

**SERO-PREVALENCE AND AWARENESS OF CONTAGIOUS BOVINE  
PLEUROPNEUMONIA IN CATTLE IN THREE SELECTED LOCAL  
GOVERNMENT AREAS OF KADUNA STATE, NIGERIA**

**BY**

**Ishaya La'ah BILLY, DVM (A.B.U) 1992  
M.Sc. /VET-MED/2252/2009-2010**

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## DECLARATION

I declare that the work in this thesis entitled “**Sero-Prevalence and awareness of Contagious Bovine Pleuropneumonia in Cattle in three selected Local Government Areas of Kaduna State, Nigeria**” has been performed by me in the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree, diploma or certificate at any institution.

Ishaya La’ah BILLY  
Name of student

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Signature

-----  
Date

## CERTIFICATION

This thesis entitled “**SERO-PREVALENCE AND AWARENESS OF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN CATTLE IN THREE SELECTED LOCAL GOVERNMENT AREAS OF KADUNA STATE, NIGERIA**” by Ishaya La’ah BILLY meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

<b>Prof. A. K.B. Sackey</b> Chairman Supervisory Committee	..... Signature	..... Date
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<b>Prof. L. B. Tekdek</b> Member Supervisory Committee	..... Signature	..... Date
---	--------------------	---------------

<b>Dr. S. N. A. Saidu</b> Member Supervisory Committee	..... Signature	..... Date
---	--------------------	---------------

<b>Prof. L. B. Tekdek</b> Head, Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.	..... Signature	..... Date
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<b>Prof. A.Z. Hassan</b> Dean, School of Postgraduate School, Ahmadu Bello University, Zaria.	..... Signature	..... Date
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## **DEDICATION**

This Thesis is dedicated to my dear wife Mrs Elizabeth Hauwa, daughters, Miss Joy Amams and Late Blessing Mama and my late parents Mr and Mrs Billy La'ah.

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## ABSTRACT

This study was conducted to determine the sero-prevalence and awareness of Contagious Bovine Pleuropneumonia (CBPP) in cattle in 3 selected Local Government Areas (LGAs), (Ikara, Chikun and Kauru) of Kaduna State, involving 900 heads of cattle, using Latex Agglutination Test (BoviLAT PA 6223), developed by Ayling *et al* (1999). Ninety structured questionnaires corresponding to the 90 herds sampled were also administered to herdsmen. Selection and sampling was done using the simple random sampling without replacement technique. An overall sero-prevalence of 26.0% (234/900) was achieved, comprising of 46.0% (138/300), 17.0% (51/300) and 15.0% (45/300) for Kauru, Ikara and Chikun LGAs respectively. Kauru LGA had a higher sero-prevalence than the other 2 LGAs, with statistical significant difference ( $P < 0.05$ ). The sero-prevalence was highest (30%) in the age group of >6 years old and lowest (19.3%) in the age group <1-3 years old. The difference in the infection of CBPP by age was statistically significant ( $P < 0.05$ ). Female cattle had a sero-prevalence of 27.1%, while, male cattle had 21.9% sero-prevalence. The difference in sero-prevalence was not statistically significant ( $P > 0.05$ ). The Sokoto Gudali showed a sero-prevalence of 57.1%, followed by the Red Bororo and White Fulani with sero-prevalence of 50% and 25.3% respectively. Though the sero-prevalence showed a reasonable level of difference but, there was no statistical significant difference in the CBPP infection among the breeds sampled ( $P > 0.05$ ). The study also revealed the level of awareness to CBPP in the Pastoralists to be 80(88.9%) and formal education of 11(12.2%). Knowledge of CBPP in the communities showed 66(73.3%). Those that experienced outbreaks as well as lossed animals to CBPP were 25(27.8%) and 17(18.9%) respectively. Of the respondents, 67(74.4%) indicated

vaccinating their cattle at various times, which is not regular, thereby achieving a low vaccination coverage of 36.7%, while, 23(25.5%) do not vaccinate at all. Forty-six (51.1%) acquired the CBPP vaccines from NVRI, Vom, Plateau State, while, 21(23.3%) from the open market. Personnel used in the administration of vaccine were, 14(15.6%) Veterinarians, 18(20%) Animal health workers, 18(20%) drug vendors and 40(44.4%) herdsmen. The use of antibiotics in treatment of CBPP cases was found to be a general practice. CBPP is present in the study area, and low level of formal education (12.1%) and the use of unqualified personnel (66.4%) in the administration of vaccine are some of the factors that might be responsible for the low vaccination coverage as revealed in this study. A comparative study between this method (BoviLAT) and other serum analysis techniques recommended by OIE, should be carried out for adoption of this method as a routine test for CBPP epidemiological study on a wider scale. Pastoralists are encourage to intensify the pursue for formal education.

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## ABBREVIATIONS AND SYMBOLS

ABU	Ahmadu Bello University
Ag	Antigen
AGID	Agar gel Immunodiffusion Test
AU	African Union
BoviLAT	Bovine Latex Agglutination Test
CBPP	Contagious Bovine Pleuropneumonia
CCPP	Contagious Caprine Pleuropneumonia
C-ELISA	Competitive Enzyme-linked Immunosorbent Assay
CFT	Complement Fixation Test
CPS	Capsular Polysaccharides
DGIT	Disk Growth Inhibition Test
DLPCS	Department of Livestock and Pest Control Services
DNA	Deoxynucleic acid
ELISA	Enzyme-linked Immunosorbent Assay
EMPRES	Emergency and prevention system for transboundary Animal and plants pests/diseases
EU	European Union
FAO	Food and Agricultural Organizations of United Nations
FAT	Flourescent Antibody Test
FCT	Federal Capital Territory
FDL	Federal Department of Livestock
FMD	Foot and Mouth Disease
G	Gauge

IAEA	International Atomic Energy Agency
IBAR	Inter African Bureau for Animal Resources
IBT	Immunoblotting Test
IFA	Immunoflourescent Antibody
IFAT	Immunoflourescent Antibody Test
IgA	Immunoglobulin Antibodies
IgG	Immunoglobullin subclass G
IgM	Immunoglobullin subclass M
IHC	Immunohistochemistry
ISCOMS	Immune Stimulating Complexes
IU	International Units
JP-28	Joint Project 28
KADP	Kaduna State Agricultural Development Project
kDa	kildalton
KDSG	Kaduna State Government
LAT	Latex Agglutination Test
LGA	Local Government Area
LPPQ	Lipiprotein
Mabs	Monoclonal Antibodies
<i>MCCP</i>	<i>Mycoplasma Capri</i> subsp. <i>capripneumonia</i>
MDGs	Melinium Development Goals
MF-dot	Membrane Filtration dot
ml	Millilitres
<i>MmmLC</i>	<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> Large Colony
<i>MmmSC</i>	<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> Small Colony

NADIS	National Animal Disease Information System
NIAS	Nigerian Institute of Animal Science
NLR	National Livestock Reports
NLR	National Livestock Resources
NO <sub>2</sub>	Nitrogen (iv) oxide
NVMA	Nigerian Veterinary Medical Association
NVRI	National Veterinary Research Institute
OAU-IBAR	Organisation of African Unity/Inter African Bureau for Animal Resources
°C	Celsius
OIE	Office Internationale des Epizootics. World Organisation for Animal Health
PANVAC	Pan-African Veterinary Vaccine Center
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reactions
PM	Post-mortem
PPLO	Pleuropneumonia-like Organisms
RNA	Ribonucleic acids
rRNA	ribosomal Ribonucleic acids
SADC	South African Disease Control
SAT	Slide Agglutination Test
TADs	Trans boundary Animal Disease
Th 1	Thymus cell 1
Th 2	Thymus cell 2
TMB	Tetramethyl Benzidine
TM	Terramycin
UAES	Unified Agricultural Extension System

<i>UI</i>	Microlitres
US	United States
USAHA	United States Animal Health Association
VNTR	Variable-Number Tandem Repeat
>	Greater than
<	Less than
%	Percentage

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Historical Background

Contagious bovine pleuropneumonia (CBPP) is an important highly contagious mycoplasma disease with devastating consequences, caused by *Mycoplasma mycoides* subsp. *mycoides* Small Colony (*MmmSC*) on cattle (Egwu *et al.*, 1996; Tambi *et al.*, 2006; Tambuwal *et al.*, 2011a; Thiaucourt *et al.*, 2011).

The first mollicute to be isolated, which became the type of *Mycoplasma*, was isolated from cattle affected by contagious bovine pleuropneumonia (Nocard and Roux, 1898). It was later named *Mycoplasma mycoides* (Edward and Freundt, 1956).

The disease has been known to occur in Europe since the 16<sup>th</sup> century, but it gained a world-wide distribution only during the second half of the 19th century, because of increased international trade in live cattle (Nicholas and Bashiruddin, 1995).

