SEROEPIDEMIOLOGY OF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN NORTHERN AND CENTRAL PARTS OF KADUNA STATE, NIGERIA

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By

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SEPTEMBER, 2012

DECLARATION

I declare that the work in this thesis titled "Seroepidemiology of Contagious Bovine Pleuropneumonia in Northern and Central Parts of Kaduna State, Nigeria" was performed by me in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, under the supervision of Dr. M. Bello, Prof. L.T. Zaria and Dr. A.A. Dzikwi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been previously presented for another degree or diploma at any institution.

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CERTIFICATION

This thesis, titled "Seroepidemiology of Contagious Bovine Pleuropneumonia in Northern and Central Parts of Kaduna State, Nigeria" meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

My effort in this work is dedicated to the memory of my late brother, Ibrahim Suleiman, a Batch A 2009 Youth Corps Member and Graduate of Fisheries from Federal University of Technology, Minna, whose sad event took place on Wednesday, July, 29, 2009 following a ghastly motor accident along Pandogari-Birnin Gwari road in Niger State. May Aljannah-al-Firdaus be his final abode, Ameen.

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ABSTRACT

Contagious Bovine Pleuropneumonia (CBPP), a respiratory disease of cattle caused by *Mycoplasma mycoides* subspecie *mycoides* Small Colony (*Mmm*SC) type and transmitted by infectious aerosol inhalation, is at present the most economically important livestock disease in Africa due to its considerable effects on production and rural economy. Nigeria suffers from CBPP directly through deaths of cattle, and indirectly by exclusion from participation in international livestock products trade. Even though past control efforts had reduced the incidence of CBPP in the country, the disease is presently endemic with most outbreaks occurring in the Fulani pastoral herds of the North where most of the cattle are located. CBPP control is particularly difficult in Nigeria due to lack of epidemiological data for sustained control measures which are currently estimated to cost 1.5 million US Dollars annually. This cross-sectional study on 516 animals from 190 proportionately selected herds was designed to determine herd and animal level seroprevalence of CBPP, identify risk factors for seroprevalence at both levels and assess the awareness of cattle owners about the disease in Chukun, Giwa, Igabi, Kaduna South, Sabon Gari and Zaria Local Government Areas of Northern and Central parts of Kaduna state, Nigeria. A competitive enzyme linked immunosorbent assay (cELISA) revealed that 58.4% of the herds had at least one seropositive animal and 50 (45%) of the seropositive herds had 40-59% seropositive cattle. Odds Ratio and 95% CI on the Odds Ratio analyses of the CBPP seroprevalence showed that herd size, livestock contacts (animals brought into herds through gifts, purchase, transfers, borrowing for service and brought back from markets when animals were not reasonably priced), herd dynamics (mixing at grazing and watering points) and reported CBPP outbreaks were positively correlated to seroprevalence. The odds for

having a CBPP seropositive herd were found to be only 0.04 in vaccinated herds relative to their unvaccinated counterparts. Similarly, prompted recall of CBPP clinical signs and control measures based on an 8-point score revealed that the likelihoods of having a seropositive herd significantly decreased with increasing level of awareness about the disease by the corresponding herdsmen. CBPP vaccination efforts, particularly in villages that reported outbreaks of the disease and those with comparatively larger herd sizes in Kaduna state should be intensified as it offers a protective advantage against the infection while correct and relevant animal health enlightenment campaigns on topics of livestock diseases would likely reduce the spread of CBPP in the state. It was also recommended that more grazing and watering facilities should be made available to cattle herds especially in dry season when such points are unlikely to be available and or accessible.

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ABBREVIATIONS

°C	Degree Celsius
µL	Microlitre
Bg	Bovine group
CBPP	Contagious Bovine Pleuropneumonia
CFT	Complement Fixation Test
CFU	Colony Forming Units
CI	Confidence Interval
CIRAD	La Recherché Agronomique Pour Le Devéloppement (Agricultural Research for Development)
DFZ	Disease Free Zone
ECOWAS	Economic Community of West African States
FAO	Food and Agricultural Organisation
FDL	Federal Department of Livestock
g	Gravitation
g/L	Gramme per Litre
HPAI	Highly Pathogenic Avian Influenza
IBT	Immunoblotting Test
IgG1	Immunoglobulin G1

IHC	Immunohistochemistry
ISCOM	Immune Stimulating Complex
JP	Joint Project
Kb	Kilo base pair
KDa	Kilodalton
KDSG	Kaduna State Government
LAT	Latex Agglutination Test
LGA	Local Government Area
mL	Millilitre
MLSA	Multilocus Sequence Analysis
*Mmm*SC	*Mycoplasma mycoides* subspecies *mycoides* Small Colony
NLPD	National Livestock Projects Department
OD	Optical Density
OIE	Office Internationale des Epizooties (World Organisation for Animal Health)
OR	Odds Ratio
PACE	Pan African Programme for the Control of Epizootics
PCR	Polymerase Chain Reaction
PCR-LIF	Polymerase Chain Reaction-Laser Induced Fluorescence

PI	Percentage Inhibition
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
TMB	3,3',5,5'-Tetramethylbenzidine
US	United States of America

CHAPTER 1

INRODUCTION

1.1 BACKGROUND OF THE STUDY

Contagious Bovine Pleuropneumonia (CBPP) caused by *Mycoplasma mycoides* subspecie *mycoides* Small Colony (*Mmm*SC) variant, is a transboundary cattle disease of great economic importance at both herd and national stages (Mtui-Malamsha, 2009). It is the only bacterial disease mentioned in list A (a list of diseases that pose a major threat to animals, thus requiring special international measures) of the Office International des Epizooties (OIE) (Provost *et al.,* 1987; Egwu *et al.,* 1996; Lefevre, 2000). Although CBPP has been eradicated in the United States of America, Europe and some parts of Asia, its re-emergence in Europe in the late 90s suggests that the disease is still a threat to countries that have successfully eradicated it in the past.

CBPP is primarily a lung disease of cattle (*Bos indicus* and *B. taurus*) transmitted through inhalation of infectious aerosols by a susceptible animal (Provost *et al.,* 1987). The main reservoir of *Mmm*SC is cattle, from which the organism has been recovered during both clinical disease (Kusiluka *et al.,* 2000) and after recovery (Windsor and Masiga, 1977a). Active cases are usually the source of new infections and spread through cattle movements (Mariner *et al.,* 2006).

Manifestation of CBPP shows a wide range of severity and signs (Provost *et al.,* 1987). It could vary from persistent coughing and laboured breathing as a result of pneumonia and pleurisy in adult animals to lameness in calves due to arthritis (FAO, 2008). Some animals appear to be resistant to infection by *Mmm*SC and subclinical cases are also common (Mtui-Malamsha, 2009).

CBPP, which was first described in 1550 (Provost *et al.,* 1987), has remained one of the most important cattle diseases in history. In Africa, the disease was first reported in 1854, when an infected bull was imported into South Africa from the Netherlands (Mtui-Malamsha, 2009) and has since then been endemic in large areas of sub-Saharan Africa almost 150 years later with serious implications for cattle production and major constrains to food security in affected countries (Amanfu, 2009).

Presently, CBPP is the most important cattle disease in Nigeria because of its considerable effects on livestock production and rural economy. The country suffers from both direct losses through deaths of cattle, and indirect losses by exclusion from participation in international livestock products trade (Fasanmi, 2003). Prevalence of the disease in Nigeria varies with the diagnostic tests applied and can be as high as 32% based on competitive ELISA (Aliyu *et al.,* 2003).

1.2 STATEMENTS OF RESEARCH PROBLEM

Contagious bovine pleuropneumonia is a major obstacle to cattle production in Africa and is regarded as the most important infectious disease of cattle especially with the recent eradication of rinderpest from the continent (Mariner et *al.,* 2006*)*. Annual losses of up to €30.1 million due to CBPP were estimated in 12 endemic sub-Saharan African countries (Tambi *et al.,* 2006). Losses are not only attributed to mortality and reduction in beef and milk production and draft power but also to reduced fertility and lost market opportunities as a result of trade bans (Rweyemamu and Bankirane, 1996; Karimuribo *et al.,* 1997).

Many countries within the infected zones of East, Central and West Africa have over the last few years reported an increase in the incidence of the disease due mostly to reduced surveillance and control programmes (Amanfu, 2009). In Nigeria for instance,

the number of reported CBPP outbreaks had increased from 4 in 1999 to 31 in 2001 (Fasanmi, 2003).

The recent concentration of available resources on the prevention and control of highly pathogenic avian influenza (HPAI) has also led to decreased efforts in CBPP control activities. It has particularly resulted in reduced funding for vaccination. So also was the termination of externally funded combined rinderpest/CBPP vaccination campaigns. These may have led to the resurgence of the disease in Nigeria and other previously known endemic areas of West Africa (Nawathe, 1992; Amanfu, 2009).

Field based evidence for CBPP prevalence is the pillar upon which efforts on control activities depend (Thomson, 2005). Unfortunately, such activities as field, abattoir and laboratory surveillance have been abandoned. This, in addition to reduced mobility and failure of field veterinary and other extension personnel that should implement cattle movement regulations, may have also contributed to the deteriorating status of the disease in West Africa as manifested by the rising incidence of the disease in the region (Amanfu, 2009).

1.3 JUSTIFICATION OF THE STUDY

Even though the Joint Project 28 (JP 28) eradication programme of the early 70s had significantly reduced the incidence of CBPP in Nigeria, the disease unfortunately regained its previous status due to collapse of essential surveillance systems in the country (Anonymous, 2003).

Previous and present reports on CBPP indicated that the disease is still endemic in the country with most outbreaks occurring in the northern part where most of the cattle population is located (Nwanta and Umoh, 1992; Aliyu *et al.,* 2000; Bello and Ibrahim, 2010; Okaiyeto *et al.,* 2011). More than 70% of cattle in Nigeria are owned by nomadic

Fulanis who move for long distances thereby enhancing the spread of the disease (Aliyu, *et al.,* 2000; Useh *et al.,* 2008).

CBPP control, particularly in Nigeria, appears to be very difficult due to lack of plausible epidemiological data needed for sustained control measures and other eradication strategies such as effective management of cattle movements and mass vaccination. Both of these have serious financial, logistic and technical implications (Thomson, 2005; Amanfu, 2009).

Of the 15.2 million cattle in Nigeria, only about 1.45 million (9.4%) are vaccinated against CBPP annually. This figure translates to an estimated annual expenditure of about 1.5 million US Dollars for CBPP vaccination alone (Thomson, 2005). Overall economic losses due to CBPP in Nigeria were estimated to be in excess of 3.6 million US Dollars (Osiyemi, 1981).

Kaduna state has an estimated cattle population of 1,099,686 (FDL, 2010), the largest in North-western Nigeria. The White Fulani (*Bos indicus*) breed makes up about 90% of this population (Mbuk *et al.,* 2011). Extensive husbandry system, where the pastoralists move around with their animals in search of pastures, enhancing the spread of CBPP, is widely practised. A thorough understanding of the epidemiology of CBPP in the state is therefore justified in order to plan for an effective control of the disease and design a rational plan for sustained control and eventual eradication of CBPP from the country.

1.4 RESEARCH QUESTIONS

What is the prevalence of contagious bovine pleuropneumonia in Kaduna state for its control efforts to be justified?

Are cattle owners in the state informed about CBPP for such programmes to be well accepted?

1.5 AIM

The aim of the study was to determine the herd and animal level seroprevalence of CBPP in Northern and Central parts of Kaduna state

1.6 OBJECTIVES

The objectives of the study were to determine CBPP seroprevalence in cattle herds by competitive Enzyme Linked Immunosorbent Assay (cELISA), assess the level of awareness of herdsmen about CBPP and identify possible risk factors associated with transmission of the disease among herds, through administration of questionnaires to herd owners.

CHAPTER 2

LITERATURE REVIEW

Contagious bovine pleuropneumonia (CBPP), caused by *Mycoplasma mycoides* subsp. *mycoides* biotype Small Colony (*Mmm*SC), is one of the most serious cattle diseases in Africa. 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 (Karimuribo *et al.*, 1997; Rweyemamu and Benkirane, 1996).CBPP is included in the list A of the Office International des Epizooties (OIE), a list of animal diseases requiring official declaration. Public health concerns may also be associated with CBPP because incidence of childhood malnutrition increases in areas experiencing large-scale cattle deaths due to the disease (Boonstra *et al*., 2001).

2.1 AETIOLOGY

2.1.1 General Characteristics and Classification

CBPP is caused by *Mycoplasma mycoides* subspecies *mycoides* Small Colony variant (*Mmm*SC), an extracellular bacterium that lives in close contact with its host cells (Mtui-Malamsha, 2009). This organism of genome size of 1,211 Kb lacks a cell wall and has the ability for self replication (Westberg *et al.,* 2004). *Mmm*SC is a member of the '*Mycoplasma mycoides* cluster', a group of six mycoplasmas with similarities in many biochemical, immunological and genetic characteristics (Petterson *et al.,* 1996). *Mycoplasma mycoides* subspecies *mycoides* Large Colony, *Mycoplasma capricolum* subspecies *capricolum, Mycoplasma capricolum* subspecie *capripneumonia*, *Mycoplasma mycoides* subspecies *capri* and Mycoplasma specie bovine bio group 7 constitute the other members of the cluster.

Only two of these organisms cause disease in large ruminants; viz: *MmmSC* which is the cause of CBPP, and *Mycoplasma* bovine group 7 (Bg 7) which may cause bovine arthritis and bovine mastitis. The remaining four, two subspecies within *Mycoplasma mycoides* species and two subspecies within *Mycoplasma capricolum* species are responsible for goat respiratory and other diseases. *Mmm*SC belongs to the class Mollicutes, Order *Mycoplasmatales* and family *Mycoplasmataceae* which contains two genera, the urea negative *Mycoplasma* and urea positive *Ureaplasma*. Sequence analyses and phylogenetic studies of the 16S ribosomal RNA genes showed that mycoplasmas are closely related to the Bacillus-Lactobacillus-Streptococcus group, suggesting that they may have evolved from Gram positive bacteria (Weisnburg *et al.,* 1989).

Recently described multi-locus sequence analysis (MLSA) distinguishes three main lineages of *Mmm*SC which correlate with their geographical origins (Europe, Southern Africa and rest of Africa). The strains of European origin can be differentiated from African ones by molecular methods, and are not able to oxidise glycerol, which may account for an apparent lower pathogenicity. African strains seem to be more diverse. The sequence of the complete genome of the reference strain PG1 has been published (Vilei and Frey, 2001).

Due to absence of cell walls, Mycoplasmas are therefore pleomorphic and resistant to antibiotics of the beta lactamine group, such as penicillin. Growth of mycoplasma is relatively fastidious and requires special media rich in cholesterol with addition of horse serum (OIE, 2008).

2.1.2 Resistance to Physical and Chemical Action

*Mmm*SC does not survive for long periods in the environment and transmission often involves close contact, though, under favourable atmospheric conditions of humidity and wind, aerosols can transport the organism over longer distances (OIE, 2008).

Temperature: Inactivated in 60 minutes at 56°C or 2 minutes at 60°C

Chemicals/Disinfectants: Inactivated by many of the routinely used disinfectants such as 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. *Mmm*SC may survive more than 10 years frozen (OIE, 2008).

2.1.3 Host Specificity

Under natural conditions, *Mmm*SC is found in cattle of the species *Bos* and allied animals including buffalo, yak, bison and even reindeer (Hutyra *et al.,* 1938). Experimental work in Australia showed that buffaloes could be infected by artificial means but did not spread CBPP to in-contact buffaloes (Newton, 1992). However, Santini *et al.* (1992) reported pulmonary lesions and isolated *MmmSC* from seropositive buffaloes which had been in contact with CBPP-affected cattle in Italy. Buffaloes are susceptible although at a low level and further research would be necessary to clarify the role of buffaloes as a reservoir of infection for cattle (Santini *et al.,* 1992).

