that are thermostable until reconstituted, but after reconstitution they have to be used within a short period of time, approximately one hour. This is a serious disadvantage when undertaking vaccination in areas with poor infrastructure and relative small herds (Nicholas *et al*., 2009).

2.10. Economic importance: CBPP 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, trade bans and reduced investment in livestock production (Radiostits *et al*, 2007). In addition to these, it leads tin imposition of rigorous limitations to international trade soon CBPP affected countries in accordance with world organization of Animal Health (OIE) regulations (Bonnet and Lesnoff, 2008; Muuka *et al*., 2011).

The OIE Terrestrial Animal Health Code provides standards for trade in live animals, semen and embryos but specifies that trade in milk and milk products, hides and skins, and meat and meat products other than lung should be free of any restrictions (OIE, 2003). This international recommendation may be over ridden by more stringent national standards. For example, the 1995 outbreak of CBPP in Botswana resulted in loss of access to the Europe market, although the product involved was deboned beef. To regain access as soon as possible Botswana resorted to

mass culling with compensation to the cattle owners. More than 300,000 cattle were destroyed in the affected area and on the southern boundary of which additional veterinary cordon fences were erected with high costs. The massive cull and movement control resulted in eradication of the outbreak; but socio-economic assessments indicated that the effects on the human population were negative and included malnutrition in children who were under the age of five years at the time owing to a total lack of cow's milk. Apart from its negative effects, such an approach would be financially impossible for most African countries (Tambi *et al.*, 2006).

Table 3: Estimated value of annual losses in cattle and cattle products caused by contagious bovine pleuropneumonia in Africa

| Country | Cattle death in number | Value of loss (1,000 Euros) | | |
		Beef (metric tons)	Milk (metric tons)	Drought power (1,000 ox days)
Burkina Faso	1,606	216	1,312	365
Chad	3,335	299	1,927	506
Côte d'Ivoire	930	83	537	141
Ethiopia	10,112	1,350	8,500	1,645
Ghana	474	64	387	108
Guinea	1,395	188	1,140	317
Kenya	3,033	373	2,316	494
Mali	2,606	350	2,129	593
Mauritania	556	75	476	126
Niger	785	106	672	179
Tanzania	4,499	526	3,527	641
Uganda	1,542	180	1,209	220
Total	**30,873**	**3,810**	**24,132**	**5, 33**

Source: (Tambi *et al.*, 2006)

Table 4: Estimated annual economic cost of contagious bovinepleuropneumonia (1,000 Euros)

| Country | Value of production losses | Disease control costs | | Total economic cost |
		Vaccination	Treatment	
Burkina Faso	1,601	660	178	2,439
Chad	2,547	1,333	247	4,126
Côte d'Ivoire	710	377	69	1,156
Ethiopia	10,294	3,597	1,097	14,987
Ghana	480	235	53	768
Kenya	3,437	1,015	329	4,781
Mali	2,598	1,115	289	4,003
Mauritania	565	228	62	855
Niger	798	323	87	1,208
Tanzania	4,256	1,412	488	6,156
Guinea	3,437	549	155	2,095
Uganda	1,459	645	167	2,271
Total	**30,136**	**11,489**	**3,221**	**44,846**

Source: (Tambi *et al.*, 2006)

2.11. Status of CBPP in Ethiopia: In Ethiopia CBPP has been suspected to occur for a long period, especially in areas found at the vicinity of endemic areas of Kenya and Sudan. CBPP has been reported from almost all regions of Ethiopia including Tigray, Afar, SNNP, Gumbela, Oromia, Benishangul-Gumuz, Amhara and Somali regional states. It is more prevalent in the arid and semi-arid low land of rift valley, Gambella, Benishangul-Gumuz and other pastoral areas of Ethiopia (Gulima, 2011).

Table 5: Distribution of CBPP in different regions of Ethiopia

Region	No of zone	No of district	Total sample	Negative	Positive	Prevalence (%)
Afar	3	3	1080	1070	79	7.13
Amhara	9	12	4320	4260	59	1.29
B.Gumuz	2	2	720	633	87	12.05
Gambella	1	2	720	578	142	19.72
Oromia	11	40	7140	6730	410	5.74
SNNP	8	8	2700	253	147	5.44
Somali	2	3	1110	1099	11	0.9
Tigray	2	4	1140	1352	88	6.11
Total	38	54	19230	18210	1020	5.63

Source: (Gulima, 2011)

Table 6: **Prevalence of CBPP in different part of Ethiopia**

Study area	Prevalence (%)	Animal species	Reference
Export quarantine center Adama	0.4	Bovine	Ermias *et al.,* 2014
West Welega	4	Bovine	Fikru, 2001
Shinille and Jijiga (Somali Region)	39	Bovine	Gedlu, 2004
Borena	0.4	Bovine	Gezahegn *et al.,* 2014
Borena	5.1	Bovine	Issa, 2004
Export quarantine center Adama	9.5	Bovine	Kassaye and Delil, 2012
Bishofitu quarantine center	7.8	Bovine	Biruhtesfa *et al*., 2015
Southern part of Tigray	11.5	Bovine	Teshale *et al*., 2015
Bishofitu quarantine center	10	Bovine	Molla and Delil, 2015
West Wellega	28.5	Bovine	Daniel *et al*., 2016

Source**:** veterinary research Journal, DVM thesis and Msc thesis (2001-2015)

3. Conclusions and recommendations: Contagious bovine pleuropneumonia (CBPP) is an acute, subacute or chronic respiratory disease of cattle caused by *Mycoplasma mycoides subspecies mycoides* small colony (MmmSC). Transmission of the disease occurs through direct contact between an infected and a susceptible animal which becomes infected by inhaling droplets disseminated by coughing. The epidemiology of CBPP is dominated by different factors. These are; cattle are the only species affected, transmission is through the direct contact of susceptible animal with clinical cases or chronic carriers and cattle movements play a very important role in the maintenance and extension of the disease. The tools currently available for CBPP diagnosis include clinical signs, identification and isolation of the agent, immunoblotting, serology and PCR techniques. Commonly used antibiotics include tetracyclines, tylosin, erythromycin, lincomycin, spectinomycin, and tilmicosin. 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. The disease has serious implications for food security and peoples' livelihoods in affected countries. The disease is posing a major threat to cattle in many parts of the country thereby causing considerable economic losses through morbidity and mortality. The occurrence of such disease may cause restriction on the trade of animals and animal products internationally, affecting the export earnings of the country, thereby threatening the livelihood of the farmers and national agricultural economy.

Based on the above conclusion the following recommendation was forwarded:

➢ Research in the improvements of vaccine should continue and include the possibility of differentiation between vaccinated and non-vaccinated animals.

➢ Strategic control of CBPP should be progressive and based on impact assessment and cost benefit analysis done with appropriate methods including participatory techniques to cover regional, national, and zone and district level.

➢ A veterinarian should well train to perform diagnosis of the disease through the available diagnostic tools.

➢ Research should be done on the epidemiological situation of the disease.

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