In natural conditions, *MmmSC* affects only the ruminants of the *Bos* genus. The organism has been isolated from buffaloes (*Bubalus bubalus*) in Italy (Santini *et al.*, 1992; Halle *et al.*, 1998). Among wild animals, only a single case has been reported in American buffaloes (*Bison bison*). The organism has similarly been isolated from sheep and goats in Africa, and more recently in Portugal and India (Srivastava *et al.*, 2000). Wild animals do

not seem to play any significant role in the epidemiology of the disease (Santini *et al.*, 1992).

It was eradicated from many countries such as Western Europe, the United States of America (USA) and Japan by the end of the 19th and beginning of 20th century, through vigorous control efforts, involving stamping out and strict control of animal movement (Provost *et al.*, 1987). Australia succeeded in eradicating the disease in 1973, after being present in the country for 100 years (Regalla *et al.*, 1996). The last outbreak was reported in Portugal in 1999, although its re-emerging potential is ardent (Nicholas *et al.*, 2000). It has been reported in recent times from India, Bangladesh and Myanmar (OIE, 2008). In Italy the disease reappeared in 1990, but was eliminated in 1993, while, in Spain, the last case was recorded in 1994 (FAO/OIE, 1995).

There have been no reported outbreaks in Europe since 1999 (Srivastava *et al.*, 2000). Although CBPP has been eradicated from most continents, the disease still persists in Africa, especially West, Central, East and parts of Southern Africa and, has remained endemic in Nigeria, with massive spread across the country (Tambi *et al.*, 2006, Anon, 2008; Thiaucourt *et al.*, 2011).

It is a major threat for cattle health and production and also one of the most significant epidemic disease of cattle in Africa, where it has been reported in 17 countries in 2001 (OIE, 2002) and 27 countries in 2002 (Tambi *et al.*, 2006).

The first incidence of CBPP in Nigeria was recorded in 1924, and since then, it has remained endemic in the country (Foluso, 2004). The disease has been reported in Kano, Katsina, Borno, Sokoto and Kaduna states of Nigeria (Nawathe, 1992). In Nigeria “a live with the disease” attitude has always prevailed in the last few years. Farmers hardly report cases but resort to treatment with antibiotics like any other bacterial disease (Chima *et al.*, 1999). Transmission occurs through direct, close, repeated contacts between diseased and healthy susceptible animals in shared accommodation or at water points, dip tanks, markets, common grazing or gathering places (Okaiyeto *et al.*, 2011).

In groups of susceptible cattle, the morbidity approaches 90%, while, case mortality rate may be as high as 50% and that 20% of the infected cattle may remain carriers with or without clinical signs (Radostits *et al.*, 2003). Source of infection is often provided by recovered ‘carrier’ animals in which, a pulmonary sequestrum preserves a potential source of the organisms for periods as long as 3 years (Brandao, 1995).

The traditional way of rearing cattle in Nigeria, like in other developing countries, has over the years contributed to the spread of the disease, especially the interaction of animals during grazing and watering (Okaiyeto *et al.*, 2011). During this interaction, infectious and air borne diseases, such as CBPP are transmitted between and within herds (Okaiyeto *et al.*, 2011).

There is considerable variation in the severity of clinical disease, from hyper-acute to acute to sub-acute and chronic forms (Radostits *et al.*, 2003).

Several diagnostic techniques have been developed for the diagnosis of CBPP. These include; culture and isolation of the causative agent, post-mortem/necropsy findings of lungs of suspected animals and serology using the Modified Complement Fixation Test (CFT), (OIE 2002; 2008). Others include Biochemical Assay (Rice *et al.*, 2000), ELISA and Immunoblot techniques (Timothy *et al.*, 2005) and Polymerase Chain Reaction (PCR) (Bashiruddin *et al.*, 1994a, 1999a; Persson *et al.*, 1999).

In Nigeria, pastoralists have adequate knowledge/experience, about common health problems affecting their livestock including disease treatment and prevention (Adekunle *et al.*, 2002). This knowledge is, however, based on oral transmission, shared information and life experience of individual herders on their livestock over the years (Catley and Mariner, 2002).

## **1.2 Statement of the Research Problem**

To provide adequate animal protein to the increasing human population, especially with regards to the FAO recommendations, on animal protein intake per head, might not be achieved, as a result of low productivity caused by CBPP (FAO/OIE, 1997). This is due to the inability to diagnose the disease in the field, which could have help in early introduction of control measures to avoid the mortality, reduced milk production, meat quality/quantity, and draft power caused by CBPP.

Diagnosis of CBPP in most developing countries of Sub-saharan Africa is presently based on culture and isolation of the causative agent, serology- Modified Complement Fixation Test (CFT), ELISA, Polymerase Chain Reaction (PCR) and post-mortem (PM)

examination of lungs of suspected animals (Nicholas *et al.*, 1996). This can be time consuming and require complex, non-mobile laboratory settings, trained personnel and are capital intensive (Timothy *et al.*, 2005). Application for CBPP diagnosis in the field using these techniques are impracticable.

Cattle keepers/owners in Nigeria are mostly the pastoralists who practice mostly nomadism and transhumance which makes it difficult for them to benefit from the current diagnostic techniques for the diagnosis of CBPP, hence the increase in the spread of the disease.

Veterinary staff cannot facilitate herd screening in efforts to control and prevent the spread of the disease in the field, due to lack of easy-to-use, rapid, field diagnostic kit.

There are many livestock diseases endemic in the area of study. Therefore, identification and understanding of the nature of CBPP need to be investigated in the pastoralists.

### **1.3 Justification**

Contagious bovine pleuropneumonia (CBPP) is believed to be the most important transboundary animal disease after rinderpest, and now endemic in the Sub-saharan Africa. It is consistently ranked amongst the most important livestock diseases, by regional and international authorities and cattle keepers alike. It is a list A disease that need early detection for control measures to be put in place. Both Food and Agriculture Organization (FAO) and African Union-International Bureau for Animal Resources (AU-IBAR) consider improved diagnostic tests and vaccines for CBPP to be a research priority (OIE, 2008).

The disease is rapidly spreading over new areas in the Sub-saharan Africa as a result of unrestricted cattle movements, this call for early diagnosis (OIE, 2009). The disease was first reported in 1924, in Nigeria, and has remain endemic with records of pockets of outbreaks, occurring in different parts of the country, particularly the northern part, where most of the cattle populations are located (Osiyemi, 1981; Fayomi and Aliyu, 1992; FAO/OIE, 1995; Nwanta and Umoh, 1995; Ameh *et al.*, 1998).

Most cattle in Nigeria are owned by nomadic Fulani, who are mainly on transhumance during the greater part of the year. Their movement that could be for long distances can be said to enhance the spread of the disease (Aliyu *et al.*, 2000). The disease causes severe economic losses to farmers in Nigeria, and to the livestock industry as a whole, in spite of years of attempts at vaccination which has been inconsistent (Aliyu, *et al.*, 2000). The direct economic losses due to the disease in Nigeria, has been shown to affect mostly the beef sector without sparing the dairy sector, in the form of mortality and reduction in beef and milk production and draft power, reduced fertility and loss of market opportunities, due to trade bans. Such losses have been put at US\$3.6 Million annually (Osiyemi, 1981; Rweyemamu and Benkirane, 1996). In northern Nigeria alone it is put at US\$1.5Million annually (Egwu *et al.*, 1996).

Considering the devastating economic implications of CBPP in Nigeria, and with the escalating trend of the highly contagious disease, it is necessary that more attention be given to it by improving on the early diagnosis of the disease, so as to reduce the economic impact of the disease.

The need for up to date information on CBPP in Kaduna State, as it is the most important cattle disease after eradication of rinderpest cannot be overemphasized. Therefore, this study is designed to evaluate the level of CBPP infection in the field using BoviLAT Latex Agglutination Test as an alternative to the existing ones, in three selected Local Government Areas of Kaduna State, Nigeria.

#### **1.4 Aim of the Study**

The study is aimed at determining the sero-prevalence of CBPP in cattle in three selected Local Government Areas of Kaduna State, Nigeria, using the BoviLAT Latex Agglutination Test.

#### **1.5 Objectives of the Study**

- a. To determine the sero-prevalence of CBPP in cattle using the BoviLAT Latex Agglutination Test, in three selected Local Government Areas of Kaduna State, Nigeria.
- b. To determine the awareness, knowledge, attitude and practices of pastoralists on CBPP using structured questionnaire, in three selected Local Government Area of Kaduna State, Nigeria.

#### **1.6 Research Questions**

- a. Can the use of BoviLAT Latex Agglutination test detect the presence of antibodies to CBPP in cattle in the field?

b. Do pastoralists in the three selected Local Government Areas have the awareness of  
CBPP in cattle?