Small ruminants, especially goats, have also been reported to harbour *Mmm*SC (Hudson *et al.,* 1971; Egwu *et al.,* 2012). *Mmm*SC had been isolated from milk of sheep with mastitis and goats with pneumonia in Europe and Africa (Brandao 1995; Egwu *et al.,* 2012). However, experimental studies on goats that were in contact with cattle infected by the African strain of *Mmm*SC (Afadé), which is genetically different from European

strains, suggested that goats were not susceptible to CBPP (Belli *et al.,* 1990, Cheng *et al.,* 1995).

2.2 PATHOGENESIS AND IMMUNOPATHOLOGY

Contagious Bovine Pleuropneumonia is often associated with an inconspicuous and insidious nature which could lead to an underestimation of the threat. CBPP cannot be easily reproduced under experimental conditions. Vaccines currently in use against CBPP do not confer a satisfactory immunity and little is known about the type of immunity they confer and generally about the immunopathology of the disease (Dedieu, 2008; Mtui-Malamsha, 2009).

The disease is associated with pneumonia and sero-fibrinous pleurisy. Transmission by direct close contact with animals excreting droplets by coughing may be facilitated by favourable winds over distances of few kilometres (Abdo *et al*., 2000). The danger of CBPP spread can be increased by the fact that the disease has a prolonged incubation period (three weeks to six months) and may occur in an asymptomatic form in about 20% of infected cattle (Masiga *et al.,* 1996).

Pathological changes in CBPP are characterised by the involvement of mostly one lung and its gross macroscopic lesions are clearly known and are used for disease surveillance at abattoirs. However, mechanisms of the disease process are not well understood. It has been suggested that autoimmune reactions are essential for lesions to develop. The immunological mechanism involved during infection and ability of the pathogen to evade the immune system need also to be elucidated (Ayling *et al*., 1999a). It is also unclear how the different and sometimes variably severe pathological lesions observed in disease affected cattle are produced. This may partly be due to the fact that target animal experiments are very expensive while a valid laboratory animal model is

not available. This lack of knowledge has consequences for *in vitro* diagnostic tests, assessing immune response and for development of an improved CBPP vaccine.

Not much is known about the different immunogenic components of *Mmm*SC during CBPP infection. Several major proteins of *Mmm*SC appear to be specifically recognised by antibodies from cattle with CBPP, and not by those from cattle infected with other members of the *M. Mycoides* cluster (Dedieu, 2008). Recent reports have shown that a surface exposed lipoprotein of 72 kDa (named P 72) was identified and characterised; this lipoprotein was observed to induce an early and persistent humoral immune response in cattle with CBPP (Chambaud *et al.,* 1999; Luhrmann *et al*., 2002).

Western blot techniques have also identified major immunogenic antigens for different strains (Abdo *et al*., 1998). Bronchial immune responses (IgA) were shown to be strong but seemed to be directed to fewer antigens than those in serum samples and directed to antigens assumed to be surface exposed lipoproteins (Abdo *et al*., 1998). Some lipoproteins from other mycoplasmas may play an important role in virulence (Kostyal *et al*., 1994; Rawadi and Romanroman, 1996). Similarly, un-adjuvanted and Immune Stimulating Complexes (ISCOM) incorporated *MmmSC* membranes were observed to induce pro-inflammatory cytokines (Abusugra *et al*., 2001).

The role of capsular galactan in inducing pathological lesions has also been suggested and the biochemical link between capsular galactan and pneumogalactan has been suspected to be the cause of autoimmune reactions. Precipitation of immunocomplexes, observed around lung capillaries, seems to support the theory of autoimmune reactions as components of the pathology of CBPP (Dedieu, 2008).

The insidious and inconspicuous nature of CBPP, the variable individual susceptibility of cattle, the pathological changes generally characterised by the involvement of only one lung and its gross macroscopic aspects, the inability of the traditionally

manufactured vaccines to induce protective immunity, are some of the problems to be faced in designing any CBPP control programme. The obvious but mostly controversial difference in pathogenicity between European and African strains is associated with the ability of the later to oxidise glycerol and produce hydrogen peroxide (Nicholas *et al*., 2000).

2.3 MODE OF TRANSMISSION

CBPP is primarily a disease of cattle (*Bos indicus* and *B. taurus*) without any apparent wildlife reservoir (Provost *et al*., 1987), although one single case has been reported in American buffaloes (*Bison bison*) and not in African buffaloes (*Syncerus caffer*) or other wild ruminants (OIE, 2008). Direct contact has been described as the principal mode of transmission. This is by way of inhalation of infectious aerosols by a susceptible animal. Alternative routes such as wind-borne and indirect transmission cannot be excluded (Masiga *et al.,* 1996; Regalla *et al.,* 1996a).

The main reservoir of *Mmm*SC infection is cattle, from which the organism can be isolated from both clinical disease (Windsor and Masiga, 1977b; Kusiluka *et al*., 2000). The bacteria have been isolated from nasal discharges and lung sequestra. Isolation of *Mmm*SC from the foetus of an infected dam has been reported, suggesting a possible trans-placental transmission (Stone *et al*., 1969; Masiga *et al*., 1972). *Mmm*SC has also been isolated from urine of cattle in acute phase of CBPP (Scudamore, 1976). In addition, the recent isolations of *Mmm*SC strains from sheep and goats at different times and places, including CBPP-free countries (Brandão, 1995; Regalla *et al*., 1996; Srivastava *et al*., 2000; Kusiluka *et al*., 2001; Egwu *et al.,* 2012) suggested that non-bovine ruminants should also be included in surveillance programmes as potential reservoirs.

The organism has also been reported to be isolated from semen and prepucial washings of infected bulls (Gonçalves, 1994). The roles of infected urine and semen in the transmission of CBPP remain largely unclear (Mtui-Malamsha, 2009), although transmission by fomites and contaminated feed has been reported under experimental conditions (Windsor and Masiga, 1977a). Water buffaloes were shown to be susceptible to CBPP, but transmission of CBPP from buffaloes to cattle has never been reported (OIE, 2008).

2.4 CLINICAL MANIFESTATION

Severity of signs observed in cattle affected by CBPP varies considerably. In natural disease, incubation period may vary from 3 to 24 weeks (Provost *et al.,* 1987; Masiga *et al*., 1996). In an experimental infection via bronchial intubation, clinical signs and serological responses were observed from 40 days after contact with intubated animals (Regalla *et al.,* 1996b). In a naive herd, CBPP usually results in high morbidity (100%) and almost 50% mortality (Masiga *et al.*, 1996). Relative to *Mmm*SC isolated from African and Australian CBPP outbreaks, *Mmm*SC isolated from CBPP cases in Europe have been reported to be less pathogenic (Nicholas *et al.*, 1996; Pilo *et al.*, 2007). The clinical signs of CBPP may take a variety of forms, depending on host susceptibility and virulence of the pathogen.

2.4.1 Peracute form

Clinical signs observed in this form of CBPP are much accelerated. Pathological lesions of the pulmonary tissues are usually characterized by pronounced pleural adhesion accompanied by exudative pericarditis (Provost *et al.,* 1987). Death of affected animals occurs within a week of exhibiting classical respiratory signs.

2.4.2 Acute form

The early stages of CBPP are indistinguishable from any severe pneumonia with pleurisy (Mtui-Malamsha, 2009). Animals appear dull, anorexic and feverish with irregular rumination and signs of respiratory disease. Coughing is usually persistent and is slight or dry. In severe cases, fever goes up to 40 – 42°C, and the animal becomes recumbent with difficulty of movement.

The clinical signs become more pronounced as typical lung lesions develop with increased frequency of coughing and the animal stands with the back arched, head extended and elbows abducted. In calves, classical respiratory signs may be evident but localisation of *Mmm*SC in joint capsules results in lameness due to arthritis. Complications in calves may also include valvular endocarditis and myocarditis (Martel *et al.,* 1983).

2.4.3 Subacute/chronic form

In the subacute form, clinical signs are often limited to a mild cough evident only when the animal is exercised. CBPP in Africa, unlike in Europe where mortality rates are typically 0-10%, is characterised by high morbidity (75-90%) and mortality (10-70%) with many of infected cattle showing chronic lesions (Masiga *et al.,* 1996; Regalla *et al.,* 1996b).

These differences may be due to the fact that European cattle are healthier in general, better fed, less stressed, and are mostly permanently housed throughout the year and usually experience strains of lower virulence than the African (Abdo *et al.,* 1998). The use of antibiotics and anti-inflammatory drugs may help mask clinical signs and accelerate the formation of chronic lesions. In Africa, up to a third of cases that recover from acute disease become potential carriers. This figure is probably higher in Europe where there is far more widespread use of antimicrobials (Abdo *et al.,* 1998).

2.5 DIAGNOSES

Diagnosis of CBPP is based on a combination of history of contact with an infected animal or herd, physical examination, post-mortem findings and laboratory examination which relies on cultural methods and serological tests (FAO, 2008). Protein and nucleic acid-based molecular techniques are also utilized (Gonçalves, 1994; Bashiruddin *et al.*, 1999a).

2.5.1 Clinical signs

Clinical diagnosis of CBPP can be achieved during the acute phase of the disease particularly if signs are typical. However, no clinical signs are pathognomonic for CBPP (Mtui-Malamsha, 2009). Therefore, it is important to eliminate other diseases presenting similar signs from the differential diagnosis. Such diseases include acute bovine pasteurellosis, haemorrhagic septicaemia, acute East Coast fever (theileriosis), traumatic pericarditis, bovine ephemeral fever in acute forms of the disease and ecchinococcosis (hydatid cyst), actinobacillosis, abscesses, tuberculosis or bovine farcy in chronic CBPP (FAO, 2008).

2.5.2 Postmortem Lesions

Gross pathological lesions in the acute stage are typified by fibrinous exudates and deposits on the parietal surfaces of lungs in which distended interlobular spaces are filled with the straw coloured serofibrinous exudates (Trichard *et al.*, 1981). In majority of cases, lesions are usually unilateral, restricted to the diaphragmatic lobe and present a distinct marbling appearance (Masiga *et al.*, 1996; Provost *et al.*, 1987). Structural changes are detectable on palpation. If incised, regions of red and grey hepatisation are revealed.

In subacute cases, pathologic changes are characterised by necrosis organized within lobules and interlobular septa and early sequestrum formation (Provost *et al.*, 1987).

Typical lesions in the chronic stage of CBPP include well-defined sequestra surrounded by fibrous capsules and adhesions which connect the thickened viscera to the parietal pleura (Trichard *et al.*, 1981; Nicholas *et al.*, 1996; FAO, 2008).

2.5.3 Histopathology

Microscopically, the earliest pulmonary lesions consist of foci of catarrhal bronchiolitis, with distension of the lymphatics in the interlobular septa and thickened alveolar walls (FAO, 2012). Soon after, blood vessels and lymphatics become thrombosed, and alveoli are filled with fluid and cells (alveolar macrophages and sometimes polymorphonuclear leucocytes). There is proliferation of the cells in lymphatic follicles and an increase in the population of mononuclear cells (FAO, 2012). The detection of these lesions is an important factor in identifying cattle infected subclinically. The "organising centres" observed in the interlobular septa of lungs with lesions are considered pathognomonic for CBPP (Ferronha *et al.,* 1990; Di Francesco *et al.,* 1998).

2.5.4 Detection and Identification of *Mmm*SC

2.5.4.1 Culture

Sample collection for culture: Samples from the living animal include nasal swabs, secretions, tracheal, bronchoalveolar washings and pleural fluid. Occasionally, blood, urine and synovial fluid may be obtained (FAO, 2008). Sample from the dead animal include pleural fluid, portions of affected lungs and lung sequestra (scrapings from inside the capsule) and lung-associated lymph nodes, and kidneys.

Tissues transportation should be carried out in insulated containers at temperatures between 0 and +4ºC, and must reach the laboratory within 48 hours post collection. For a period of time over 48 hours before dispatch, the tissues must be frozen at –20ºC even though some loss of viability of the organism occurs. Swabs should be inoculated onto suitable medium (such as pleuropneumonia like organism; PPLO broth) and refrigerated

between 0 and +4ºC, to arrive at the laboratory within 48 hours after collection. These conditions are very important and often overlooked in the success of mycoplasma isolation particularly in hot climates (FAO, 2008).

Isolation and transport media: Isolation media for *Mmm*SC are also used as transport media and are based on conventional growth media with the addition of inhibitors to stop the growth of cell-walled bacteria (FAO, 2008). The maximum concentrations of inhibitors, allowing normal growth of *Mmm*SC (i.e. having no effect on cell yield or growth rate) in a medium are: ampicillin, 1.0 g/L; bacitracin, 0.2 g/L; penicillin G, 0.05 g/L; polymyxin B, 0.05 g/L and thallium acetate 0.5 g/L. These inhibitors may be combined to produce a highly selective medium (FAO, 2008).

Growth media: Unlike many other fastidious mycoplasmas such as *Mycoplasm capricolum* subspecies *capripneumonia*, *Mmm*SC is not intrinsically difficult to grow but needs a fully functioning bacteriological laboratory with access to special mycoplasma media. Many media have been described which enable the growth of *Mmm*SC (e.g., Hudson, 1971; Freundt, 1983). A medium (PRM, named after the developers) specifically made to give optimum growth and yield of small colony strains, has been described by Nicholas *et al.* (2000). Pyruvate is included in the medium as a supplementary source of energy to glucose and increases the growth rate.

The growth of *Mmm*SC normally takes up to 10 days. In specific culture media (agar and broth), growth is visible within 3–10 days as a homogeneous cloudiness with whirls when shaken; on agar, small colonies develop, 1 mm in diameter, with the classical 'fried-egg' appearance. *Mmm*SC is then identified routinely with immunological tests (growth inhibition, immunofluorescence or dot immunobinding on a membrane filter [MF-dot] test). Definitive identification is best done by an OIE Reference Laboratory (OIE, 2008), using biochemical tests combined with immunological assays.

2.5.4.2 Immunobinding

Immunobinding assays are one of the most reliable tests for routine identification of *Mycoplasma* species isolated from clinical materials (Poumarat, 1998). These tests are based on the detection of *Mycoplasma* surface antigens which are known to be highly specific. However, sensitivity and specificity can be affected in certain circumstances. Because the number of *Mycoplasma* species is increasing, each isolate must be tested with several sera for definitive identification. Immunobinding assays involving mycoplasma colonies or imprints of colonies are becoming highly laborious. In addition, immunobinding assays with broth culture bound onto nitrocellulose paper can be affected by a high level of background staining (Poumarat, 1998).

2.5.4.3 Immunohistochemistry

Immunoperoxidase has been shown to be a robust test in the diagnosis of CBPP, especially where *Mmm*SC, cannot be recovered, for instance, due to long transport distances, where the animal died of acute disease or where serology cannot be performed and or is inconclusive (Ferronha *et al.,* 1990; Scanziani *et al.,*, 1997). Sensitivity of this test using polyclonal serum can be low and non-specific results have been shown to occur frequently (Bashiruddin *et al.,* 1999b).

Ayling *et al.,* (1998) have described a monoclonal antibody, M92/20, for use in IHC confirmation of suspected CBPP cases. This monoclonal antibody shows some cross reactivity with other *Mycoplasma*s from the "*M. Mycoides* cluster". IHC is the test of choice when the diagnostic laboratory is presented with a carcass in which lung lesions are suggestive of CBPP while serum is not available and mycoplasma culture from lung is unreliable. Samples are taken from lung, lymph nodes, or lung tissues with suspected PM lesions, fresh or even already formalin fixed and embedded in paraffin wax (Ayling *et al.,* 1998).