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 General Perspective of CBPP

Nigeria has the highest cattle population of about 16.3 million in West Africa (Ikhatua, 2011), and is constantly threatened with contagious bovine pleuropneumonia (CBPP) (Ajuwape *et al.*, 2004; Tambuwal, 2009). The disease is highly contagious in cattle and is caused by *Mycoplasma mycoides* subsp. *mycoides* small colony (*MmmSC*) (Mbulu *et al.*, 2004; Yaya *et al.*, 2008). The disease is usually spread by movements of animals across international boundaries with devastating consequences on cattle, particularly in severe outbreaks (Egwu *et al.*, 1996; Huebschle *et al.*, 2004; Tambi *et al.*, 2006; Thiaucourt *et al.*, 2011 and Tambuwal *et al.*, 2011b). On account of its contagiousness, the potential for its rapid transboundary spread and the associated economic impacts, CBPP is now the most important amongst the OIE list A diseases after rinderpest (OIE, 2008). For similar reasons, CBPP is included in the list of 6 priority diseases for FAO's EMPRES-Livestock programme (FAO/OIE, 1995). In addition, the second priority vaccine for the Pan African Veterinary Vaccine Centre (PANVAC) is that for CBPP (PANVAC, 1991; OIE, 1996).

#### 2.2 Distribution

##### 2.2.1 Contagious bovine pleuropneumonia in Europe

Historically, CBPP was a disease of Europe and Asia (Tambi *et al.*, 2006). It has been known to occur in Europe since the 16th century but it gained a world-wide distribution only during the second half of the 19th century because of increased international trade in

live cattle (Egwu *et al.*, 1996). In spite of strict sanitary control measures, it re-emerged in Europe in the 1980s and 1990s in countries like Spain and Portugal, thus posing a serious threat to animal health and livestock production around the world (Masiga *et al.*, 1999; Regalla, 2011). However, effective control policies made eradication a success (Nicholas and Basiruddin, 1996).

### **2.2.2 Contagious bovine pleuropneumonia in Africa**

The disease was reported to have been introduced into East, Central and West Africa from India in the 19th century (Provost *et al.*, 1987). It is currently endemic in most parts of these countries and is spreading fast towards the Southern part of Africa, especially Zambia and Namibia, where it is responsible for huge economic losses (Musisi *et al.*, 2011). Endemic infection extends throughout the pastoral herds of much of Western, Central and Eastern Africa, with Angola and northern Namibia in Southern Africa (Mtui-Malamsha, 2009). In the 1990s, newly infected areas included much of Uganda, parts of Kenya, the Democratic Republic of the Congo and most of Tanzania, with the disease spreading alarmingly to Rwanda (1994), Burundi (1997), Botswana (1995) and Zambia (1997) (OIE, 2002). By 2001, it was reported in 17 African countries and has spread to 27 countries in 2002 (OIE, 2009). Of the 27 countries that reported cases of CBPP between 1995 and 2002, 13 were in West Africa, 2 in Central Africa, 6 in East Africa and the rest in Southern Africa. In West, Central and East Africa where the Pan-African Programme for the Control of Epizootics (PACE) programme was being implemented, a total of 2,719 outbreaks were reported between 1995 and 2002 (PACE, 2003). Countries in East Africa reported 66% of the total outbreaks of which 58% was in Ethiopia and Tanzania and 8% in the other countries in the region (PACE, 2003). Countries in West and Central Africa accounted for

33% and 1% of the total number of outbreaks respectively (OIE, 2002, Tambi *et al.*, 2006). Between 2007 and 2008, CBPP was wide spread in 12 countries in Africa ([http://www.oie.int/wahis/public.php?page=disease\\_status\\_lists](http://www.oie.int/wahis/public.php?page=disease_status_lists) as accessed on 2nd April, 2009). The countries were Uganda, Tanzania and Ethiopia in East Africa and Nigeria, Niger, Chad, Benin, Ghana, Burkina Faso, Togo, Democratic Republic of Congo and Angola in Western and Central Africa. The disease had a zonal distribution as a result of its control in countries such as Burundi, Namibia, Zambia, Kenya and Mali (Thomson, 2003).

### **2.2.3 Contagious bovine pleuropneumonia in Nigeria**

The first incidence of CBPP in Nigeria was recorded in 1924 (Foluso, 2004). It is an endemic disease in Nigeria at present with pockets of outbreaks occurring in the Northern part of the country, where most of the cattle populations are located (Osiyemi, 1981; Fayomi and Aliyu, 1992; FAO/OIE, 1995; Ameh *et al.*, 1998). A prevalence of 0.29% was recorded by Aliyu *et al.* (2000), in a study of prevalence of CBPP in 5 Northern States of Nigeria. Danbirni *et al.* (2010) reported a prevalence of 47%, in a herd of cattle with concurrent infection of CBPP and bTB in Igabi LGA, Kaduna State, using LAT. Similarly, Okaiyeto *et al.* (2011) reported a prevalence of 16.7% and 17.5% for adults and young cattle respectively, in a herd of cattle with CBPP outbreak in Kafur LGA Katsina State, using LAT. A recent report by Ikhatua, (2011) has indicated that outbreaks of the disease still occur in Nigeria.

## **2.3 Epidemiology**

*Mycoplasma mycoides* subsp. *mycoides* Small Colony type (*MmmSC*) was isolated from a case of contagious bovine pleuropneumonia (CBPP) for the first time in France (Nocard

and Roux 1898). Since then it has been reported in different parts of the world (Das 1967; Hudson 1971; Cottew *et al.*, 1987; Guadagnini *et al.*, 1991). Outbreaks of this disease tend to exhibit two distinct epidemiological trends in Africa. The first is reflected in cases of epidemic outbreaks in areas hitherto considered to be CBPP-free. Botswana is a good example. Following the eradication of CBPP in 1939, the disease reappeared in 1994 (OIE, 2009). In 1995, the Government of Botswana once again eradicated the disease by slaughtering all infected and in-contact stock and compensated their owners. Other examples of epidemic outbreaks include those in Burundi and Zambia in 1997, Ethiopia in 1998, Guinea in 1995, Rwanda in 1994 and Tanzania in 1996 and 1999 (Thompson, 2003). They attributed these outbreaks to uncontrolled entry of cattle from known infected populations due to poor movement control and surveillance (Radostits *et al.*, 2003).

The second trend of CBPP outbreaks is spartial, and is attributed to the increased number of areas in which the disease has become endemic. Mortality rates can be as high as 50%, after some time however, the disease will have a less explosive character, the severity of the symptoms will decline and many animals will recover or become chronic carriers. In East Africa, Rwanda, Burundi, most parts of Tanzania, Southern Sudan, Ethiopia and Somalia have remained endemically infected (OIE, 2008). Neighbouring countries such as Malawi, Mozambique and Zambia are at risk. The disease has become endemic in Eastern Guinea (since its introduction into the north in 1974), Mali, Niger and Mauritania and is a threat to disease-free Senegal and Sierra Leone (Windsor and Masiga, 1977a). In Southern Africa, the presence of large endemic areas in Angola and Zambia constitutes a potential risk to Zimbabwe, Lesotho, Swaziland, Botswana and South Africa (OIE, 2008).

## 2.4 Aetiology

*Mycoplasma mycoides* subsp. *mycoides* Small Colony (*MmmSC*) belongs to the Genus; *Mycoplasma*, in the Family; *Mycoplasmataceae*, Order; Mycoplasmatales, Class; Mollicutes (Nicolet, 1996). *MmmSC* is the causal agent of contagious bovine pleuropneumonia (CBPP). It is an extra-cellular pathogen that lives in close association with the host cells. The organism is capable of self-replication and has a genome size of 1,211 kb and lacks a cell wall. It is pleomorphic and resistant to antibiotics of the beta-lactamase group, such as penicillin (Westberg *et al.*, 2004). Growth of the organism is relatively fastidious and requires special media that are rich in cholesterol (addition of horse serum) (OIE, 2009).

### 2.4.1 Classification of *MmmSC*

*Mycoplasma mycoides* subsp. *mycoides* biotype Small Colony (*MmmSC*) shares many biochemical immunological and genetic properties with six other mycoplasmas grouped under the so-called *Mycoplasma mycoides* cluster (Pettersson *et al.*, 1996). The members of the *M. mycoides* cluster which are specially difficult to differentiate due to phenotypic and genotypic features that cross react serologically, they include; i. *Mycoplasma capricolum* subsp. *Capricolum* that causes contagious agalactiae of sheep and goats by (*Mcc*), ii. *Mycoplasma mycoides* subsp. *capri*, (*Mmc*), including the recently reclassified serovar iii. *Mycoplasma mycoides* subsp. *mycoides* biotype Large Colony (*MmmLC*), iv. contagious bovine pleuropneumonia of cattle by *Mycoplasma mycoides* subsp. *mycoides* Small Colony (*MmmSC*) and v. contagious caprine pleuropneumonia of goats by *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) (Songer and Post, 2005). The sixth, recently reclassified *M. mycoides* cluster member, *Mycoplasma leachii* sp. nov. (formerly *Mycoplasma* sp.

*bovine group 7* of Leach) has been isolated from calves with pneumonia (Manso-Silvan *et al.*, 2009; Tardy *et al.*, 2009; Shahram *et al.*, 2010). Specific identification of *MmmSC* can now be achieved by polymerase chain reaction (PCR) or the use of specific monoclonal antibodies (MAbs). Although *MmmSC* has been considered to be a very homogeneous biotype, recent molecular techniques have identified differences among strains (Lorenzon *et al.*, 2001). The strains of European origin can be differentiated from the African ones by molecular methods, and are also not able to oxidise glycerol, which may account for an apparent lower pathogenicity (Miles *et al.*, 1994). African strains seem to be more diverse (Songer and Post, 2005). The list of *Mycoplasma mycoides* clusters is presented (Appendix II).

#### **2.4.2 Genus: Mycoplasma**

This group of organisms are widely distributed in mammals, birds, reptiles, fish, and plants, and require intimate association with the host cell surfaces for growth (Songer and Post, 2005). Until recently, these agents were considered to be strict pathogens of the mucous membranes, associated mainly with respiratory, arthritic, or genitourinary tract diseases (Songer and Post, 2005). That dogma has changed with recognition of a new group, the hemotrophic mycoplasmas or hemoplasmas, which parasitize erythrocytes and are refractory to cultivation on solid media (Weinsburg *et al.*, 1989).

The genus *Mycoplasma* comprises of more than 100 species some of which cause chronic diseases in animals and humans (Neimark *et al.*, 2001). The majority of mycoplasmas have species-specific host-organism associations or tropisms for particular anatomic sites (Nicholas and Ayling, 2003). Lack of a cell wall account for their plasticity and allows them to pass through filters with pore sizes as small as 450 nm, despite cell size ranging

from 0.3 to 0.8  $\mu\text{m}$  mycoplasmas are structurally simple, in that they consist of ribosome and DNA bound by a trilaminar cytoplasmic membrane composed of sterols, phospholipids, and proteins (Nicholas *et al.*, 2000).

Sequence analysis and phylogenetic studies of the 16S ribosomal RNA (16S rRNA) genes have shown that mycoplasmas are closely related to the Bacillus-Lactobacillus-Streptococcus group, suggesting that they have descended from Gram positive bacteria (Weisburg *et al.*, 1989). It is not clear whether the presence of *MmmSC* in sheep and goats was associated with the observed diseases (Brandão, 1995; Kusiluka *et al.*, 2000a; Srivastava *et al.*, 2000).

Mycoplasmas have complex requirements because of their limited capabilities and depend on *in-vivo* host microenvironment. Most genes for amino acid and co-factor biosynthesis were lost during evolution. Exogenous fatty acids are required. The latter separates the family Mycoplasmataceae from similar organisms and is useful in their identification because it renders them sensitive to digitonin (Nicholas and Ayling, 2003). In recent years, more than 20 species of *Mycoplasmas* have been isolated from cattle with different diseases (Razins, 1991). It is generally believed that Mycoplasmas play a secondary role in infections. However, it has been shown that *Mycoplasma bovis* (*M. bovis*) can play a primary role. This organism *M. bovis* is considered as one of the more pathogenic species that causes pneumonia, mastitis, and arthritis in cattle (Nicholas, *et al.*, 2000).

## 2.5 Host Specificity

Under natural conditions, CBPP occurs in cattle and perhaps in other allied animals including buffalo, yak, bison and even reindeer (Hutyra *et al.*, 1938). However, Provost (1988), reviewing the literature could find no evidence to show that the domestic buffalo *Bubalus bubalis* was susceptible under natural or experimental conditions. 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), observed pulmonary lesions and isolated *MmmSC* from sero-positive buffaloes which had been in contact with CBPP affected cattle in Italy. They concluded that buffaloes were susceptible albeit at a low level and that further research was necessary to clarify the role of buffaloes as a reservoir of infection for cattle. Infections have also been reported from Asian buffalo (*Bubalus bubalis*), captive bison (*Bison bison*) and yak (*Poephagus grunniens*, formerly *Bos grunniens*) (Santini *et al.*, 1992). The African water buffalo (*Syncerus caffer*) is resistant to *MmmSC* (Provost, 1988). Wild bovids and camels seem to be resistant, and, so far, do not appear to be important in the spread of CBPP (Santini *et al.*, 1992). Small ruminants, in particular goats, have also been shown to harbor *MmmSC* (Hudson, 1971). Brandao (1995) isolated *MmmSC* from the milk of sheep with mastitis, as well as from goats with pneumonia in Portugal outside the endemic region of CBPP. Experiments with goats in contact with cattle infected by the African strain Afadé showed clear genetic differences with European strains, suggest a lack of susceptibility in the goat (Cheng *et al.*, 1995). Egwu *et al.* (2012), isolated mycoplasmas with special reference to *MmmSC* from the pneumonic lesions of sheep and goats. They demonstrated that *M. mycoides* subsp. *mycoides* SC can be harboured by small ruminant species such as sheep and goats,

indicating the need for these species to be considered during epidemiological control of CBPP, especially when they are herded in close contact with cattle (Egwu *et al.*, 2012).

## **2.6 Transmission**

Transmission occurs mainly by aerosol through close contact with infected animals within herds and from herd to herd through direct contact and repeated contact between sick and healthy animals, and occasionally from latent carriers shedding intermittently *Mycoplasma* organisms from sequestrated lung lesions (Radostits *et al.*, 2003). Direct close contact between animals excreting flugge-type droplets by coughing ensures the rapid spread of the infection (Jores *et al.*, 2009). The main reservoir of *MmmSC* infection is cattle, from which other bacteria have been isolated during the course of clinical disease (Kusiluka *et al.*, 2000b) and after clinical recovery (Windsor and Masiga, 1977b). Trans-placental transmission has been suggested following isolation of *MmmSC* from the foetus of an infected dam (Stone *et al.*, 1969; Masiga *et al.*, 1972).

In addition, *MmmSC* has been isolated from urine of acutely diseased cattle, with titres ranging between  $10^2$  and  $10^8$  organisms per millilitre of urine (Scudamore, 1976). The organism has also been isolated from semen and sheath washings of two bulls (Gonçalves, 1994). The importance of infected urine or semen in the natural transmission of CBPP is unknown. Transmission through fomites and contaminated fodder has been suggested under experimental conditions (Windsor and Masiga, 1977a). Although water buffaloes are susceptible to CBPP, transmission of the disease from buffaloes to cattle has never been reported (Aliyu *et al.*, 2000).

## 2.7 Pathogenesis and Immunopathology

Following the infection with sufficient large infective dose of Mycoplasmas, the organism invades the lungs of cattle and causes a mycoplasmaemia; this result in localization in numerous other sites including the kidneys and brain (Radostits *et al.*, 2003). An essential part of the pathogenesis of the disease is thrombosis in the pulmonary vessels (Niang *et al.*, 2007). The mechanism of development of the thrombosis is not understood, but there is no general increase in blood coagulability, and no generalized tendency to spontaneous thrombosis (Radostits *et al.*, 2003). Mycoplasmas have evolved unique strategies that allow them to survive and replicate in their hosts (Jores *et al.*, 2009). Some of their inherent properties may cause damage to the host cell, their inability to synthesize many essential nutrients forces them into competition with host cells, altering cellular integrity and function. For example, non-fermenting mycoplasmas use the arginine dihydrolase pathway to generate ATP, and host cell protein synthesis grinds to a halt when arginine reserves are depleted (Niang *et al.*, 2007). Intimate association of mycoplasmas with the host cellular surface may lead to a build up in the local concentration of cytotoxic metabolites and cytolytic enzymes. Hydrogen peroxide production is thought to play a role in the pathogenicity (Nicholas *et al.*, 2000).

Cytadhesins are among the major virulence determinants of Mycoplasmas. Their production may induce oxidative stress in host cells, resulting in damage to the cellular membrane. Many *Mycoplasma* species have membrane-bound phospholipases that may release cytolytic lysophospholipids capable of disrupting host cell membranes (Nicholas *et al.*, 1996). Mycoplasmas produce at least three different types of modulins (molecular

moieties that induce cytokine synthesis with pathologic consequences). Mycoplasmal lipoproteins play a key role in pathogenesis, stimulating monocytes and inducing secretion of pro-inflammatory cytokines and interleukins (Nicholas *et al.*, 2000). The resulting pulmonary inflammation is characteristic of mycoplasmal pneumonia. Infections are further characterized by initial infiltration of neutrophils, followed by an influx of macrophages and lymphocytes. The extent of neutrophil infiltration is directly correlated with disease severity because neutrophil attraction is controlled by chemotactic cytokines. Mycoplasmas have the genetic capability to alter, at high frequencies, the structure and expression of membrane lipoproteins that are exposed to the host immune system. The exact mechanism underlying lipoprotein variation is not precisely understood. Another virulence factor, which may be important in infections by *M. mycoides* subsp. *mycoides* and *M. dispar*, is a polysaccharide capsule, which can afford protection from phagocytosis (Nicholas *et al.*, 2000; Tulasne *et al.*, 1996).