2.5.5 Detection and Quantification of Antibodies

Antigenic cross-reactivity among *Mycoplasma* species, especially those of the "*M. Mycoides* cluster", has been widely observed, raising practical problems for CBPP serodiagnosis (Mtui-Malamsha, 2009). The first conventional test described is the complement fixation test (OIE, 2008).

2.5.5.1 Complement fixation test (CFT)

The CFT, the approved OIE test, is specific but lacks sensitivity (Regalla, 1995). With a positive result being any reaction at 1/10 or higher, the CFT is also not robust. In addition, it requires highly trained staff to perform it accurately and consistently (Regalla, 1995). In a thorough examination of the CFT in which over 33,000 sera from healthy herds were tested between 1991 and 1994 in Italy, Bellini *et al.*(1998) reported that the CFT was 98% specific while its sensitivity, based on 600 cattle with specific lesions from 11 infected herds, was about 64%. It then means that by using the CFT as a screening test, some CBPP affected cattle or herds, in the early or later stages of infection, are most likely to be missed.

2.5.5.2 Latex Agglutination Test (LAT)

The latex agglutination test gives results in less than two minutes, using sera or whole blood. This test was developed for screening cattle in the field (Ayling *et al.,* 1999a). The test uses a "specific" polysaccharide antigen extracted from the *Mmm*SC capsule, which is then bound to latex beads ("specific"means: found to be specific by empirical means, testing it against different antigens until the one was found that did not cross-react or gave false-positive results). LAT is as sensitive as the internationally recognised complement fixation test, but is far simpler and more rapid to perform. This test may have high potential in parts of Africa where there are long distances between the

outbreaks locations which in most instances nomadic herds and diagnostic laboratories, enabling control measures to be implemented rapidly (Ayling *et al.,* 1999a).

2.5.5.3 Enzyme Linked Immunosorbent Assays (ELISAs)

Indirect ELISAs have been described for CBPP in the past using crude antigens (Onoviran and Taylor-Robinson, 1979; Le Goff, 1986; Poumarat *et al.,* 1989; Nicholas *et al.,* 1996). These assays all seemed sensitive but may lack specificity. A blocking ELISA, with a monoclonal antibody which was designed to target *Mmm*SC but recognised all members of the cluster, was subsequently developed for detecting antibodies in cattle during the Italian CBPP eradication campaign (Mia *et al.,* 1993). There was 96% agreement between the test and CFT on over 300 positive and negative sera. Nevertheless, a small number of sera from apparently healthy herds were positive in the CFT but gave negative results in the ELISA and in both pathological and cultural examinations. Brocchi *et al.* (1993) reported monoclonal antibodies which recognized a 70kDa protein from the PG1 strain of *Mmm*SC but did not react with the type strains of other members of the *M. Mycoides* cluster.

Competitive(c) ELISAs using monoclonal antibodies were more sensitive than the CFT for detecting antibodies in affected herds and were generally more specific when used in negative herds (OIE, 2008). A cELISA was developed by Le Goff and Thiaucourt (1998) and validated in Africa by Thiaucourt *et al.* (1999). This test utilizes a mouse monoclonal antibody IgG1 that recognises an epitope located on a protein with an apparent molecular weight of 80 kDa.

This test has considerable advantages in terms of ease of testing and standardisation of results and has a sensitivity level similar to that of CFT (Thiaucourt *et al.,* 1999). However, more sensitive techniques, such as ELISAs that utilize better antigens thus

allowing mass screening of herds are necessary. For confirmatory diagnosis of CBPP, this test should be backed by highly specific tests such as immunoblotting tests.

A Tween 20 treated *Mmm*SC cell fraction shows increased specificity in the indirect ELISA compared to conventional whole cell antigens and at the same time maintaining sensitivity (Ayling *et al.*, 1999b). In addition, it was shown that incubation times could be reduced thus, allowing a rapid generation of results without necessarily interfering with the quality of the information. In addition, a study with 88 cattle that show CBPP lesions, the ELISA detected antibodies in 92%, IBT detected 91% and CFT only 82% (Ayling *et al.*, 1999b).

Research on the development of new tests and improvement of existing ones is clearly needed because significant numbers of infected cattle still remain seronegative in most tests: about 8% of field sera from CBPP affected cattle are negative, even when using the most sensitive tests like IBT and ELISAs (OIE, 2008).

2.5.5.4 Immunoblotting test (IBT)

Western (immuno) blotting analysis has been applied with success to the evaluation of antibody response in a number of diseases. IBT allows the analysis of the host humoral immune response in relation to the electrophoretic profile of *Mmm*SC antigens, thus solving the problems associated with non-specific binding in other immunoassays. Studies on the immunogenicity of *Mmm*SC have led to the development of an immunoblotting assay for the detection of antibody responses in sera from CBPP infected cattle (Regalla *et al.,* 1996b). An immunological pattern composed of a combination of antigens that have immunodominant properties and apparent molecular weights of 110 (p110), 98 (p98), 95 (p95), 62/60 (p62/60) and 48 (p48) kDa was identified in a preliminary analysis with sera from both experimentally and naturally infected cattle (Regalla *et al.,* 1996b). Detection of the core profile from CBPP positive

animals only confirmed the immunodominance of these five proteins and indicated the longevity of the specific response which was present in these animals regardless of the stage of the disease (Regalla *et al.,* 1996b).

2.5.6 Polymerase Chain Reaction (PCR)

PCR is a rapid and sensitive diagnostic technique and allows direct detection of *Mmm*SC in clinical samples such as lungs, bronchial lymph nodes, nasal swabs, pleural fluid and blood. Pre-incubation for 24 hours of clinical specimens in growth medium has been reported to increase sensitivity of PCR (De Santis *et al.,* 1997). If used for the identification of new isolates it reduces drastically the time required which is 24-48 hours compared to 2-3 weeks. The test was used as a diagnostic technique to detect *Mmm*SC during the course of 1990s European CBPP outbreaks (Bashiruddin *et al.,* 1999a).

Detection of the *Mmm*SC from bovine samples is one way to confirm a suspected CBPP case but isolation and serological or biochemical identification tests are time-consuming which could lead to delays. To solve this problem, both single and nested PCR systems have been described for identification of *Mmm*SC (Bashiruddin *et al.,* 1999a; Dedieu *et al.,* 2010). Primers are designed based on the sequence of CAP gene of *M. Capri* to amplify a 0.5 kb region of *Mmm*SC, *Mmm*LC and *M. capri* but do not amplify DNA from other members of the cluster. Identification is carried out by restriction fragment analysis of the amplicons with *Asn*I (Bashiruddin *et al.,* 1994).

Nested PCR is more sensitive than conventional PCR (Miserez *et al.,* 1997). Both reactions were highly specific to *Mmm*SC. Two nested pairs of oligonucleotide primers which were designed by evaluation of the most heterologous segments between the P72 gene of *Mmm*SC and the closely related lipoprotein P67 gene of *Mycopalsma* sp. bovine group 7 (PG50) have been used by Miserez *et al.* (1997). Based on the 16S rRNA

genes, two PCR techniques which are highly sensitive and specific have also been developed to identify *Mmm*SC from clinical samples (Johansson *et al.,* 1998; Persson *et al.,* 1999). Both systems amplify the genes from all members of the *M mycoides* cluster. In one of the systems, restriction digest analysis with *Asn*I is done for the final identification. The other system (PCR-Laser Induced Fluorescence, PCR-LIF) which is based on a sequence length difference between the two 16S rRNA genes of *Mmm*SC utilizes fragment analysis of fluorescent labelled amplicons with a DNA sequencer for the final identification of *Mmm*SC (Persson *et al.,* 1999).

Investigation of outbreaks of CBPP mostly requires analysis of large numbers of samples to detect the occurrence of *Mmm*SC. The traditional method for the analysis of PCR products is agarose gel electrophoresis which is useful if a small number of samples are involved but colorimetric PCR has overcome the problem. In this method, specific dsDNA produced by PCR is captured and detected enzymatically in a 96 well microtitre plate (Bashiruddin *et al.,* 1999a). Results of this PCR product correlate well with those obtained by gel electrophoresis. In this way, analysis time is reduced by 50%. In addition, colorimetric PCR allows the use of standard equipment available for ELISA serology. A comparatively large number of samples can also be handled by PCR-LIF (Persson *et al.,* 1999).

2.6 CBPP CONTROL

Contagious bovine pleuropneumonia has been eradicated in the USA, Japan and Western Europe by stamping out and strict animal movement control (Provost *et al.*, 1987; Masiga and Domenech, 1995). In Africa, Botswana has also been able to eradicate the disease through stamping out (Amanfu *et al.*, 1998). However, stamping out may not be economically possible in sub-Saharan Africa. Vaccination based on T1/44 live attenuated *Mmm*SC and animal movement control are the most commonly

used control strategies. Vaccination is only effective if it is carried out repeatedly, initially at short intervals and later annually for 3 to 5 years because the duration of immunity offered by this vaccine is rather short (FAO, 2008).

For eradication purposes, slaughter and disposal of all affected and exposed animals will be appropriate, with strict quarantine (Mtui-Malamsha, 2009). However, eradication programs are difficult to accomplish, especially in the context of nomadic livestock production systems. Therefore, intervention strategies to deal with CBPP and other diseases in the country are increasingly focused on control.

Antibiotic therapy, particularly with tylosin and oxytetracycline is widely practised by farmers to treat CBPP and it is unclear whether the dosages that cattle receive result in plasma concentrations consistent with the desired control and whether withdrawal periods are observed (Danbirni *et al.,* 2010; Tambuwal *et al.,* 2011). CBPP chemotherapy is not part of any official control strategy because of the fear that use of antibiotics may promote developments of sequestra, increase the number of carrier animals, facilitate development of resistant strains and conceal the occurrence of clinical disease (Provost *et al.*, 1987; OIE, 2008). This makes disease diagnosis difficult and often contributes to unrecognized infections and CBPP transmission. Nevertheless, antibiotics are widely used in Fulani communities (Mariner *et al.*, 2006). At a meeting of international experts organized by FAO in 2003 it was recommended that chemotherapy be reconsidered for CBPP control (cited by Mtui-Malamsha, 2009).

2.7 CBPP IN NIGERIA AND KADUNA STATE

In 2003, the Nigeria Animal Diseases Information System under the Pan-African Programme for the Control of Epizootics (PACE) classified Nigeria as an endangered zone based on the country's CBPP status (Fadiga *et al.,* 2011). North-western part of the country had been divided into exposed (comprising Kaduna, Kano, Katsina and Jigawa

States) and enzootic (Zamfara, Sokoto and Kebbi States) zones (Fiure 2.1) (Fadiga *et al.,* 2011).

2.7.1 Prevalence

Reported prevalence of CBPP rates in Nigeria vary with sample size, type as well as diagnostic methods. In Kaduna state, Danbirni *et al.* (2010) reported a within herd prevalence of 47% using Latex Agglutination Test while in a retrospective abattoir study based on records of lungs condemned at post mortem inspection due to lesions suggestive of CBPP at Kano abattoir in Kano state, Fayomi and Aliyu (1997) reported a prevalence rate of 0.21% during a five-year period (1985-89). Another abattoir-based survey at the Sokoto Metropolitan Abattoir conducted between 1990 and 1994 reported a CBPP prevalence of 2.1% (Onu, 2004). From 1988 to 1997 Aliyu *et al*. (2000) found the prevalence of post-mortem CBPP-like lung lesions from 1,936,015 slaughtered cattle in 81 slaughterhouses from five states in Northern Nigeria to be 0.29%. In the report, CBPP prevalence varies between states, but there was no much difference between years (Aliyu *et al*., 2000).

Even though some of the studies reported lower prevalences, it must be noted that in most of the abattoirs in Nigeria, organs (lungs) are only condemned when they are 'so badly damaged', thus lungs with obvious lesions of CBPP which did not completely involve the whole lungs could have missed being condemned (Aliyu *et al.,* 2003).

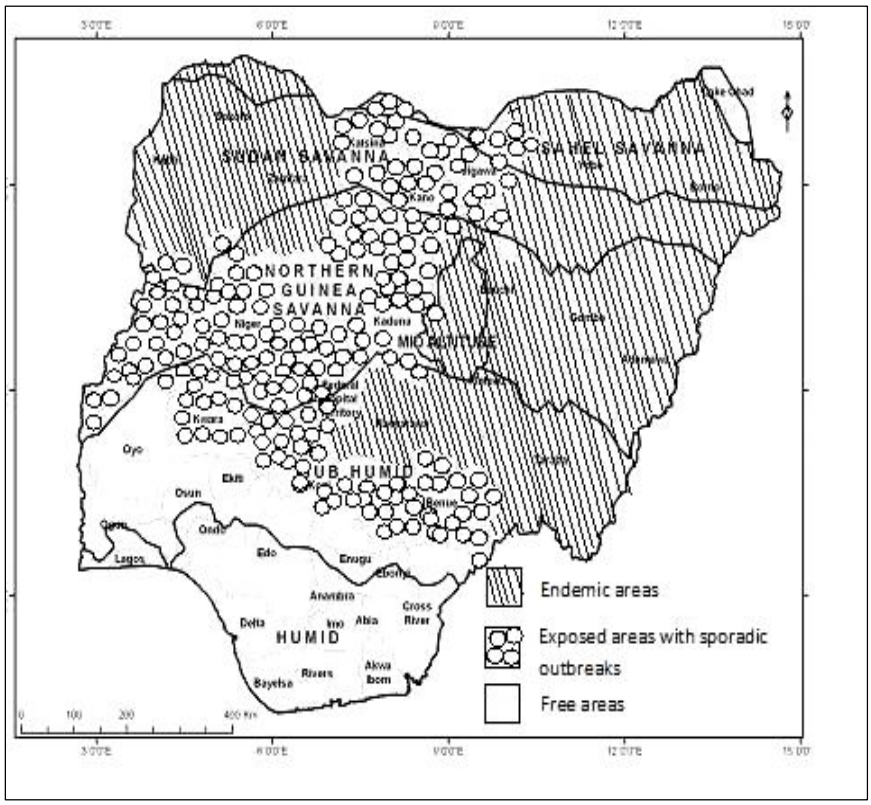


Figure 2.1: Map of Nigeria showing the distribution of contagious bovine pleuropneumonia

Source; Fadiga *et al.* (2011)

2.7.2 Socio-economic Impacts

A spreadsheet model of the economic impact of CBPP which included several of Nigeria's neighbours reported the Benefit-Cost Ratio of CBPP control to be 1.91 in Burkina Faso, 1.61 in Chad and 1.95 in Niger (Tambi *et al*., 2006). However, vaccination and treatment were the only control measures considered in the model. Therefore, it may have likely underestimated the costs of CBPP as other control measures like chemotherapy were being used in the study areas. Another study on the prevalence of CBPP in only five of the Northern states of Nigeria showed that an average of 30 outbreaks were reported annually from all the states, and based on available data the annual economic loss due to CBPP in the study area was estimated at $3.2 million (Aliyu 2002).

Based on direct and indirect financial burden of inaction including costs of death of animals, weight loss, decreased milk production, draught power and treatment during illness and costs of targeted interventions including treatment, vaccination, surveillance and sanitary measures, the Integrated Animal and Human Health Management Project reported the national CBPP burden to be in excess of $12 million (□ 1.9 billion) (Fadiga *et al.,* 2011).