It has been suggested that autoimmune and hypersensitive reactions are essential in the development of lesions (Tulasne *et al.*, 1996). The immunological mechanism involved during infection and ability of the pathogen to evade the immune system, has been highlighted (Ayling *et al.*, 1999a). Several major proteins of *MmmSC* appear to be specifically recognised by antibodies from cattle with CBPP, and not by those from cattle infected with other members of the *MmmSC* cluster. An immunodominant protein of 98 kDa is not common in all strains, and was lacking from most strains detected in the Italian outbreaks between 1990 and 1993. In recent studies, a surface exposed lipoprotein of 72 kDa (named P 72) was identified and characterized. This lipoprotein was shown to induce an early and persistent humoral immune response in cattle with CBPP (Jores *et al.*, 2009).

Some major immunogenic antigens have been identified by Western blot technique for different strains (Nicholas *et al.*, 2000). Bronchial immune responses (IgA) were strong but appear 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). Lipoproteins from other mycoplasmas are suggested to play an important role in virulence (Kostyal *et al.*, 1994; Rawadi and Romanroman, 1996). Recent studies indicated that non-adjuvanted and ISCOM incorporated *MmmSC* and membranes induced pro-inflammatory cytokines (Abusugra *et al.*, 2001). The role of capsular galactan in inducing pathological lesions has been suggested and the biochemical relationship 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 (Thiaucourt *et al.*, 20011).

The apparent but debatable difference in pathogenicity between African and European strains has also been associated with the ability of the former to oxidise glycerol and produce hydrogen peroxide (Nicholas *et al.*, 2000). Little is known about the different immunogenic components of *MmmSC* upon CBPP infection. It is relevant to study the immunopathology of CBPP and to elucidate the pathogenic mechanisms of *MmmSC*, by identifying the role of the microorganism and its immunogenic components in eliciting pro-inflammatory and inflammatory reactions (Filipa *et al.*, 2010). Cellular elements of inflammatory nature like macrophages, monocytes, lymphocytes and neutrophils that may liberate inflammatory products such as Nitrogen (IV) Oxide (NO<sub>2</sub>), myeloperoxidases or cytokines are to be identified (Tulasne *et al.*, 1996; Nicholas *et al.*, 2000).

## **2.8 Pathology**

### **2.8.1 Gross pathology**

The pathological lesions of the disease are generally confined to the thoracic cavity and lungs. The lesions are usually unilateral, showing no preference for the right or left lung although the diaphragmatic lobe is more commonly affected than the cranial lobe (Nunes Petisca *et al.*, 1990).

In acute CBPP, there is a severe fibrinous pneumonia with copious exudate. The latter is a striking feature, and there may be up to 30 litres of yellow exudate, containing clots, in the chest cavity (Ter Laak, 1992). One or both lungs may be partially or completely consolidated, giving a characteristic marbled appearance. Affected areas are swollen, vary from pink to dark red, have a moderately firm consistency and exude clear fluid and sometimes blood from cut surfaces (Provost *et al.*, 1987). The interlobular septa are grossly thickened. Pleural surfaces over affected areas are enlarged, oedematous and may contain areas of necrosis (Hudson, 1971).

In chronic cases, necrotic lung tissue becomes encapsulated to form sequestrum of 1 to 20cm diameter (Martel *et al.*, 1983). The tissues within the sequestrum tend to retain much of the architecture of the acute lesions, but may eventually become calcified or liquified (Trichard *et al.*, 1989). The lesions may either break open to release viable mycoplasmas or be resorbed. Pleural adhesions are commonly found in chronic lesions (Scanziani *et al.*, 1993).

### **2.8.2 Histopathology**

Microscopically, the early pulmonary lesions consist of foci of catarrhal bronchitis with extension of lymphatics in the interlobular septa and thickened alveolar walls (Nicholas *et al.*, 1996). At the same time, or soon after, blood vessels and lymphatics become thrombosed, and alveoli are filled with fluid and cells (alveolar macrophages). There is proliferation of the cells around the bronchioles. There is also lymphatic oedema, with distension of subpleural lymphatics (Scanziani *et al.*, 1997). Necrosis can occur early and tends to have a lobular distribution. It is often demarcated from living tissue by a zone of leucocytes and nuclear debris (Bashirruddin *et al.*, 1999a). A connective tissue capsule develops rapidly, but the necrotic material may persist for many months. Resolution of the pneumonia is by connective tissue replacement of damaged tissue. This starts around blood vessels. A layer of mononuclear cells borders the connective tissue on the necrotic side, and connective tissue gradually moves in to replace the dead tissue (Scanziani *et al.*, 1999). The list of *Mycoplasma spp* along with hosts and associated diseases is shown (Appendix 1).

### **2.9 Clinical Signs**

Following infection, the disease may take a variety of forms, depending on host susceptibility and virulence of the pathogen. Calves have been observed to get mild infections involving tendons and joints (Aliyu *et al.*, 2003). Yearlings and those up to three years show higher susceptibility than calves with lesions characterized by lung involvement (Masiga and Windsor, 1975).

Compared to *MmmSC* isolated from CBPP outbreaks in Europe, *MmmSC* isolated from CBPP outbreaks in Africa and Australia have been reported to be highly pathogenic (Pilo *et*

*al.*, 2007; Nicholas *et al.*, 1996). The incubation period of the natural disease may range from 5 to 207 days, although Turner and Campbell (1937) reported a range of 29-58 days while, Provost *et al.* (1987) reported the incubation period to be 20-40 days. In experimental bronchial intubation infections, Regalla *et al.* (1996b) reported signs of this disease appearing in cattle about 40 days after contact with inoculated animals. Serological responses appeared also around 40 days after contact. The severity of the clinical signs observed in cattle affected by CBPP vary, ranging from, hyperacute through acute to subclinical and chronic forms. Respiratory distress and coughing, are evident on stimulation of resting animals, are the main signs of CBPP (Radostits *et al.*, 2003).

### **2.9.1 Hyperacute form**

The clinical signs observed in the hyperacute form are much more accelerated (Provost *et al.*, 1987). Affected animals may die without exhibiting classical respiratory signs. The pathology associated with the signs is usually characteristic with marked pleural adhesion accompanied by exudative pericarditis (Radostits *et al.*, 2003).

### **2.9.2 Acute form**

Signs due to the acute form of the disease have been reported by Provost *et al.* (1987). Following the incubation period, there is a sudden onset of high fever (40°C; 105°F), a fall in milk yield, anorexia and cessation of rumination. There is severe depression and the animals stand apart or lag behind in pasture during grazing. Coughing, at first only on exercise, and thoracic pain are evident (Radostits *et al.*, 2003). Affected animals are

disinclined to move, standing with the elbows out, the back arched and neck extended. Respirations are shallow, rapid and accompanied by expiratory grunting. Pain is evidenced on percussion of the chest. Auscultation reveals pleuritic frictional sounds in the early stages of acute inflammation, and dullness, fluid sound and moist gurgling crackles in the later stages of infection. Dullness of areas of the lung may be detectable on percussion (Martel *et al.*, 1983). Oedematous swelling of the throat and dewlap may occur and swelling of large moveable joints may be present. In calves, valvular endocarditis and myocarditis may occur. In fatal cases, death occurs after a variable course ranging from several days to 3 weeks (Egwu *et al.*, 1996).

### **2.9.3 Subacute/chronic form**

Signs due to this form of the disease have been reported by Radostits *et al.* (2003). Recovered animals may be clinically normal but in some, an inactive sequestrum forms in the lungs, with a necrotic centre of sufficient size to produce a granuloma causing unthriftiness, chronic cough and mild respiratory distress, only noticeable when the animal is exercised (Aliyu *et al.*, 2000). The sequestrum commonly breaks when the animal is exposed to environmental stress and cause an acute attack of the disease. In Africa, up to one-third of the acute cases that recover become potential carriers. It is much higher in places where the use of anti-microbial is widely spread (Brandao, 1995).

In Europe, unlike in Africa where mortality rates are typically 10-70% during epizootics, the disease is characterised by low morbidity and low or non-existent mortality with the majority of infected cattle showing chronic lesions (Masiga *et al.*, 1996; Regalla, 1996b).

These differences may be due to the fact that European cattle are healthier in general, better fed, subjected to less physical stress, and are often permanently housed throughout the year and probably experience strains of lower virulence than in Africa (Abdo *et al.*, 1998). In Italy, during the early 1990s, CBPP forms with mild or without clinical signs were frequently observed in cattle, with lesions of CBPP observed at slaughter in infected herds and mortality were usually around 2-3% (Guadagnini *et al.*, 1991).