It is very difficult to fully evaluate the economic impacts of the disease in Kaduna state and other parts of the country where the disease is endemic due to lack of accurate data on the disease situation. In addition, available data on the impact of the disease is limited to incidence rather than the effects on livelihoods (Aliyu *et al.,* 2000). Complete socio-economic impacts of CBPP can only be appraised if the disease effects on such social parameters as Fulani migration and conflicts rates, number of animals

Table 2.1: Estimated physical losses caused by contagious bovine pleuropneumonia in Sudan Savannah (North-Western) zone of Nigeria

Losses	**Quantity**
Cattle deaths (Head)	1568
Loss of beef (Tonnes)	111
Loss of milk (Tonnes)	694
Loss of draught power (Thousand Ox-Days)	61

Source; Fadiga *et al.* (2011)

sold for CBPP treatment, stigma, social pressure and discrimination against access to drinking or grazing facilities are clearly understood.

2.7.3 CBPP Control Efforts in Nigeria

In the past, Nigeria had made several efforts to control CBPP. These included the Joint Project (JP 28) of 1960s and the 3-Year National CBPP Programme in the early 90s (Fasanmi, 2003). Under the JP 28, control of CBPP was undertaken through a policy of compulsory vaccination in the endemic parts of the country. This was followed by test and slaughter of affected animals. In the 1970s, refusal of herdsmen to allow vaccination of their animals due to post-vaccination reactions experienced then was a major obstacle to the successful implementation of the project. Later, efforts were directed on disease reporting, slaughter and compensation for infected animals in addition to vaccination of herds at risk. Successes recorded were however not encouraging.

A National CBPP Programme was later introduced in 1992 by the Federal Government of Nigeria but was terminated owing to lack of funding (Anonymous, 2003). The failure of past efforts by governments to achieve desired objectives have been ascribed to lack of sustainability especially in relation to continuous and sufficient funding, post-vaccination reactions encountered in some animals with attendant refusal of most pastoralists to present their animals for further vaccination, inability of governments to adequately compensate for condemned animals or parts thereof and inadequate policy framework on livestock disease control in the country (Fasanmi, 2003).

The new CBPP control strategy put in place by the government in 2001 comprised of both containment and eradication phases. The containment phase was aimed at reducing the incidence of CBPP to about 10% through 90% vaccination of the national herd. As at 2005, vaccination coverage stood at 9.4% (Thomson, 2005). This phase involved

compulsory annual vaccination of cattle for a five year period, control of cattle movement and the introduction of ECOWAS transhumance certificates, strengthening of epidemiological surveillance networks which is being carried out through Nigerian component of PACE, as well as improving the epidemiological and economic knowledge of CBPP in the country.

The next phase envisages total eradication of CBPP from 10% infection rate to zero and involves compulsory test, slaughter with compensation in designated areas to be classified as Disease Free Zones (DFZ). Actions to be taken in CBPP free zones include establishment of sanitary cordon (buffer zone and sero-surveillance zone) to protect the DFZ through active surveillance in the DFZ and surveillance zones, mass vaccination of cattle in the buffer zones and implementation of strict procedures for admittance of animal within the DFZ (Fasanmi, 2003).

2.8 THE FULANI HERDSMEN IN KADUNA STATE

The Fulani, custodians of the national cattle herd, are an ethnic group of West African pastoralists. They move over long distances and have infiltrated many cultures. They are known by different names in different parts of West Africa; they are called Peul in Wolof, Fula in Bambara, Fulaata in Kanuri, and Fulani in Hausa. The word Fulbe was first used by the German writers to refer to the Fulani (de St Croix, 1945).

More than eighty percent of people in Kaduna state and perhaps many parts of the country depend on the Fulani for meat, milk, cheese, honey, butter, manure, animal blood and bone, and hides and skins. In the villages, the Fulani provide the bulls used for carting, ploughing, and hauling (Ibrahim, 1984). Many Nigerians either totally or partly make a living from selling, milking, butchering, or transporting cattle. The

government earns revenue from cattle trades. The Fulani, therefore, play an important role in the economy and nutrition of the state in particular and Nigeria at large.

2.8.1 Herding System

Because they raised livestock for centuries, Fulani have evolved a herding system that withstands time, weather, social changes and government interventions (Khazanov, 1990). The movement of Fulani over the years has led to a pastoral calendar in which their location and the grazing habits can be predicted. The name Fulani has become synonymous with grazing and cattle ownership. The primary occupation of the Fulani is herding and sometimes farming. Non-herding jobs are seasonal and opportunistic. For example, during the wet-season, the Fulani take advantage of the abundant rain and manure to plant corn, millet, sorghum, and home gardens in their backyards (de St Croix 1945; Khazanov, 1990).

A Fulani household is the simplest, full-time, cattle-breeding unit. Every member of the household contributes and benefits from raising cattle (Iro, 2011). Labour is specialized, and by assigning it to gender and age groups, the pastoral Fulani optimize their production methods (Iro, 2011). The Fulani share the herding task, mens' work differs from womens', likewise adults' work differs from childrens'. Irrespective of age or gender, a member of the household learns all the herding skills (Iro, 2011). Herding is a huge task for the Fulani who are always trying to get the best grazing condition for their animals. Contrary to popular belief, moving with animals is not the delight of the pastoralists. The migrant Fulani in Northern Nigeria move because they have no choice (Khazanov, 1990).

At sunrise, the Fulani free the animals from the tether, milk the lactating cows and take them out to graze. In the afternoon, the herders take the animals to a stream, pond or

any other sources for watering. At sunset, the animals are taken back to the camp. In the night, the pastoral Fulani must keep vigil on the animals, protecting them from night marauders. Daily herding tasks vary according to seasonal changes (Ibrahim, 1984).

2.8.2 Herd Size and Composition

Accurate livestock statistics are difficult to get. Even in the best responses, the Fulani should be expected to underreport their livestock size. Despite the waiver of the cattle tax, questions about herd count raise suspicion and anxiety among the Fulani (Iro, 2011). In addition, many variables must be included to estimate the optimum herd size for an area and for a population. Theoretically, optimum herd size takes account of the prevailing environmental conditions, biological capacity (performance) of the species, herd management practice, and resource use and distribution. For the Fulani of Northern Nigeria, all of these factors are dynamic.

Cattle are the dominant ruminant in a Fulani herd. The camel is the least popular domestic stock of the Fulani. The female constitutes 69-73% in each herd type (Khazanov, 1990). The advantages of keeping more female variety in the herd are obvious. The Fulani maintain a balanced, functional stock composition, consisting of beefers, milkers, breeders, carriers, laborers, cash generators and stock beautifiers. The lyre-horned White Fulani cattle are the mainstay of Fulani holdings in Kaduna state (FDL&PCS, 1992; Iro, 2011). This breed, though less hardy, has higher milk and beef yields compared to the Sokoto Gudali (Iro, 2011).

The Fulani, most of whom are Muslims, do not raise pigs because Islam forbids the eating of swine. However, many Fulani keep sheep and goats for many reasons. Goats require less water and care, mature quickly, reproduce rapidly and produce high quality protein from low-quality feed (Cisse, 1980; and Waters-Bayer, 1986). The slaughter of

a small ruminant can prevent the slaughter of a whole cow that cannot be stored or consumed by a small festive group.

In Kaduna state and most parts of Northern Nigeria, sheep rather than goats are used for religious sacrifices, naming ceremonies and marriage festivities (Iro, 2011). Sheep and goats also provide the initial capital for the purchase of supplementary feeds, foundation cows to rebuild a herd or pay for veterinary services or compensation to farmers for crop damages (Bates and Conant, 1980; Iro, 2011).

2.9 AWARENESS OF CBPP AMONGST CATTLE OWNERS IN KADUNA STATE

In Nigeria, pastoralists and nomads have wealth of knowledge and experiences about common health problems affecting their stock, including disease treatment and prevention (Adekunle *et al*., 2002). This knowledge is however based on oral transmission, shared information and life experiences of individual herders on their livestock over the years (Ameri *et al.,* 2009). If properly utilized, Fulani's knowledge of CBPP could be used to develop appropriate surveillance systems and could form solid foundation for sustainable control programmes in Kaduna state (Adekunle *et al*., 2002).

In a questionnaire-based appraisal of CBPP awareness amongst 274 pastoralists, settled and semi-settled herdsmen in Sokoto and Kebbi states of North-western Nigeria, Tambuwal *et al.* (2011) reported that 65% of the respondents knew CBPP, 22.3% could recognise signs of the disease in their herds and 65% treated CBPP cases with antibiotics. The study concluded that herdsmen in the North-west geopolitical zone of Nigeria were not only aware of the existence of CBPP but familiar with its clinical manifestation, control strategies and widely used antibiotics for the treatment of cattle with a suspected case of CBPP.

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY AREA

The study was conducted in Kaduna State which occupies an area of approximately 48,483.2 Km^2 and has a human population of 6,066,562 (2006 Census). The State, which is located in a central position in Northern Nigeria (with Kaduna town as its capital), shares borders with Katsina, Kano, Bauchi, Plateau and Niger States and the Federal Capital Territory, Abuja. Globally, the State lies between Longitude 30" East and Latitude 0900 and 11º 30" North (KDSG, 2011). The entire land structure consists of undulating plateau with semi arid northern part and sub humid southern part (FDLPCS, 1992). There are two marked seasons in the state; dry windy season and rainy (wet) season with an average rainfall of 1,016mm. The vegetation extends from the Guinea Savannah in the southern part to the Sudan Savannah in the northern part (FDLPCS, 1992).

Kaduna State, which is divided into 23 Local Government Areas (LGAs) (Figure 3.1) and three Senatorial Districts, is a pluralist society with a total of 36 ethnic groups. Hausa is the general language common to all the groups (Kayode and Ogunleye, 2008). About 80% of the state's population is engaged in peasant farming including cattle production (KDSG, 2011).

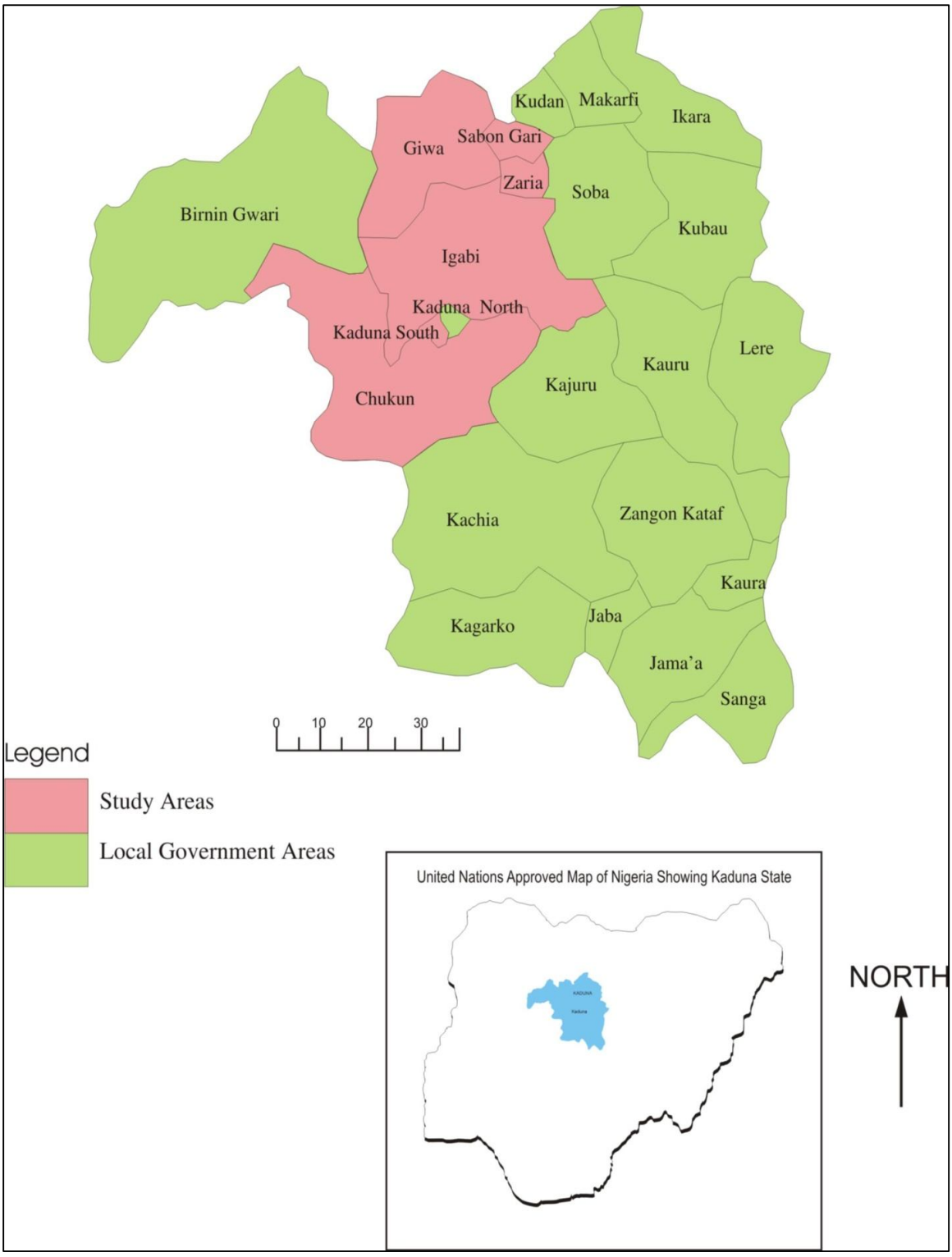


Figure 3.1: Map of Kaduna state showing the 6 local government areas involved in a study of CBPP seroepidemiology.

Inset: United Nations approved map of Nigeria showing Kaduna State

Source: Modified from KDSG (2011)

3.2 STUDY DESIGN

A Cross-Sectional Survey was carried out in Northern and Central parts of Kaduna State, Nigeria. Serological examination and participatory epidemiological techniques were used to determine prevalence of CBPP and identify risk factors to the transmission of the disease in the study population.

3.3 SAMPLING

3.3.1 Sample Size

A sample size of 171 herds was estimated from an expected seroprevalence of 85.1% (Mtui-Malamsha, 2009) as described by Thrusfield (2007). However, to optimize the utility of the cELISA for CBPP test kit, 190 herds were selected for bleeding.

3.3.2 Sampling Procedure

The study was conducted in Chukun, Giwa, Igabi, Kaduna South, Sabon Gari and Zaria LGAs. Outbreaks of CBPP were reported in some villages in Zaria LGA eight months prior to the commencement of the study. Percentage distributions of herds across the LGAs were estimated based on the quantity of CBPP vaccine doses allocated to the LGAs for the 2010 Kaduna state CBPP vaccination campaigns. Number of herds sampled per selected LGA was determined by proportional sampling method (Thrustfield, 2007) based on the estimated number of herds in the area. Villages and herds were selected based on herdsmen's willingness to participate in the study after pre-sampling sensitization and enlightenment. Sampling units comprised of single herds and 10% of animals in each herd were bled.

3.4 HERD INCLUSION CRITERIA

For the purpose of this study, a herd was defined as a group of cattle consisting of 10 or more animals of all ages and both sexes kept together in one enclosure. To eliminate seropositivity from vaccination, only herds that were not vaccinated against CBPP in the last four months prior to sampling were included in the study (Thiaucourt *et al.,* 2000).

3.5 GENERAL CHARACTERISTICS OF THE STUDY POPULATION

A total of 190 herds were bled and 100 herdsmen were interviewed but analyzable data from the interviews was available only for 77 of the 100 herds, i.e. 17, 6, 8, 6, 6 and 34 from Chikun, Igabi, Giwa, Kaduna South, Sabon Gari and Zaria LGAs respectively. Herd size varied from 10 to 89 cattle with a median of 21 (Table 3.1). In total, 516 animals were bled from the 190 herds, with a range of 1 to 9 animals per herd and a median of 2 animals. The cattle population studied comprised of both male and female animals of 3 months to 9 years of age with a median of three years (Table 3.2).

3.6 COLLECTION OF SERA

For each animal selected, sex, age and herd/animal number were recorded (Appendix I). About 6 ml of blood was drawn from the jugular vein using sterile 21 gauge needles and 9 ml plain vacutainer tubes, labelled with herd and animal numbers. Samples collected were transported in cooler boxes to Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria.

Table 3.1: General characteristics of cattle herds participating in the study of CBPP seroepidemiology in Northern and Central parts of Kaduna State

Variable	**Variable Level**	**Herds (%)**
Herd Size	< 20	91(48)
	20-40	65 (34)
	> 40	34 (18)
Local Government Area	Chukun	38 (20)
	Giwa	44 (23)
	Igabi	11 (6)
	Kaduna South	6 (3)
	Sabon Gari	18 (10)
	Zaria	73 (38)

Table 3.2: General characteristics of animals participating in a study of CBPP seroepidemiology in Northern and Central parts of Kaduna state

Variable	**Variable Level**	**Number of Cattle (%)**
Age (Years)	< 3	188 (36)
	3-5	176 (34)
	> 5	152 (30)
Gender	Male	202 (39)
	Female	314 (61)
Local Government Area	Chukun	120 (23)
	Giwa	76 (15)
	Igabi	24 (5)
	Kaduna South	19 (4)
	Sabon gari	46 (9)
	Zaria	231 (44)

The clotted blood were centrifuged at 1500g for 10 minutes (Henry, 1979) and the harvested sera were collected in serum tubes and stored at -20ºC until examined.

3.7 EXAMINATION OF SERA

Serum samples were analysed as described by Le Goff and Thiaucourt (1998) using the cELISA kit for CBPP developed and supplied by La Recherché Agronomique Pour Le Devéloppement (CIRAD), Montpellier, France. There were two plates per sample run, the U-bottom dilution plate and *Mmm*SC pre-coated plate. Wells in the first two columns (A1-H1 and A2-H2) were used for control samples while the remaining wells (A3-H12) were used for test sera. Each plate had a negative control sample dispensed in wells H1 and H2; strong positive control sample dispensed in wells B1, B2, C1 and C2 and weak positive control sample dispensed in wells D1, D2, E1 and E2. Wells F1, F2, G1 and G2 were used for monoclonal control sample while A1 and A2 were used for conjugate control.

One hundred microlitres of dilution buffer and 11 µl of each test sera or control samples were dispensed into all wells of the dilution plate to give approximately 1:10 dilution. Subsequently, 110 µl of a 1:120 dilution of monoclonal antibody (Mab) was added to all the wells with the exception of A1 and A2 in which 110 µl of kit dilution buffer were added. One hundredmicro litres of the contents in each well of the dilution plate were transferred to the equivalent wells of the *Mmm*SC pre-coated plate.

The plates were then incubated at 37ºC for one hour with gentle agitation. The plates were washed twice using wash buffer. Thereafter, 100 µl of a 1:100 dilution of horseradish peroxidise-conjugated rabbit anti-mouse IgG was added to each well and incubated at 37ºC for 30 minutes. After three more washes with wash buffer, 100 µl of

TMB substrate (3,3',5,5'-Tetramethylbenzidine, supplied with the kit as a ready to use solution) was added to each well and the plates incubated at 37ºC for 20 minutes. One hundred microlitres of 0.5M sulphuric acid were added to each well to stop the reaction. Optical densities (OD) of the separate reactions were measured at 450nm with the aid of APPAR Plate Reader (UniEquip GmbH®, Martinsried, Munich, Germany, Serial Number 1587) and the percentage inhibition (PI) value for each test serum calculated using the following formula as instructed by the kit manufacturer;

$$PI = \left\{ \frac{OD_{mab} - OD_{test\ serum}}{OD_{test\ serum} - OD_{conjugate}} \right\} x100\%$$

Where;

OD_{mab}= optical density of monoclonal antibody

$OD_{test\ serum}$= optical density of test serum

$OD_{conjugate}$=optical density of the conjugate

Any test serum that had a *PI* of 50% or higher was considered positive for CBPP antibodies (Le Goff and Thiaucourt, 1998).

3.8 QUESTIONNAIRE DEVELOPMENT, PRE-TESTING AND ADMINISTRATION

Structured questionnaires (Appendix II) were administered to herdsmen visited in order to determine their knowledge of livestock diseases (CBPP in particular), livestock contacts and herd dynamics. One herdman (household head, where possible) was interviewed for each herd visited and personal observations (such as presentation of

CBPP vaccination certificates for vaccinated herds) where necessary were used to validate herdsmen's responses. To facilitate data analyses, improve precision of responses and minimize variations, the questionnaire contained mostly close-ended questions. Pre-testing of the questionnaires was initially carried out in five selected herds at Anguwan Dankali, Zaria LGA to evaluate its performance in terms of time taken to complete the interviews, ambiguity and precision of responses and necessary modifications were made.

3.9 DATA ANALYSES

Seroprevalence of CBPP was calculated from the formula;

$$Seroprevalence = \frac{Number\ of\ Positive\ Samples}{Total\ Number\ of\ Samples}\ X\ 100$$

Descriptive charts were constructed with Microsoft Excel® 2007. Odds ratio (OR) and 95% confidence interval (C.I) on the ORs were calculated to measure strengths and statistical significance of associations between variables and CBPP serostatus using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

Herdsmen's knowledge of CBPP was assessed with four items each of clinical signs and control measures (Appendix II) using a prompted recall format (Waller *et al.,* 2004) based on an 8-point score. Herdsmen's responses were summed up into 4 categories based on their scores: those with scores of 0 to 2 were classified as having Very Limited Awareness, while those with scores of 3-4, 5-6 and 7-8 were classified as having Limited Awareness, Good Awareness and Very Good Awareness respectively. Variables that were considered for analyses and their descriptions are given in Table 3.3.

Table 3.3: Description of variables considered for statistical analyses of a study of CBPP seroepidemiology in Northern and Central parts of Kaduna state

Variable	**Description**
Age (Years)	□ 3; 3-5; □ 5
Brought back from markets	Animals not sold at the markets brought back home (Yes/No)
Brought in	Animals brought into the herd (as bought, gift, hire) in the last 12 months (Yes/No)
CBPP outbreak	CBPP outbreak reported by interviewed herdsmen in the last 3 years (Yes/No)
CBPP vaccination	CBPP vaccination carried out in the last 12 months
Herd size	Number of animals in a herd (<20; 20-40; □40)
Herdsmen's knowledge of CBPP	Very limited, Limited, Good, Very good
Local Government Area	Administrative units
Mixing at grazing	Herds mix at grazing point (Yes/No)
Mixing at watering point	Herds mix at drinking point (Yes/No)
Number of animals sampled	Number of animals tested per herd
Sex	Animal gender (Male/Female)

CHAPTER 4

RESULTS

4.1 HERD LEVEL SEROPREVALENCE OF CBPP AND UNIVARIABLE RISK FACTOR ESTIMATES

Of the 190 herds sampled, 111 had at least one animal that tested positive by cELISA, giving a herd level seroprevalence of 58.4%. Herd seroprevalence was significantly highest in herds with more than 40 cattle (76.5%) and lowest in herds with fewer than 20 cattle (48.4%) (Table 4.1). Lowest and highest seroprevalence estimates of 52.3% and 83.3% were observed in Giwa and Kaduna South Local Government Areas respectively. However, there was no significant difference ($P < 0.05$) in herd level prevalence across the LGAs studied (Table 4.2).

Herd level seroprevalence of CBPP was also lesser among herds that were vaccinated against CBPP (48%) in the last 12 months prior to sampling than unvaccinated herds (96.3%) (Table 4.3). Eighty percent of herds that had signs suggestive of CBPP outbreaks within the last three years prior to sampling had at least one animal that tested positive to CBPP antibodies (Table 4.4). Ninety six percent of the herds to which new animals were introduced within the last 12 months prior to sampling were seropositive (Table 4.5). The seroprevalence estimate was also significantly higher for herds that mixed with others at grazing or watering points (89.4%) than for herds that grazed or were watered alone (24.4%) (Table 4.5).

Table 4.1: Distribution of CBPP seropositive herds according to herd size in Northern and Central parts of Kaduna State

Herd Size	**Herds Sampled**	**Positive Herds**	**Prevalence (%)**	**OR**	**95% CI on OR**
Less than 20	91	44	48.4	0.45	0.248-0.806*
20 to 40	65	41	63.1	1.34	0.725-2.484
More than 40	34	26	76.5	2.27	1.157-6.370*
Total	**190**	**111**	**58.4**		

*Significant at 95% CI

Table 4.2: Distribution of CBPP seropositive herds according to Local Government Areas in Northern and Central parts of Kaduna State

LGA	Herds Sampled	Positive Herds	Prevalence (%)	OR	95% CI on OR
Chukun	38	17	44.7	0.50	0.244-1.025
Giwa	44	23	52.3	0.72	0.366-1.422
Igabi	11	9	81.8	3.40	0.713-16.18
Kaduna South	6	5	83.3	3.68	0.421-32.14
Sabon Gari	18	14	77.8	2.71	0.856-8.561
Zaria	73	43	58.9	1.03	0.570-1.870
Total	**190**	**111**	**58.4**		

Table 4.3: Distribution of CBPP seropositive herds according to vaccination status in Northern and Central parts of Kaduna State

		Herds Sampled	Positive Herds	Prevalence (%)	OR	95% CI on OR
CBPP Vaccination	Yes	50	24	48.0	0.04	0.005-0.282*
	No	27	26	96.3		
Total		**77**	**50**	**64.9**		

*Significant at 95% CI

Table 4.4: Distribution of CBPP seropositive herds according to reported CBPP outbreaks in Northern and Central parts of Kaduna State

		Herds Sampled	**Positive Herds**	**Prevalence (%)**	**OR**	**95% CI on OR**
CBPP Outbreak	Yes	35	28	80.0	3.64	1.303-10.15*
	No	42	22	52.4		
Total		**77**	**50**	**64.9**		

*Significant at 95% CI

Table 4.5: Distribution of CBPP herds according to herd contacts and dynamics in Northern and Central parts of Kaduna State

Variable	Variable Level	Herds Sampled	Positive Herds	Prevalence (%)	OR	95% CI on OR
Brought in	Yes	44	42	95.5	65.63	12.90-334.0*
	No	33	8	24.2		
Congregating at grazing/watering points	Yes	47	42	89.4	23.10	6.744-79.11*
	No	30	8	26.7		

*Significant at 95% CI

Within the seropositive herds, seroprevalence ranged from 7% to 100% (Figure 4.1) and a bimodal distribution of within-herd seroprevalence was observed. Eighty three of the herds had no or only one test-positive animal, indicated by a within-herd seroprevalence of 0 to 19%. The remaining herds had a wide range of within-herd seroprevalence with a mode at 40 to 59% which, depending on the number of animals sampled within a given herd, would represent 2 or more seropositive animals.

4.2 ANIMAL LEVEL SEROPREVALENCE OF CBPP

Of the 516 animals sampled, 160 tested positive from the 111 cELISA-positive herds, resulting in an animal-level seroprevalence of 31% in all herds. The proportion of cELISA-positive animals was higher among female cattle (103) than male animals (57) (Table 4.6). The odds of being positive for CBPP were higher in cattle 3 to 4 years old (1.15) but this association was not significant (Table 4.7). Animal level seroprevalence estimates for cattle from Igabi (41.7%) and Chukun (25%) LGAs were highest and lowest respectively (Table 4.8). There was no significant difference in point animal seroprevalence across the LGAs studied.

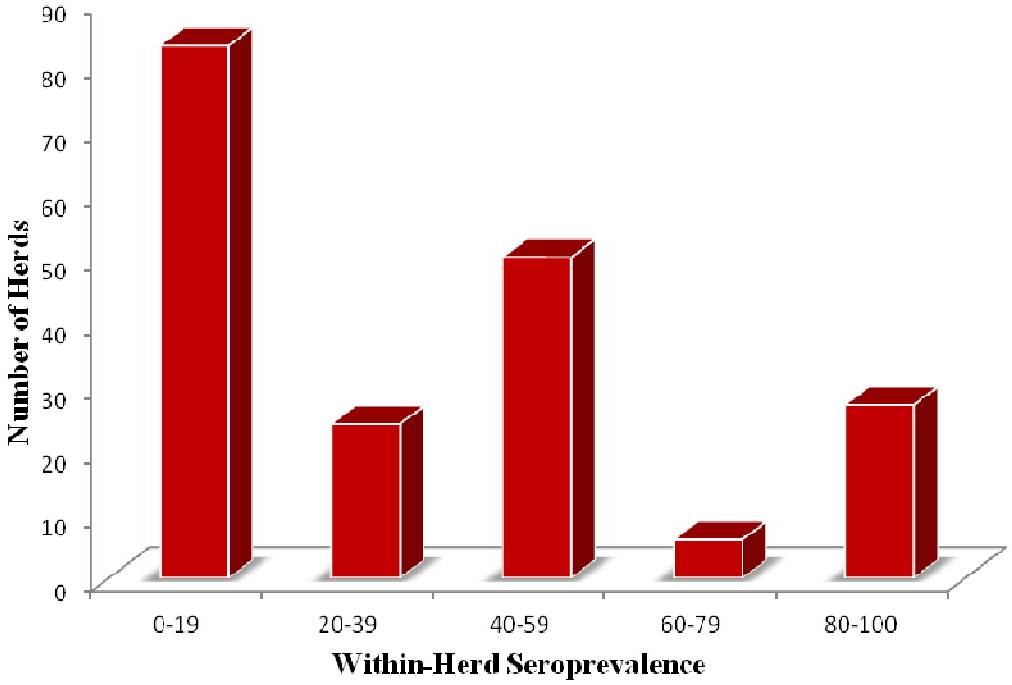


Figure 4.1: Within-herd seroprevlance of contagious bovine pleuropneumonia in Northern and Central parts of Kaduna State

Table 4.6: Distribution of CBPP seropositive animals according to gender in Northern and Central parts of Kaduna State

Sex	**No. of Animals Sampled**	**No. Positive**	**Prevalence (%)**	**OR**	**95% CI on OR**
Male	202	57	28.2	0.81	0.547-1.185
Female	314	103	32.8	1.24	0.844-1.828
Total	**516**	**160**	**32.0**		

Table 4.7: Distribution of CBPP seropositive animals according to age group in Northern and Central parts of Kaduna State

Age	**No. of Animals Sampled**	**No. Positive**	**Prevalence (%)**	**OR**	**95% CI on OR**
Younger than 3 years	188	60	31.9	1.08	0.733-1.586
3 to 5 years	176	58	33.0	1.15	0.776-1.695
Older than 5 years	152	42	27.6	0.86	0.524-1.209
Total	**516**	**160**	**31.0**		

Table 4.8: Distribution of CBPP animals according to Local Government Areas in Northern and Central parts of Kaduna State

LGA	No. of Animals Sampled	No. Positive	Prevalence (%)	OR	95% CI on OR
Chukun	120	30	25.0	0.68	0.429-1.084
Giwa	76	26	34.2	1.19	0.709-1.989
Igabi	24	10	41.7	1.63	0.707-3.750
Kaduna South	19	7	15.4	1.31	0.506-3.397
Sabon Gari	46	16	34.8	1.08	0.572-2.043
Zaria	231	71	30.7	0.98	0.671-1.422
Total	**516**	**160**	**31.0**		

4.3 ASSESSMENT OF HERDSMENS' AWARENESS OF CBPP IN NORTHERN AND CENTRAL PARTS OF KADUNA STATE

Of the 190 herds visited, 100 herdsmen agreed to participate in the interview initially. Twenty three more herdsmen withdrew during the course of the interview and so their data could not be included in the analysis. Thirty nine percent (30/77) of the respondents listed pneumonia or CBPP in the top three diseases they often encountered in their herds. The percentage of CBPP seropositive herds was higher (93.3%) in herds where the herdsmen listed CBPP or pneumonia among the three most common health problems affecting their animals than in those herds where pneumonia or CBPP was not considered to be a major problem (46.8%). The animal level seroprevalence was observed to decrease with increasing level of herdsmen's awareness of CBPP (Table 4.9). Majority of the herdsmen reported acquiring their knowledge of CBPP and livestock disease control options from a combination of mass media, fellow herdsmen, extension agents and oral traditions (Figure 4.2).