## **2.10 Differential Diagnosis**

Due to the economic importance and transboundary nature of contagious bovine pleuropneumonia, there is the need to routinely differentiate it from other diseases.

### **2.10.1 Foot and mouth disease (FMD)**

This is a viral disease that can be confused with CBPP in terms of its signs like salivation, lameness and fever. However, in FMD there are blisters inside the mouth, excessive secretion of stringy and drooling saliva, blisters on the feet along with swelling of the testicles in mature male cattle (OIE, 2009).

### **2.10.2 Haemorrhagic septicaemia (HS)**

The lung lesions seen in animals that survive the longest can appear very similar to the marbling lesion of CBPP. There may be yellow fluid in the chest and the affected lung may adhere to the inside of the rib cage. Thus, in the individual case distinguishing between HS and CBPP can be difficult. This is a very acute disease and most affected animals die within 6 to 72 hours after the onset of clinical signs. Buffaloes are particularly susceptible. Oedema of the throat and neck to the brisket is often very pronounced (Gordon, 2005).

### **2.10.3 Bacterial or viral broncho-pneumonia**

Clinical signs of certain bacterial or viral broncho-pneumonia may resemble closely those of acute CBPP. Post mortem examination shows usually both lungs to be affected, fibrinous exudate may be present but not to the same extent as in CBPP. While dark, solid areas of lung may be seen, these are usually restricted to the anterior lobes (not the diaphragmatic lobe as in CBPP) and marbled lungs are not often seen (Radostits *et al.*, 2003).

### **2.10.4 Abscesses**

At post-mortem examination, abscesses can be mistaken for sequestra. When cut open the contents of abscesses may have an offensive smell, and be purulent or liquid in nature. A total destruction of the lung tissue occurs. In sequestra the contents often retain typical lung structure (Barlett *et al.*, 2005).

### **2.10.5 Tuberculosis**

Tubercular nodules can superficially resemble sequestra but they are degenerative cheese-like lesions, sometimes calcified. The lung tissue is destroyed. The same lesions are also seen in lymph nodes in the chest. The capsule of the tubercular nodules is not well defined when compared to that of sequestra. There is no marbling and adhesions of the lung to the rib cage (Abubakar *et al.*, 2011).

### **2.10.6 Bovine farcy**

The lung lesions of bovine farcy differ from the CBPP sequestra as they are filled with foul smelling purulent material as described for abscesses. Similarly lymph node lesions are always present (Hamid, 2012).

### **2.10.7 Actinobacillosis**

This is characterized by the presence of granulomas with pus containing small, hard yellow to white granular lesions which are generalized and seldom present in lungs (Glenn, 2005). In additions, lesions will be found in the soft tissues of the head, especially the tongue.

### **2.10.8 Echinococcal (hydatid) cysts**

Here the cysts have a double wall and contain a clear liquid, often calcified when old which can be differentiated from sequestra due to CBPP (Daryani *et al.*, 2007)

### **2.10.9 Foreign body (traumatic) pericarditis**

Although the two condition present clinical and pathological similarities, only one animal is usually affected (Athar *et al.*, 2012). Furthermore, at posmortem, the heart and abdominal organs especially the reticulum is always involved.

## **2.11 Diagnosis**

Diagnosis of contagious bovine pleuropneumonia relies on a combination of clinical examination, post-mortem and laboratory examination of suspected samples based on cultural methods and serological tests (FAO, 2002). Protein and nucleic acid-based molecular techniques are also available (Gonçalves, 1994; Bashiruddin *et al.*, 1999a).

### **2.11.1 Samples for laboratory diagnosis**

Samples to be taken from live animals include; nasal swabs or nasal discharges, broncho-alveolar lavage or transtracheal washing and pleural fluid collected aseptically by puncture made in the lower part of the thoracic cavity between the seventh and eighth ribs. Blood

may also be cultured (Hudson, 1971). Samples to be taken at necropsy include; lungs with lesions, pleural fluid ('lymph'), lymph nodes of the broncho-pulmonary tract, and synovial fluid from those animals with arthritis. The samples should be collected from lesions at the interface between diseased and normal tissue (Rodastits *et al.*, 2003).

### **2.11.2 Post-mortem lesions/necropsy**

Gross pathological lesions in the acute stage are characterized by fibrinous deposits on the parietal surfaces of the lungs and distension of the interlobular septa with straw coloured sero-fibrinous exudate (Trichard *et al.*, 1981). Lesions are usually unilateral, localized in the diaphragmatic lobe and present a characteristic marbling appearance (Provost *et al.*, 1987; Masiga *et al.*, 1996). Lesions are detectable on palpation, and upon incision, red and grey areas of hepatization are revealed. In subacute cases, lesions are characterized by necrosis organized within lobules and interlobular septa and early sequestrum formation. Lesions in the chronic stage are characterized by well-defined sequestra surrounded by fibrous capsules. Adhesions, connecting thickened viscera and parietal pleura, are common (Trichard *et al.*, 1981; Nicholas *et al.*, 1996; Amanfu *et al.*, 1998). In affected calves, exudative peritonitis, arthritis, bursitis and fibrinous arthritis of carpal and tarsal joints may be present (Egwu *et al.*, 1996).

Histologically, in the early stages, the typical lesion consists of bronchiolar necrosis and oedema, progressing to exudative sero-fibrinous bronchiolitis with extension to the alveoli, and adjacent lymphatics. This process extends to the tracheobronchial lymph nodes and pleural lymphatics, the mediasternal, sternal, aortic and intercostal lymph nodes are enlarged, oedematous and hemorrhagic. Lymphatics become thrombosed and fibrosed. The

pulmonary lobules become consolidated with alveolar oedema, fibrin and inflammatory cells. Coagulation necrosis is common and the organism can be demonstrated in these lobules by immunohistochemistry (Bashiruddin *et al.*, 1999a, b).

Perivascular organization foci or organizing centres in the interlobular septa are considered typical of CBPP (Radostits *et al.*, 2003). They consist of a centre occupied by a blood vessel with proliferation of connective and inflammatory cells surrounded by a peripheral zone of necrotic cells. Type I foci contain more proliferative cells in the central zone, which is larger than the peripheral zone. In type II foci, the proliferative cells are scarce and the peripheral zone is relatively larger. Immunoreactive antigen is visible in the central zone inside blood vessels (Abusugra *et al.*, 2001). Immunocytochemical tests can be used to detect the organism in tissue sections and provide valuable confirmatory diagnosis after slaughter. Stained antigen is visible in the smaller bronchioles and alveoli and within the interlobular septa of the lung. Immunofluorescent staining of impression smears of lungs may be more sensitive and rapid than culture (Egwu *et al.*, 1996).

### **2.11.3. Culture and isolation**

The causal organism can be isolated from samples taken either from live animals or at necropsy. The samples must be kept cool at 4°C if stored for a few days or frozen at or below –20°C for a longer period. For laboratory to laboratory transfer, lung fragments or pleural fluid can also be freeze-dried (OIE, 2008). When dispatching samples to the laboratory, it is advisable to use a transport medium that will protect the mycoplasmas and prevent proliferation of other bacteria. The organism is not intrinsically difficult to grow, unlike many other fastidious mycoplasmas such as the one causing contagious caprine

pleuropneumonia (CCPP) but requires a fully functioning bacteriological laboratory with access to special mycoplasma media (Provost *et al.*, 1987). Many media have been described which enable the growth of *MmmSC* (Hudson, 1971; Freundt *et al.*, 1979; Nicholas and Baker, 1998). A medium (PRM, named after the developers) specifically formulated to give maximum growth rate and yield of SC strains, has recently been described by Nicholas *et al.* (2000) as a result of a recent EU-COST action. Pyruvate is included in the medium as an additional energy source to glucose and increases the growth rate. Bacteriological identification of the agent is more complex and can be done by biochemical tests, nucleic acid recognition methods and immunological methods (OIE, 2008).

#### **2.11.4 Immunobinding test**

Immunobinding assays are diagnostic techniques that are based on the detection of Mycoplasma surface antigens which are believed to be highly specific (Totte *et al.*, 2008). However, sensitivity and specificity can be affected in certain circumstances. The aetiological agent or its antigens can be demonstrated by immunochemical tests on infected tissues, tissue fluids and/or cultures of the organism. The advantages of these methods is that, is the most reliable test for routine identification of *Mycoplasma spp* isolated from clinical material (Poumarat, 1991). However, as some of these tests are dependent on a minimum number of organisms being present in the sample, only positive results are taken into account. Since the number of *Mycoplasma spp* is increasing, each isolate has to be tested with several sera for complete identification and immunobinding assays involving mycoplasma colonies or imprints of colonies are becoming highly laborious and capital intensive (Aliyu *et al.*, 2003).