Table 4.9: Distribution of CBPP seropositive herds according to herdsmen's level of CBPP awareness in Northern and Central parts of Kaduna State

Level of Awareness	**No. of Responding Herdsmen/Herds**	**Positive Herds**	**Prevalence**	**OR**	**95% CI on OR**
Very Limited	26	25	96.2	26.00	3.270-206.7*
Limited	21	18	85.7	5.06	1.345-19.06*
Good	10	5	50.0	0.49	0.128-.1.868
Very Good	20	2	10.0	0.02	0.004-0.106
Total	**77**	**50**	**64.9**		

*Significant at 95% CI

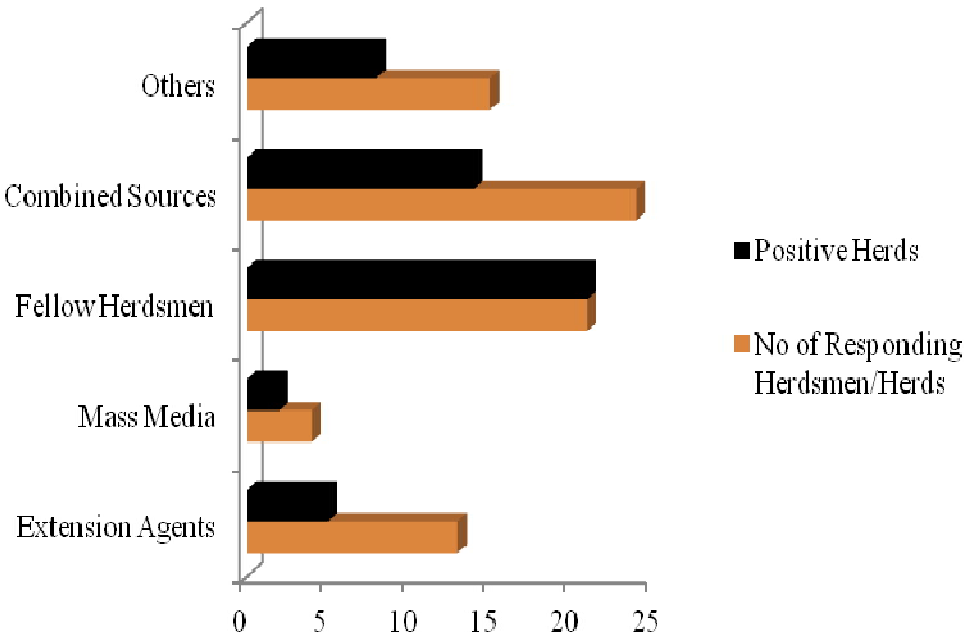


Figure 4.2: Herdsmens' sources of information on livestock diseases in Northern and Central parts of Kaduna State

CHAPTER 5

DISCUSSION

There have been few studies which estimated the CBPP prevalence in Nigeria (Aliyu *et al.,* 2000) and most of such reports were exclusively based on abattoir surveys with focus on mostly the north eastern part of the country (Aliyu *et al.,* 2003; Bello and Ibrahim 2010; Tambuwal *et al*., 2011). The present study was aimed at providing an estimate of seroprevalence, associated risk factors and level of herdsmens' awareness of CBPP in cattle herds of Northern and Central parts of Kaduna state, Nigeria.

Higher seroprevalence of CBPP was observed in this study to be significantly associated with herds of more than 40 animals. CBPP is a contagious disease, in which the effective reproduction number of the disease is not only determined by the pathogenicity and virulence of the *Mmm*SC strain alone, but also by the rate and duration of contact between infected and susceptible animals (Kusiluka and Sudi, 2003; Mariner *et al*., 2006). Close proximity of animals in herds is therefore likely to increase with increasing herd size as observed in the present study where the odds for having a seropositive herd was 2.3 more in herds with more than 40 animals relative to herds with fewer cattle. Studies on herd level seroprevalence of CBPP based on cELISA are limited. The herd level seroprevalence of CBPP reported here was much lower (58%) than 85% found in the Masaai ecosystem of South-Western (Mtui-Malamsha, 2009). This difference may not be unconnected with the fact that the area studied by Mtui-Malamsha (2009) was classified as 'recently infected' with CBPP (Wanyoike-Kairu *et al*., 2004) prior to the survey. It is not surprising therefore, that CBPP point seroprevalence at herd level recorded lower estimates in the present study.

At both herd and individual animal levels, CBPP seroprevalence was not significantly associated with local government areas from which herds were selected (Tables 4.2 and 4.8). Contact structures, herd size and herding practices were generally similar in all the local government areas sampled. More than 80% of cattle population in Nigeria are owned by the Fulani herdsmen whose herding practices and social dynamics are generally similar (Aliyu *et al*., 2000). It is therefore likely that CBPP seroprevalence does not have spatially significant distribution in the studied area.

This study observed that seroprevalence of CBPP was significantly higher in herds that did not vaccinate their cattle against the disease. The odds for having CBPP seropositive herds were found to be only 0.04 in vaccinated herds. This finding agreed with the report of Thiaucourt *et al.* (2003) in which administration of live attenuated vaccine (T1/44) played a significant role in reducing the disease prevalence.

An analysis of reported CBPP outbreaks in a herd was shown to be positively associated with seroprevalence at the herd level. Herds that reported outbreaks of diseases with signs suggestive of CBPP in the last 3 years prior to commencement of this study were 3.6 times more likely to be CBPP seropositive than herds that did not report such outbreaks. The strength of this association might have been underestimated due to inadequacy of data which was only based on the interviewed herdsmen's ability to recall signs suggestive of a CBPP outbreak. Most herdsmen can only recognise such signs as coughing and lagging behind while grazing which are evident only during the acute stage of the disease. Therefore, subclinical or chronic CBPP forms may likely have been passed unnoticed and thus unreported. This finding was in agreement with the report of Mtui-Malamsha (2009) where herdsmen were unable to recognize subclinical CBPP even in an enzootic area.

The present study established that CBPP seroprevalence was significantly associated with risk factors describing aspects of livestock contacts and herd dynamics (Table 4.5). Herd exposure to other animals have been reported to determine CBPP distribution patterns in many African pastoral systems (Mariner *et al*., 2006) and risk factors for the disease spread were shown to mainly include high-density confinement in night housings and use of common pastures and watering places (Provost *et al*., 1987).

The fact that CBPP is a contagious disease is an indication that a true positive herd is likely to have more than one seropositive animal unless it is at the onset of the outbreak or a seropositive animal is just brought into an uninfected herd (Mtui Malamsha, 2009). The within herd prevalence observed in the present study showed a bimodal distribution, one at 0-19% and another at 40-59%. It is possible that herds with 0-19% within herd seroprevalence represent seronegative herds, while majority of the positive herds have a 40-59% within herds CBPP seroprevalence estimate.

The animal level seroprevalence of CBPP estimated in this study was 31%. This point prevalence, even though slightly lower, compared well with the 32% reported in an abattoir study of CBPP seroprevalence on 500 cattle determined by cELISA in the Song area of Adamawa state, north eastern Nigeria (Aliyu *et al.,* 2003). It was also higher than 10.5% estimated from 13,251 cattle by CFT in Kenya (Wanyoike, 1999) and 11.8% CBPP seropositive rates based on Latex Agglutination Test (LAT) (Okaiyeto *et al.,* 2011) in Katsina, Nigeria. The slightly higher abattoir-based seroprevalence reported in Adamawa state might be attributable to the influence of sick animals being sent for slaughter as there was not much regional difference in herd dynamics and herding practises of the Fulani pastoralists who own about 80% of the national cattle herd (Aliyu *et al.,* 2000; Aliyu *et al.,* 2003).

In addition, the lower estimates of CBPP seroprevalence recorded in Kenya and Nigeria (Wanyoike, 1999; Okaiyeto *et al.,* 2011) could have been possibly due to differences in the serologic tests applied. The sensitivity of CFT for instance, in detecting a chronically infected animal is low (Nicholas *et al.,* 1996) and the assay is reported to be unable to discriminate between natural and vaccinal exposures (Aliyu *et al.,* 2000).

The odds for having a seropositive herd were observed to significantly decrease with the increasing level of awareness about CBPP by the herdsmen. In a questionnaire based survey in Sokoto and Kebbi states of north western Nigeria, Tambuwal *et al*. (2011) reported that 65% of herdsmen were aware of the existence of CBPP outbreaks in the studied area. It is therefore likely that the higher the level of CBPP awareness by the herdsmen, the more they accept and implement CBPP control options such as vaccinations which has been shown to effectively reduce the number of seroposiive animals (Thiaucourt *et al.,* 2003; Mariner *et al.*, 2005)

This study has also observed that although mass media was the least source of information regarding livestock diseases and prevention strategies, it is still one of the most effective means of correct information dissemination to herdsmen; second only to agricultural extension agents (Figure 4.2). This may be explained by the fact that jingles concerning animal health enlightenment on the media are more carefully planned, well understood by the intended audience and broadcasted in their local dialects. This is in contrast to information obtained by fellow herdsmen which were based on hearsay and folktales and therefore unlikely to be useful in making an informed choice on CBPP control.

CHAPTER 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY AND CONCLUSIONS

The present study estimated herd-level and animal level CBPP seroprevalence of 58.4% and 31.0% respectively in Northern and Central parts of Kaduna State, based on cELISA. The study has shown that higher seroprevalence is associated with herd size and livestock contacts and dynamics. The study therefore concludes that larger herds in the state may be at an increased risk of being CBPP seropositive due possibly to increased frequency and duration of contact between infected and susceptible animals.

Congregation of cattle at pastures and or watering points could be one of the major determinants of CBPP seropositive herds in Kaduna State. This may be particularly evident during dry seasons when grazing and water sources are limited. Such exposures may lead to CBPP transmission between infected and susceptible herds especially in herds located at relatively urban areas where pastures have been overtaken by physical developments. Such other important risk factors, as previous outbreaks of CBPP, though underestimated in the present study by herdsmens' inability to identify and report chronic or subclinical phases of the disease, were associated with high levels of seropositivity. On the other hand, CBPP vaccination, even though it offers immunity against the disease for only short periods (6-12 months), is one of the few control options in the state, as it still offers protection against the disease.

From the herdsmens' perspective, CBPP is still one of the most important disease problems in their herds. Herdsmen with good level of awareness about CBPP appeared to have fewer seropositive animals

6.3 RECOMMENDATIONS

CBPP vaccination efforts in Kaduna state should be intensified because it still offers a protective advantage against the disease. These efforts should be especially directed to villages that reported CBPP outbreaks and those with comparatively larger herd sizes as these focal points might be at increased risk of the disease.

Correct and relevant public health enlightenment campaigns must be reinvigorated as poor information dissemination especially on topics of livestock diseases are likely to enhance the spread of CBPP in particular and other livestock diseases in general. In addition, enhanced knowledge of the disease is likely to improve herdsmen's acceptance of CBPP control measures.

Whenever possible, more grazing and watering facilities for livestock production should be made available to cattle herds particularly in dry season when such points are not only unavailable but generally inaccessible. This will undoubtedly reduce the frequency and duration of contacts between herds of different CBPP status.

REFERENCES

Abdo, E. M., Nicolet, J., Miserez, R., Goncalves, R., Regalla, J., Griot, Ch., Bensaide, A., Krampe, M. and Frey, J. (1998). Humoral and bronchial immune responses in cattle experimentally infected with *Mycoplasma mycoides* subspecies *mycoides* SC. *Veterinary Microbiology* 59:109-122.

FAO, (2012). Preparation of contagious bovine pleuropneumonia contingency plans. Accessed 20 October, 2012 from http://www.fao.org/docrep/005/Y4143E/y4143e05.htm

Abdo, E., Nicolet, J. and Frey, J. (2000). Antigenic and genetic characterization of lipoprotein LppQ from *Mycoplasma mycoides* subsp. *mycoides* SC. *Clinical and Diagnostic Laboratory Immunology,* 7 (4): 588-595.

Abusugra, I., Langella, V., Lelli, R., Pinni, A., Hübshle, O. Olofsson, A. and Morein, B. (2001). Proinflammatory cytokines induced by *Mycoplasma mycoides* subsp. *mycoides* SC (*Mmm*SC) non-adjuvanted membranes and incorporation of these membranes into ISCOMs. *In:* Poveda, J., Fernández, A., Frey, J. and Johansson, K-E. (Eds.), *Mycoplasmas of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics* Vol 5, Office for official publications of the European Communities, Luxembourg. Pp. 166-169.

Adekunle, O.A., Oladele, O.I. and Olukaiyega, T.D. (2002). Indigenous control methods for pests and diseases of cattle in Northern Nigeria. *Livestock Resources and Rural Development,* 14 (2): 1 – 4.

Aliyu, M.M. (2002). Contagious bovine pleuropneumonia (CBPP) in some states of Northern Nigeria: Status, Immunological Techniques and Polymerase Chain Reaction in the Diagnosis of the Disease, Unpublished PhD Thesis, University of Ibadan, Ibadan, Nigeria.

Aliyu, M.M., Egwu, G.O. and Ameh, J.A. (2003). Comparison of two immune diagnostic techniques for detection of *Mycoplasma mycoides* subspecies *mycoides* SC in cattle. *Tropical Veterinarian,* 21: 23 – 29.

Aliyu, M.M, Obi, T.U. and Egwu, G.O. (2000). Prevalence of contagious bovine pleuropneumonia in northern Nigeria *Preventive Veterinary Medicine,* 47: 263-269.

Amanfu, W. (2009). Contagious bovine pleuropneumonia (lungsickness) in Africa. *Onderstepoort Journal of Veterinary Research* 76: 13-17.

Amanfu, W., Masupu, K. V., Adom, E. K., Raborokgwe, M. V. and Bashiruddin, J. B. (1998). An outbreak of contagious bovine pleuropneumonia in Ngamiland district of northwestern Botswana. *Veterinary Record,* 143: 46-48.

Ameri, A.A., Hendrickx, S., Jones, B., Mariner, J., Mehta, P. and Pissang, C. (2009). *Introduction to Participatory Epidemiology and its Application to Highly Pathogenic Avian Influenza Participatory Disease Surveillance: A Manual for*

Participatory Disease Surveillance Practitioners. International Livestock Research Institute, Nairobi, Kenya. Pp.11-32.

Anonymous (2003). Country specific control strategies. *In*: *Proceedings of the 3rd Meeting of the FAO-OIE-AU/IBAR-IAEA Consultative Group on Contagious Bovine Pleuropneumonia.* Nairobi, Kenya, pp.20-22.

Ayling, R.D., Regalla, J., Spencer, Y., Nicholas, R. and De Santis, P. (1998). Investigations on a CBPP outbreak in Portugal. *In*: Leori, G., Santini, F., Scanziani, E. and Frey, J. (Eds), *Mycoplasmas Of Ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular Genetics* Vol II. Office for official publications of the European Communities, Luxembourg. Pp. 117-120.

Ayling, R.D., Regalla, J. and Nicholas, R.A.J. (1999a). A field test for detecting antibodies to *Mycopalsma mycoides* subsp. *mycoides* small colony type using the latex slide agglutination test. *In*: Stipkovits, L., Rosengarten, R., and Frey,J. (Eds.), *Mycoplasmas Of Ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular Genetics.* Vol III. Office for official publications of the European Communities, Luxembourg. Pp.155-158.

Ayling, R., Miles, R.J., Regalla, J. and Nicholas, R.A.J. (1999b). Development of improved serological tests for the diagnosis of CBPP. *In: Proceedings of the International Symposium on Mycoplasmas of Ruminants*. Toulouse, France. Pp.35.

Bashiruddin, J.B., Taylor, T.K. and Gould, A.R. (1994). A PCR-based test for the specific identification of *Mycoplasma mycoides* subspecies *mycoides* SC. *Journal of Veterinary Diagnostic Investigations*, 6: 428-434.

Bashiruddin, J.B., De Santis, P., Vacciana, A. and Santini, F.G. (1999a). Detection of *Mycoplasma mycoides* subsp. *mycoides* SC in clinical materials by a rapid colorimetric PCR. *Molecular and Cellular Probes*, 13: 23-28.

Bashiruddin, J.B., Santini, F.G., De Santis, P., Visaggio, M.C., Di Francesco, G., D'Angelo, A. and Nicholas, R.A.J. (1999b). Detection of *Mycoplasma mycoides* subspecies *mycoides* in tissues from an outbreak of contagious bovine pleuropneumonia by culture, immunohistochemistry and polymerase chain reaction. *Veterinary Record,* 145: 271-274.

Bates, D. and Conant, F. (1980). Livestock and Livelihood: A Handbook for the 1980s. *In*: *Proceedings of the Institute of Development Studies Conference on the Future of Pastoral People,* Nairobi, Kenya. Pp. 89-101.

Belli, P., Poumarat, F., Perrin, M., Longchambon, D. and Martel, J.L. (1990). Experimental reproduction of contagious bovine pleuropneumonia in a herd of cattle and goats: clinical and pathological aspects. *In:* Regalla, J. (Ed.), *Contagious Bovine Pleuropneumonia*, Commision of the European Communities, Luxembourg. Pp. 7-15.

Bellini, S., Giovaninni, A., di Francesco, C., Tittarelli, M. and Caporale, V. (1998). Sensitivity and specificity of serological and bacteriological tests for contagious

bovine pleuropneumonia. *Office International des Epizooties Revue Scientifique et Technique,* 17: 654-659.

Bello, M. and Ibrahim, N.D.G. (2010). Contagious bovine pleuropneumonia (CBPP) at Zango Abattoir, Zaria, Northern Nigeria. *Zariya Veterinarian,* 7(1): 47-50.

Boonstra E., Lindbaek, M., Fizdani, B. and Bruusgaard, D. (2001). Cattle eradication and malnutrition in under five's: a natural experiment in Botswana. *Public Health and Nutrition*, 4:877-882.

Brandao, E. (1995). Isolation and identification of *Mycoplasma mycoides* subsp. *mycoides* SC strains in sheep and goats. *Veterinary Record*, 136: 98-99.

Brocchi, E., Gamba, D., Poumarat, F., Martel, J-L. and De Simone, F. (1993). Improvements in the diagnosis of contagious bovine pleuropneumonia through the use of monoclonal antibodies. *Office International des Epizooties Revue Scientifique et Technique,* 12:559-570.

Chambaud, I.H, Wroblewski H. and Blanchard, A. (1999). Interactions between mycoplasma lipoproteins and the host immune system. *Trends in Microbiology,* 7:493–499.

Cheng, X., Nicolet, J., Poumarat, F., Regalla, J., Thiaucourt, F. and Frey, J. (1995). Insertion element IS 1296 in *Mycoplasma mycoides* subsp. *mycoides* SC identifies a European clonal line distinct from African and Australian strains. *Microbiology* 141:3221-3228.

Cisse, S. (1980) Sedentarization of Nomadic Pastoralists and 'Pastoralization' of Cultivators in Mali. In: *Proceedings of the Institute of Development Studies Conference on the Future of Pastoral People,* Nairobi, Kenya. Pp. 123-144.

Danbirni, S., Okaiyeto, S.O., Pewan, S.B. and Kudi, A.C.(2010). Concurrent infection of contagious bovine pleuropneumonia and bovine tuberculosis in Bunaji nomadic cows. *Research Journal of Animal Sciences,* 4(1): 23-25.

De Santis, P., Bashiruddin, J. B., Ayling, R., Nicholas,R. and Regalla, J. (1997). Sample treatment and detection of *Mycoplasma mycoides* subsp. *mycoides* SC DNA from CBPP outbreak in Northern Portugal. *In:* Leori, G., Santini, F., Scanziani, E. and Frey, J. (Eds.), *Mycoplasma Of Ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular Genetics*. Official Publication of the European Commission, EUR report 18018, Luxembourg.

de St Croix, F. (1945) *The Fulani of Northern Nigeria: Some General Notes*. Lagos: Government Printer. Retrieved 23 August, 2011 from http://www.gamji.com/fulani8.htm

Dedieu, E. L. (2008). Contagious bovine pleuropneumonia: A rationale for the development of a mucosal sub-unit vaccine. *Comparative Immunology, Microbiology and Infectious Diseases,* 31: 227–238.

Dedieu, L., Totte, P., Rodrigues, V., Vilei, E.M. and Frey, J. (2010). Comparative analysis of four lipoproteins from *Mycoplasma mycoides* subsp. *mycoides* Small Colony identifies LppA as a major T-cell antigen. *Comparative Immunology, Microbiology and Infectious Diseases,* 33: 279–290.

Di Francesco, G., Della Salda, A., D'Angelo, R., Regalla, J., De Santis, P., Bashiruddin, J.B. and Santini, F.G. (1998). Pathological findings in infections of *Mmm*SC. *In:* Leori, G., Santini, F., Scanziani, E. and Frey, J. (Eds.), *Mycoplasma Of Ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular Genetics.* Official Publication of the European Commission, EUR report 18018, Luxembourg. Pp. 137-141.

Egwu, G.O., Nicholas, R.A.J., Ameh, J.A. and Bashiruddin, J.B. (1996). Contagious bovine pleuropneumonia: an update. *Veterinary Bulletin,* 66: 875-888.

Egwu G. O., Adamu, M., Mshelia, G. D. and Bukar-Kolo, Y. M. (2012). Isolates of *Mycoplasma mycoides* subspecies *mycoides (SC)* in small ruminants in Sahel zone of Nigeria and its implications on disease control. *African Journal of Biotechnology,* 11(23): 6396-6401.

Fadiga, M., Jost, C. and Ihedioha, J (2011). Financial costs of disease burden, morbidity and mortality from priority livestock diseases in Nigeria: Disease burden and cost-benefit analysis of targeted interventions. *In*: *Final Report of the Nigeria Integrated Animal and Human Health Management Project*, International Livestock Research Institute, Nairobi, Kenya. Pp. 9-20

FAO, (2008). Recognizing Contagious Bovine Pleuropneumonia. *In: FAO Animal Health Manual*. Retrieved 20 December, 2011, from ftp://ftp.fao.org/docrep/fao/005/y4142E/y4142E00.pdf

Fasanmi, F.E. (2003). Status of contagious bovine pleuropneumonia (CBPP) in Nigeria with emphasis on control strategies. Retrieved 25 August, 2011 from http://www.fao.org/007/y5510e0u.htm.

Fayomi, A. and Aliyu, I.D. (1997). A retrospective study of the incidence of contagious bovine pleuropneumonia in Kano and environs.*In: Proceedings of the Nigerian Veterinary Medical Association (NVMA) Annual Congress,* Oshogbo, Nigeria.

FDL (Federal Department of Livestock) (2010), Ministry of Agriculture, Abuja, Nigeria. Document Available with the department

FDL&PCS (Federal Department of Livestock and Pest Control Services), National Livestock Reports. (1992). *Nigeria Livestock Resources*. Vol. III, State Reports: Kaduna State. Pp. 2-22.

Ferronha, M.H., Nunes Petisca, J.L., Sousa Ferreira, H., Machado, M., Regalla, J. and Goncalves, P.A. (1990). Detection of *Mycoplasma mycoides* subsp. *mycoides* immunoreactive sites in pulmonary tissue and sequestra of bovines with contagious pleuropneumonia. *In:* Regalla, J. (Ed.) *Contagious Bovine Pleuropneumonia*. Commission of European Communities Publications, Luxembourg. Pp 2-6.

Freundt, E. A. (1983). Culture media for classic mycoplasmas. *In:* Razin, S. and Tully, J.G. (Eds.). *Methods in Mycoplasmology,* Vol 1, Academic Press, New York, United States of America. Pp.127-135.

Gonçalves, M.R. (1994) Isolation and identification of Mycoplasma mycoides subspecies mycoides SC from bull semen and sheath washings in Portugal. *Veterinary Record*, 135: 308-309.

Henry, J.B., (1979). *Clinical diagnosis and management by laboratory methods,*Vol. 1, W.B Saunders Company, Philadelphia, United States of America. Pp.60.

Horowitz, M. (1980). Research priorities in pastoral societies: An agenda for the 1980s." In: *Proceedings of the Institute of Development Studies Conference on the Future of Pastoral People, Nairobi, Kenya.* Pp. 61-88.

Hudson, J. R. (1971). Contagious bovine pleuropneumonia. FAO, Rome. Retrieved 09 November, 2011 from http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.09_CBPP.pdf

Hutyra, F., Marek, J., and Manninger, R. (1938). *Special pathology and therapeutics of the diseases of domestic animals*. 5th Edn. Bailliere Tindall, United States of America. Pp. 455-471.

Ibrahim, M. (1984). Veterinary Traditional Practice in Nigeria. *In*: von Kaufmann, R., Chater, S. and Blench, R. (Eds.), *Livestock Systems Research in Nigeria's Subhumid Zone,* pp. 189- 208. Proceedings of the second I.L.C.A. /N.A.P.R.I. symposium, Kaduna, Nigeria, October 29-November 4, 1984.

Iro, I. (2011). Grazing reserve development: A panacea to the intractable strife between farmers and herders. Retrieved 23 August, 2011 from http://www.gamji.com/fulani8.htm

Johansson, K.E., Persson, A. and Persson, M. (1998). Diagnosis of contagious caprine and contagious bovine pleuropneumonia by PCR and restriction enzyme analysis. *In: Proceedings of the International Symposium on Diagnosis and Control of Livestock Diseases Using Nuclear and Related Techniques "Towards Disease Control in the 21st century"*. International Atomic Energy Agency, Vienna, Austria, pp. 137-158.

Karimuribo, E., Kambarage, D.M., Leman, B.E., Kazwala, R.R., Mtambo, M.M. and Kusiluka, L.J.M. (1997). Factors influencing the effective control of contagious bovine pleuropneumonia in Tanzania. *In*: *Proceedings of the 15th Scientific Conference of the Tanzania Veterinary Association,* Arusha, Tanzania, 1-3 December, 1997.

Kayode, J. and Ogunleye, T.O., (2008). Checklist and status of plant species used as spices in Kaduna state, Nigeria. *African Journal of General Agriculture.* 4(1) 13-18.

KDSG (2011). Kaduna State Government. Retrieved 9 September, 2011 from http://www.kadunastate.gov.ng.

Khazanov, A. (1990). Pastoralism in the Past, Present, and Future: A Comparative View. *In*: Oslon, P. A. (Ed.), *the Struggle for the Land: Indigenous Insight and Industrial Empire in the Semiarid World*. The University of Nebraska Press, Lincoln, United States of America. Pp. 81-99.

Kostyal, D. A., Butler, G. H. and Beezhold, D. H. (1994). A 48-kilodalton *Mycoplasma fermentans* membrane protein induces cytokine secretion by human monocytes. *Infection and Immunity*, 62: 3793-3800.

Kusiluka, L.J.M., Ojeniyi, B., Friis, N.F., Kazwala, R.R. and Kokotovic, B. (2000). Mycoplasmas isolated from the respiratory tract of cattle and goats in Tanzania. *Acta Veterinaria Scandinavica,* 41: 299-309.

Kusiluka, L.J.M., Ojeniyi, B., Friis, N.F., Kokotovic, B., Ahrens, P. (2001). Molecular analysis of field strains of *Mycoplasma capricolum* subspecies *capripneumoniae* and *Mycoplasma mycoides* subspecies *mycoides*, small colony type isolated from goats in Tanzania. *Veterinary Microbiology*, 82: 27–37.

Kusiluka, L.J.M. and Sudi, F.F. (2003). Review of successes and failures of contagious bovine pleuropneumonia control strategies in Tanzania. *Preventive Veterinary Medicine*, 59: 113-123.

Lefevre, P.C. (2000). Contagious bovine pleuropneumonia. *In*: *Manual of Standards for Diagnostic Tests and Vaccines.* OIE, 12 Rue de Prony, 75017, Paris, France. Pp.123-133.

Le Goff, C. (1986). Technique immunoenzymatique appliqué au diagnostic sérologique de la péripneumonie. Note préliminaire. *Revue d'Élevage et de Medicine Vétérinaire des Pays Tropicaux,* 3 9 (2): 171-173.

Le Goff, C. and Thiaucourt, F. (1998). A competitive ELISA for specific diagnosis of contagious bovine pleuropneumonia (CBPP). *Veterinary Microbiology,* 60: 170-191.

Luhrmann, A., Deiters, U. and Skokowa J, *et al*. (2002). *In vivo* effects of a synthetic 2-kilodalton macrophage activating lipopeptide of *Mycoplasma fermentans* after pulmonary application. *Infection and Immunity,*70 (7):3785–3792.

Mariner, J.C., McDermott, J., Heesterbeek, J.A.P., Thomson, G., and Martin, S.W. (2006) A model of contagious bovine pleuropneumonia transmission dynamics in East Africa. *Preventive Veterinary Medicine*, 73: 55-74.

Mariner, J.C., McDermott, J., Heesterbeek, J.A.P., Thomson, G., Roeder, P.L. and Martin, S.W. (2005). A heterogeneous population model for contagious bovine pleuropneumonia transmission and control in pastoral communities of East Africa. *Preventive Veterinary Medicine,* 73: 75-91.

Martel, J.L., Perrin, M., Belli, P. and Froget, F.J. (1983). The clinical aspects of contagious bovine pleuropneumonia .*In:* Hall, S.A. (Ed.), The Diagnosis of Contagious Bovine Pleuropneumonia and Other Infections with *Mycoplasma mycoides* subspecies *mycoides.* European Commission, Luxembourg. Pp.4-7.

Masiga, W.N. and Domenech, J. (1995). Overview and epidemiology of contagious bovine pleuropneumonia in Africa. *Office International des Epizooties Revue Scientifique et Technique,* 14: 611-620.

Masiga, W.N., Windsor, R.S., and Read, W.C.S. (1972) A new mode of spread of bovine contagious pleuropneumonia. *Veterinary Record*, 90: 247-248.

Masiga, W.N., Domenech, J. and Windsor, R.S. (1996). Manifestation and epidemiology of contagious bovine pleuropneumonia in Africa. *Office International des Epizooties Revue Scientifique et Technique,* 15:1283-1308.

Mbuk, E.U., Ajogi, I., Bale, J.O.O. and Umoh, J.U. (2011). Prevalence of Brucella antibodies in migratory Fulani herds in Kaduna state, Nigeria. *Nigerian Veterinary Journal,* 32(1): 26-29.

Mia, G.M., Marini, C. and Vitelli, B.T. (1993). Use of monoclonal antibodies for the serological diagnosis of contagious bovine pleuropneumonia. *Selezione Veterinaria*, 34:867-874.

Miserez, R., Pilloud, T., Cheng, X., Nicolet, J., Griot, C. and Frey, J. (1997). Analysis of clinical samples of cattle experimentally infected with CBPP, using a highly sensitive nested PCR method for the specific detection of *Mycoplasma mycoides* subsp.*mycoides* SC. *Molecular and Cell Probes*, 11:103-111.