### **2.11.5 Disk growth inhibition test**

The DGIT is based on the direct inhibition of the growth of the agent on a solid medium by a specific hyperimmune serum. However, cross-reactions within the *mycoides* cluster are common and great care should be taken to differentiate *MmmSC* (bovine biotype) from *MmmLC* (caprine biotype) (Nicolet, 1996). It is a simple test to perform, but some results require experience to be interpreted, false-negative and false-positive reactions (very rare). The quality of the hyperimmune serum used in this test is critical for good results (Nicolet, 1996).

### **2.11.6 Agar gel immunodiffusion test**

The agar gel immunodiffusion (AGID) test can detect the specific antigen present at the surface of *MmmSC* and the circulating galactan invading the haemolymph system of sick animals (Griffin, 1965). Pleural fluid, ground lung fragments or even sequestrae can be tested against a hyperimmune serum in two wells cut 5 mm apart in the gel. The test is considered to lack sensitivity and little is known about its specificity, but it has served as a screening test and only positive reactions should be taken into account. The results are better when the plate is incubated at 37°C and can be read within 24 hours. A simpler field test has been developed using impregnated paper discs instead of wells (Provost *et al.*, 1987).

### **2.11.7 Dot immunobinding on membrane filtration**

The MF-dot test can be used for routine identification tests in the laboratory (Poumarat *et al.*, 1991). Specific SC biotype specific MAbs have been developed to overcome cross-reactions within the *mycoides* cluster (Brocchi *et al.*, 1993).

### **2.11.8 Immunohistochemistry**

*Mycoplasma mycoides* subsp. *mycoides* Small Colony immunoreactive sites can be detected in lung lesions using the peroxidase–antiperoxidase method on sections of paraffin-embedded material (Ferronha *et al.*, 1990). Because the isolation of the agent is not always achieved from chronic cases and after treatment with antimicrobial drugs, this test is only supplementary in the diagnosis of CBPP. A negative result is not conclusive (Bashiruddin *et al.*, 1999a). Immunohistochemistry (IHC) has proved to be a robust assay in the diagnosis of CBPP, particularly where the causative organism, *MmmSC*, is not recoverable following long transport distances, where the animal has died of acute disease or where serology cannot be performed or is inconclusive (Ferronha *et al.*, 1990; Scanziani *et al.*, 1997). The sensitivity of IHC using polyclonal serum can be low and non-specific results occur frequently (Bashiruddin *et al.*, 1999b). Ayling *et al.* (1998) identified a monoclonal antibody, M92/20, for use in IHC confirmation of suspected CBPP cases. This monoclonal antibody shows no background noise, but some crossreactivity with other *Mycoplasmas* from the *M. mycoides* cluster” is known. Other monoclonals can be evaluated in this test. In an examination of 11 CBPP affected lungs from Portuguese cattle, IHC detected all, while PCR and culture detected 5 and 4 cases respectively. Though the sample size is small, this illustrated that IHC is a sensitive and robust test for CBPP.

### **2.11.9 Immunoblotting test**

Immunoblotting test (IBT) (Western immunoblotting), is an immune-enzymatic test that has been developed and is of diagnostic value. It has been applied with success to the assessment of antibody response in several diseases. This method allows the analysis of the host humoral immune response in relation to the electrophoretic profile of *MmmSC*

antigens. Thus overcoming problems related to non-specific binding in other immunoassays (Gonçalves, 1994). A field evaluation indicated a higher sensitivity and specificity than the CF test with a core profile of antigenic bands, present both in experimentally and naturally infected cattle are immunodominant. Thus the test overcomes problems related to nonspecific binding. It should be used primarily as a confirmatory test, after other tests and should be used in all cases in which the CF test has given a suspected false result. Therefore, the IBT can be applied to herds of low disease prevalence where both false-negative and false-positive results are often found. It is also extremely useful in the final stages of eradication as well as during serological surveillance in CBPP (Regalla *et al.*, 1999b).

#### **2.11.10 Biochemical tests**

For routine field use, the immunological tests and PCR are sufficient, but where these give doubtful results, biochemical tests may be used. These biochemical tests should be carried out by a reference laboratory. For this purpose, after two or three subcultures, antibiotics should be omitted from the medium to check if the isolate is a mycoplasma or an L-form of a bacterium that will regain its original form in the medium without inhibitors (Nicolet, 1996). Once this test is done and after cloning (at least three colonies should be selected), the organism can be identified using biochemical tests (Al-Aubaidu and Farbricant, 1971). *Mycoplasma mycoides* subsp. *mycoides* SC is sensitive to digitonin (like all members of the order Mycoplasmatales), does not produce 'film and spots', ferments glucose, reduces tetrazolium salts (aerobically or anaerobically), does not hydrolyse arginine, has no phosphatase activity, and has no or weak proteolytic properties (Songer and Post, 2005). For these tests, special media have been developed that include the same basic ingredients

(heart-infusion broth or Bacto PPLO [pleuropneumonia-like organisms] (Nicholas *et al.*, 1996). It should be noted that a pH indicator should not be added to a medium containing triphenyl tetrazolium chloride. For demonstration of proteolysis, growth is carried out on casein agar and/or coagulated serum agar (Nicholas and Baker, 1998). Once the biochemical characteristics have been checked, one of the following immunological tests can then be performed to confirm the identification of *MmmSC*; disk growth inhibition test (DGIT), fluorescent antibody test (FAT), and the dot immunobinding on a membrane filter (MF-dot) test (Poumarat *et al.*, 1991).

#### **2.11.11 Nucleic acid recognition methods**

These diagnostic techniques of radiolabelled or enzyme probes were used for the diagnosis of CBPP, but have been superseded by the more convenient and safe PCR technology. The PCR is sensitive, highly specific, rapid and relatively easy to perform. Primers specific for the *M. mycoides* cluster and for *MmmSC* have been developed, including a new technique that permits the specific identification of the T1/44 vaccinal strains (Taylor *et al.*, 1992; Bashirundin *et al.*, 1994b; Dedieu *et al.*, 1994; Miserez *et al.*, 1997 and Persson *et al.*, 1999). Using samples such as lung exudate allows the PCR to be performed directly after differential centrifugations to remove inflammatory cells and pellet mycoplasmas. For lung fragments, the PCR is applied after DNA extraction. The PCR can also be performed on urine or blood. The main advantage of the PCR is that it can be applied on poorly preserved samples (contaminated or without any viable mycoplasmas as may occur following antibiotic treatment) (Timothy *et al.*, 2005). If direct detection of DNA from the organ under test fails, specimens should be enriched by culturing them in an appropriate medium for 24–48 hours, followed by attempted detection of DNA from the culture. The PCR has

become the primary tool for identification of *Mmm*SC (Lorenzon *et al.*, 2001). If a sample is PCR positive in a CBPP-free zone, the test should be confirmed by a second and different PCR. Infection can be confirmed by the use of only one immunological test. One of the problems with PCR is the possible occurrence of contamination, if the necessary precautions and quality management system are not implemented correctly in the diagnostic laboratory (Gorton *et al.*, 2005).

Great care must be taken to respect the strict separation between those parts of the laboratory that may be contaminated with PCR products (such as the electrophoresis room) and those parts of the laboratory devoted to preparing the PCR reagents. The onset of real-time PCR assays should solve this possible troubleshooting as fluorescence resulting from genomic amplification is measured directly without opening the tubes. This technique has already been applied to *Mmm*SC detection and further developments are expected in the near future (Gorton *et al.*, 2005).

#### **2.11.12 Complement fixation**

The Campbell and Turner (1937) complement fixation (CF) test remains the recommended procedure (although the current method is slightly different from the original one), and it is widely used in all countries where infection occurs (Provost *et al.*, 1987). The CF test, as a micro method, has been harmonized in the European Union (OIE, 2002). For antigen titration and harmonization purposes, an international standard positive bovine serum is available from the OIE Reference Laboratory in Teramo, Italy. However, the CF test is still difficult to perform, requiring well trained and experienced personnel. The limitations of

the CF test are well known (OIE, 2008). With a sensitivity of 70% and a specificity of 98%, the CF test can detect nearly all sick animals with acute lesions, but a rather smaller proportion of animals in the early stages of the disease or of animals with chronic lesions (Bellini *et al.*, 1998). In addition, therapeutic interventions and improperly conducted prophylactic operations (partial slaughter of the herd) may increase the number of false-negative reactions. However, for groups of animals (herd or epidemiological unit) the CF test is capable of detecting practically 100% of infected groups. Despite the high specificity of the CF test, false-positive results can occur, of which an important cause is serological cross-reactions with other mycoplasmas, particularly other members of the *M. mycoides* cluster. The validity of the results has to be confirmed by post-mortem and bacteriological examination, and serological tests on blood taken at the time of slaughter (OIE, 2008).