Mtui-Malamsha, N.J. (2009). Contagious bovine pleuropneumonia in the Maasai ecosystem of south-western Kenya: Evaluation of seroprevalence, risk factors and vaccine safety and efficacy. Unpublished PhD thesis. The University of Edinburgh, Scotland.

Nawathe, D.R. (1992). Resurgence of contagious bovine pleuropneumonia in Nigeria. *Office International des Epizooties Revue Scientifique et Technique,* 11(3): 799-804.

Newton, L.G. (1992). Contagious bovine pleuropneumonia in Australia: some historic highlights from entry to eradication. *Australian Veterinary Journal,* 69:306-317.

Nicholas, R. A. J., Bashiruddin, J. B., Ayling, R. D. and Miles, R. J. (2000). Contagious bovine pleuropneumonia: A review of recent developments. *Veterinary Bulletin* 70: 827-838.

Nicholas, R.A.J., Santini, F.G., Clark, N.M.A., Palmer, P., De Santis, P. and Bashiruddin, J.B. (1996). A comparison of serological tests and gross lung pathology for detecting contagious bovine pleuropneumonia in two groups of Italian cattle. *Veterinary Record*, 139: 89-93.

Nwanta, J.N. and Umoh, J.U. (1992). Epidemiology of contagious bovine pleuropneumonia (CBPP) in northern states of Nigeria: An update. *Revue d'Élevage et de Medicine Vétérinaire des Pays Tropicaux,* 45: 17-20.

OIE, (2008). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Retrieved 15 October, 2011 from http://www.oie.int/eng/normes/mmanual/A_summry.htm

Okaiyeto, S. O., Danbirni, S. , Allam, L., Akam, E., Pewan, S. B. and Kudi, A. C. (2011). On-farm diagnosis of contagious bovine pleuropneumonia in nomadic herds using latex agglutination test (LAT). *Journal of Veterinary Medicine and Animal Health,* 3(5): 62-66.

Onoviran, O. and Taylor-Robinson, D. (1979). Detection of antibody against *Mycoplasma mycoides* subsp. *mycoides* in cattle by an enzyme-linked immunosorbent assay. *Veterinary Record*, 105: 165–167.

Onu, J.E. (2004). Contagious bovine pleuropneumonia and lung condemnation in Sokoto metropolitan abattoir in Nigeria. *Bulletin of Animal Health and Production in Africa,* 52: 123–126.

Osiyemi, T.O. (1981). The eradication of contagious bovine pleuropneumonia in Nigeria: prospects and problems. *Bulletin of Animal Health and Production in Africa,* 29: 95-98.

Persson, A., Pettersson, B, Bölske, G. and Johansson, K.E. (1999). Diagnosis of contagious bovine pleuropneumonia by PCR-LIF and PCR-REA based on the 16S rRNA genes of *Mycoplasma mycoides* subsp. *mycoides* SC. *Journal of Clinical Microbiology,* 37:3815-3821.

Petterson, B., Uhle, N.M. and Johansson, K.E. (1996). Phylogeny of some mycoplasmas from ruminants based on 16S rRNA sequences and definition of new cluster within the hominis group. *International Journal of Systemic Bacteriology,* 46: 1093-1098.

Pilo, P., Frey, J. and Vilei, E.M. (2007). Molecular mechanisms of pathogenicity of *Mycoplasma mycoides* subsp. *mycoides* SC. *Veterinary Journal*, 174: 513-521.

Poumarat, F. (1998). Identification of Mycoplasmas by dot immunobinding on membrane filtration (MF Dot). *In:* Miles, R.J. and Nicholas, R.A.J. (Eds.), *Methods in Molecular Biology*, Vol. 104: Mycoplasma Protocols, Humana Press Inc., Totowa, New Jersey, United States of America.

Poumarat, F., Perrin, B., Longchambon, D., Le Goff, C. and Martel, J.L. (1989). Recherches sur l'origine de fausses réactions positives dans le diagnostic sérologique de la péripneumonie contagieuse bovine. *Revue d'Élevage et de Medicine Vétérinaire des Pays Tropicaux,* 42:371-378.

Provost, A., Perreau, P., Bread, A., Le Goff, C., Martel, J.L. and Cottew, G.S. (1987). Contagious bovine pleuropneumonia. *Office International des Epizooties Revue Scientifique et Technique,* 6: 625-679.

Rawadi, G. and Romanroman, S. (1996). *Mycoplasma* membrane lipoproteins induce proinflammatory cytokines by a mechanism distinct from that of lipopolysaccharide. *Infection and Immunity*, 64: 637-643.

Regalla, J. (1995). La Réaction de fixation du complément pour le diagnostique de la péripneumonie contagieuse bovine: application et interprétation des résultats. *Office International des Epizooties Revue Scientifique et Technique*, 14: 631-644.

Regalla, J., Caporale, V., Giovanni, A., Santinin, F., Martel, J. L. Penha Goncalves., A. (1996a). Manifestation and epidemiology of contagious bovine pleuropneumonia in Europe. *Office International des Epizooties Revue Scientifique et Technique,* 15:1309-1325.

Regalla, J., Gonçalves, R., Ribeiro, N., Duarte, L. and Penha, A. (1996b). Use of immunoblotting test for carrier state definition in contagious bovine pleuropneumonia. *In:* Frey, J. and Sarris, K. (Eds.) *Mycoplasmas of Ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular Genetics,* European Commission, Luxembourg. P2p. 72-74.

Rwenyemamu, M.M. and Benkirane, A. (1996). Global impact of infections with organisms of the "*Mycoplasma mycoides* cluster" in ruminants. *In:* Frey, J. and Sarris, K. (Eds.) *Mycoplasmas of Ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular Genetics*. European Commission, Luxembourg. Pp. 1-11.

Santini, F.G., D'Angelo, A.R., Scacchia, M., Di Giannatale, E., Visaggio, M.C., Farinelli, G., Di Francesco, G. and Guarducci, M. (1992). Sequestro polomonare in un bufalo domestico da *M. mycoides* subsp. *mycoides* SC: isolamento, quadro anatomo-istopatologico ed immunoistochemico. *Veterinaria Italiana,* 4:4-10.

Scanziani, E., Paltrinieri, S., Boldini, M., Grieco, V., Monaci, C., Giusti, A.M. and Mandelli, G. (1997). Histological and immunohistochemical findings in thoracic lymph nodes of cattle with contagious bovine pleuropneumonia. *Journal of Comparative Pathology,* 117:127-136.

Scudamore, J.M. (1976). Observations on the epidemiology of contagious bovinepleuropneumonia: *Mycoplasma mycoides* in urine. *Research in Veterinary Science*, 20: 330-333.

Srivastava, N.C., Thiaucourt, F., Singh, V.P., Sunder, J. and Singh, V.P. (2000). Isolation of *Mycoplasma mycoides* small colony type from contagious caprine pleuropneumonia in India. *Veterinary Record,* 147: 520–521.

Stone, S.S., Masiga, W.N. and Read, W.C.S. (1969). Transplacental transmission of *Mmm*SC in cattle. *Research in Veterinary Science*, 10: 368-372.

Tambi, N.E., Maina, W.O. and Ndi, C. (2006). An estimation of economic impact of contagious bovine pleuropneumonia (CBPP) in Africa. *Office International des Epizooties Revue Scientifique et Technique,* 25: 999-1011.

Tambuwal, F.M., Egwu, G.O., Sharubutu, G.H., Junaidu, A.U., Rambo, U.G. Ibrahim, M.L. And Aliyu R M (2011). An Appraisal of Awareness of Contagious Bovine Pleuropneumonia (CBPP) amongst Settled and Semi-Settled Farmers and Pastoralists in the Two Cattle Producing States (Sokoto and Kebbi), Nigeria. *Nigerian Veterinary Journal,* 32 (3): 208-213.

Thiaucourt, F., Geiger, R., Pini, A., Prozeski, L., Regalla, J., Roger, F., and Yaya, A. (1999). Validation of a competitive ELISA for contagious bovine pleuropneumonia. *Mycoplasmas of Ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular Genetics*. European Commission, Luxembourg. Pp. 152-154.

Thiaucourt, F., Dedieu, L., Maillard, J.C., Bonnet, P., Lesnoff, M., Laval, G. and Provost, A. (2003). Contagious bovine pleuropneumonia vaccines, historic highlights, present situation and hopes. *In*: Brown, F. and Roth, J. (Eds.), Vaccines for OIE List A and Emerging Animal Diseases, Vol. 4. Basel, Karger. Pp. 147-160.

Thiaucourt, F., Yaya, A., Wesonga, H., Huebeschle, O.J.B., Tulanse, J.J. and Provost, A. (2000). Contagious bovine pleuropneumonia: A reassessment of the efficacy of vaccines used in Africa. *Annals of the New York Academy of Sciences,* 916: 71-80.

Thomson, G.R. (2005). Contagious bovine pleuropneumonia and poverty: a strategy addressing the effects of the disease in sub-Saharan Africa. In: *Research Report,* Animal Health Programme of the Department For International Development, Centre for Tropical Veterinary Medicine, University of Edinburgh, Scotland, Pp.1-13.

Thrusfield, M.V. (2007). *Veterinary Epidemiology*, 3rd Edn. Blackwell Publishing, Oxford, England, Pp. 178-180.

Trichard, C.J.V., Basson, P.A., Vanderlugt, J.J. and Jacobsz, E.J. (1981). An outbreak of contagious bovine pleuropneumonia in the Owambo mangetti area of south west Africa/Namibia: microbiological, immunofluorescent, pathological and serological findings. *Onderstepoort Journal of Veterinary Research*, 56: 277-284.

Turner, A.W. and Campbell, A.D. (1937). A note on the application of complement fixation test to the control of contagious bovine pleuropneumonia. *Australian Veterinary Journal,* 13:83.

Useh, N.M., Nok, A.J., Ibrahim, N.D.G., Adamu, S. and Esievo, K.A.N. (2008). Haematological and some novel biochemical changes in Zebu cattle with black leg. *Bulgarian Journal of Veterinary Medicine,* 11(3): 205-211.

Vilei, E.M and Frey, J. (2001). Genetic and biochemical characterization of glycerol uptake in *Mycoplasma mycoides* subsp. *mycoides* SC: its impact on H_2O_2 production and virulence. *Clinical and Diagnostic Laboratory Immunology,* 8:85-92.

Waller, J., McCaffery, K. and Wardle, J. (2004). Measuring cancer knowledge: comparing prompted and unprompted recall. *British Journal of Psychology* 95(2): 219-234

Wanyoike, S.W. (1999) Assessment and Mapping of Contagious Bovine Pleuropneumonia in Kenya: Past and Present. Unpublished Masters of Science

in Tropical Veterinary Epidemiology Thesis, Freie Universitat Berlin, Germany and Addis Ababa University, Ethiopia.

Wanyoike-Kairu, S.W., Bengat, A.K. and Lung'aho, W (2004). The use of the field complement fixation test in the diagnosis and control of contagious bovine pleuropneumonia the Kenyan experience – successes and failures. *In: Proceedings of the third FAOOIE- AU/IBAR-IAEA Consultative Group meeting on CBPP in Africa*, Rome, Italy. 12-14 November, 2003.

Waters-Bayer, A. (1986) Dairy Subsector of the Agropastoral Household Economy. In: von Kaufmann, R., Chater, S. and Blench, R. (Eds.), *Livestock Systems Research in Nigeria's Subhumid Zone*. Proceedings of the second I.L.C.A./N.A.P.R.I. symposium, Kaduna, Nigeria, October 29-November 4, 1984. Pp. 414-426.

Weinsburg, W.G., Tully, J.G., Rose, D.L., Petzel, J.P., Oyaisu, H., Yang, D., Mandelco, L., Sechrest, J., Lawrence, T.G. and Van Etten, J.E.A. (1989). A phylogenetic analysis of the mycoplasmas: basis for their classification. *Journal of Bacteriology,* 171: 6455-6467.

Westberg, J., Persson, A, A., Holmberg, A., Goesmann, A., Lundeberg, J., Johasson, K.E., Pettersson, B. and Uhlem, M. (2004). The genome of *Mycoplasma mycoides* subsp. *mycoides* SC type strain PG1, the causative agent of contagious bovine pleuropneumonia (CBPP). *Genome Research,* 14: 221-227.

Windsor, R.S. and Masiga, W.N. (1977a). Investigations into the epidemiology of CBPP: the persistence of *Mycoplasma mycoides* subsp. *mycoides* in placenta, urine and hay. *Bulletin of Epizootic Diseases of Africa*, 25: 357-370.

Windsor, R.S. and Masiga, W.N. (1977b). Investigation into the role of carrier animals in the spread of contagious bovine pleuropneumonia. *Research in Veterinary Science*, 23: 224-229.

APPENDIX I:

BLOOD SAMPLE COLLECTION FORM

S/No	Date Collected	Herd No	Animal No	Village	Ward	Age	Sex	Remark

APPENDIX II:

SAMPLE QUESTIONNAIRE

Serial Number_____ Date of Interview_____
Interviewer_____

Section A: General

A1; Village_____ A2; Ward_____

A3; Local Govt Area_____

Section B; Cattle Diseases

B1; What cattle diseases do you often experience in your herd?

1_____

2_____

3_____

4_____

4_____

6_____

7_____

8_____

9_____

10_____

B2; Do you know CBPP? Yes ☐ No ☐ (*ask only if CBPP is not mentioned in B1 above*)

B3; What sign(s) of CBPP do you know? 1; Respiratory signs (coughing, nasal discharges, dyspnoea) ☐ 2; Extension of head and neck ☐ 3; Emaciation ☐; Arthritis ☐

5; Others ☐(specify)_____

B4; In which year(s) did you have CBPP in your herd?

	CBPP Occurred (Yes/No)	**Number Affected**	**Number Died**	**Intervention**
3 years ago				
Last year				
This year				

Intervention: Treatment (T), Vaccination (V), Report to Vet officer (R), Nothing (N)

B5; What type of medicine do you use to treat CBPP? Antibiotics ☐ Traditional medicine ☐

Others ☐(specify)_____

B6; Do you vaccinate your cattle against diseases? Yes ☐ No ☐

(*If yes, please indicate the vaccinations done in the last 12 months*)

Disease	**Who vaccinated**	**Age Group Vaccinated**			**Cost/Animal (□)**
		□ 1 year	1-3 years	> 3 years	

Who vaccinated; Self (S), Govt vet (G), Private vet (P)

B7; Are there CBPP vaccinated animals that have CBPP? Yes ☐ No ☐

B8; Did CBPP vaccinated animals have adverse reaction(s) to the vaccines? Yes ☐ No ☐

(*If yes, what kind of reactions*)

Specific reactions (swelling, wound at injection site) ☐ Non specific reactions (weakness, lameness, abortion, mortality) ☐ Others (specify)_____

B9; Do you know CBPP control measures? Yes ☐ No ☐

(*If yes, what are these?*)

Vaccination ☐ Slaughter ☐ Quarantine ☐ Others (specify)_____

B10; How did you know these measures?

Govt agent ☐ Mass media ☐ Fellow herdsmen ☐ Others (specify)_____

Section C; Herd Data

C1; Herd size

Type of Animal	**Size (Number)**
Cattle	
Sheep	
Goat	
Dogs	

C2; What are the changes in your herd for the last 12 months?

Change	**Yes/No**
Bought in	
Sold out	
Gift in	
Brought back from market	

C3; Do your cattle mix with others at grazing (Yes ☐ No ☐

C4; Do your cattle mix with others at drinking points (Yes ☐ No ☐

C5; How do you handle animals that are not reasonably priced in markets?

Quarantine 1st before readmission ☐ Readmit into herd directly ☐

Others (specify)_____