#### **2.11.13 Competitive enzyme-linked immunosorbent assay**

A competitive enzyme-linked immunosorbent assay (C-ELISA) developed by the OIE Collaborating Centre for the diagnosis and control of animal diseases in tropical countries, has undergone evaluation (Le-Goff and Thiaucourt, 1998; Amanfu *et al.*, 1998). An indirect ELISA based on the use of a lipoprotein antigen, is currently being validated by the IAEA (Abdo *et al.*, 1998, Bruderer *et al.*, 2002). In May 2004, the C-ELISA was designated as an OIE prescribed test for international trade by the OIE International Committee. Compared with the CF test, the C-ELISA has equal sensitivity and greater specificity (OIE, 2002). Reagents for this can be obtained from the OIE Reference Laboratories for CBPP or the OIE Collaborating Centre for ELISA and Molecular Techniques in Animal Disease Diagnosis. Validation tests that have been carried out in several African and European countries would indicate that the true specificity of the C-

ELISA has been reported to be at least 99.9%, and that the sensitivity of the C-ELISA and the CF test are similar (Amanfu *et al.*, 1998; Le-Goff and Thiaucourt, 1998). Furthermore, antibodies are detected by the C-ELISA in an infected herd very soon after they can be detected by the CFT, and C-ELISA antibody persists for a longer period of time (Niang *et al.*, 2006). This C-ELISA is now provided as a ready made kit that contains all the necessary reagents including precoated plates kept in sealed aluminium foil (Le-Goff and Thiaucourt, 1998). The kit has been especially designed to be robust and offers a good repeatability. As a consequence, sera are analysed in single wells. The substrate has been modified and is now TMB (tetramethyl benzidine) in a liquid buffer and the reading is at 450 nm. The substrate colour turns from pale green to blue in the first place and becomes yellow once the stopping solution has been added. Monoclonal Antibodies (MAb) controls exhibit a darker colour while strong positive serum controls are very pale. The cut-off point has been set at 50% and should be valid in every country (Le-Goff and Thiaucourt, 1998).

#### **2.11.14 Slide agglutination test**

A rapid field slide agglutination test (SAT) with either whole blood or serum has been developed to detect specific agglutinins (Ayling *et al.*, 1998). The antigen is a dense suspension of stained mycoplasmas that is mixed with a drop of blood or serum. Due to a lack of sensitivity, the test detects only animals in the early (acute phase) stages of the disease. It should be used only on a herd basis (Turner and Etheridge, 1963).

### **2.11.15 Latex agglutination test**

This is a rapid agglutination test that has been developed that is easier to interpret than the SAT, which gives results in less than two minutes, using sera or whole blood, was developed for screening cattle in the field (Ayling *et al.*, 1999b). This 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 (John *et al.*, 2003). This test has been evaluated using CBPP negative sera from England and CBPP positive sera from Africa, Portugal and Italy (Ayling *et al.*, 1999). Sensitivity was comparable to the internationally recognized complement fixation test, but is far simpler and more rapid to perform. This test may have great potential in parts of Africa where there are great distances between the outbreaks, usually in nomadic herds, and diagnostic laboratories enabling control measures to be implemented rapidly (March *et al.*, 2003).

### **2.12 Treatment**

Mycoplasmas have no cell walls and are resistant to penicillin and related antimicrobials (Yaya *et al.*, 2004). Other Mycoplasma species such as *M. bovis* are sensitive *in-vitro* to enrofloxacin and lincomycin but there is no information available on the antimicrobial susceptibility of *M. mycoides* subsp. *mycoides*. The use of antimicrobials is discouraged in both Europe and Africa (Egwu *et al.*, 1996). However, antibiotic treatment against CBPP is widely used in pastoralist communities (Msami *et al.*, 2001; Twinamasiko *et al.*, 2004; Mariner *et al.*, 2006). It is not part of any official control strategy due to suspicion that its use could facilitate development of sequestra, increase the number of carrier animals along with the increase development of resistant strains, and mask the occurrence of clinical

disease making diagnosis difficult, which may contribute to unrecognized infections and CBPP transmission (Provost *et al.*, 1987).

At a meeting of international experts organized by the FAO in 2003, it was recommended that chemotherapy be reconsidered for CBPP control despite the fact that the effectiveness of treatment has not been adequately studied. However both *in-vivo* and *in-vitro* studies demonstrating usefulness of antibiotics for treating CBPP have been reported (Ayling *et al.*, 2000; Twinamasiko *et al.*, 2004; Yaya *et al.*, 2004; Hübschle *et al.*, 2006). In an *in-vitro* experiment, tilmicosin, danofloxacin, oxytetracyclines, florfenicol and spectinomycin were found to be effective against a variety of strains of *MmmSC* isolated from CBPP cases that had occurred in Africa and Europe (Ayling *et al.*, 2000).

In a study carried out in Namibia, it was demonstrated that naïve animals kept in-contact with danofloxacin treated animals that had CBPP had significantly fewer lesions, were less likely to die and to develop clinical disease than naïve animals kept in-contact with untreated animals with CBPP (Niang *et al.*, 2007). In the same study, *MmmSC* was isolated from a limited number of the in-contact controls kept with the treated animals suggesting low spread of infection (Hübschle *et al.*, 2006). In a different trial, long-acting tetracycline (popularly called L.A by farmers in Nigeria) was demonstrated to be effective in limiting clinical severity of the disease but ineffective in the prevention of persistence of viable *MmmSC* in treated animals, thus, the direct effect of tetracycline on the individual is positive (less clinical damage), but the indirect effect on the population may be negative (masking of signs leading to transmission) (Yaya *et al.*, 2004; Niang *et al.*, 2007).

Antibiotic treatment, such as tetracyclines or tylosin, are generally given to prevent death of animal from local reactions ('Willems reaction'), that may appear at the site of injection after 2–3 weeks after vaccination with strain T1/44. Treatment is recommended only in endemic areas because the organisms may not be eliminated although the indiscriminate use of antimicrobials may interfere with eradication efforts and complicate the epidemiology of the disease (Tulasne *et al.*, 1996).

### **2.13 Prevention and Control/Eradication**

There are four essential tools in CBPP control and eradication (Radostits *et al.*, 2003). These include; movement control, vaccination, treatment and stamping out. Each control measure acts by reducing the effective reproductive number of the agent in the population. However, not every country uses all of these control measures (OIE, 2009). The methods used for control depend on the epidemiological situation, animal husbandry methods in effect, and the availability and efficacy of veterinary services in a specific country (Radostits *et al.*, 2003).

#### **2.13.1 Cattle movement control**

The control of cattle movement is the most efficient means of limiting the spread of CBPP (Anon 1992). 'A popular husbandary method known as agro-pastoral' (a combination of agriculture and pastoralist activity), which are often impossible to avoid in Africa, does not favour cattle control movement especially in Central and West Africa (Tambi *et al.*, 2006). Cattle owners may also deliberately avoid legal livestock movement control measures, as in some countries, many associate these with taxation.

### **2.13.2 Regional and international coordination**

Regional and international coordination of stock movement is important in the control of CBPP (Hudson 1971). This practice should emphasize the exchange of disease outbreak information, cattle movement data and border harmonization of vaccination campaigns. The establishment of livestock markets on both sides of the borders would greatly facilitate stock movement control.

### **2.13.3 Vaccination**

When the disease is present in the area, two methods of control are possible. These are vaccination and eradication by test and slaughter of reactors. In Africa control of the disease is based on vaccination campaigns using attenuated *MmmSC* strains such as T1/44. These are attenuated vaccines and are currently used throughout the African continent (March, 2004). The T1/44 which is being used extensively is produced by 10 laboratories on the continent (Hudson, 1971; Provost, 1981; Anon, 1992). It offers better protection (1 year) compared to the T1SR (6 months) but has more side-effects with post-vaccinal reactions likely to occur in animals vaccinated for the first time. The possibility for post-vaccinal reactions, including the likelihood of residual virulence in the vaccine strain to cause clinical CBPP needs therefore, to be taken into account when organizing vaccination campaigns (PANVAC, 1991). Consequently, the search for new CBPP vaccines has become a major issue for African countries that are facing an increase in outbreaks of the disease. The rationale for this search is based on a better understanding of the *Mycoplasma* virulence mechanisms that could lead to a targeted attenuation of *MmmSC* strains. It is also based on a better understanding of the bovine immune response that may be driven to a pathogenic inflammatory response or conversely to a better balanced response leading to

protection. Besides safety concerns, current vaccines are relatively easy and cheap to produce and offer 80 % protection when properly used and annual vaccination is effective when using the T1/44 strain or bi-annual vaccination when using the KH3J vaccines (Davies, 1968; Gilbert *et al.*, 1970; Windsor *et al.*, 1972; Masiga and Windsor, 1975). The recommended minimum vaccinating dose of the T1/44 strain is  $10^7$  organisms. Annual mass vaccination should be performed for three to five years before genuine control of CBPP is achieved. This type of vaccination has been used with good results in East Africa and much of West Africa. More recently, this vaccination strategy has been successfully applied in Côte d'Ivoire (Gilbert and Windsor, 1971).

In outbreak areas, more intensive vaccination strategies can be instituted. Following vaccination, only one - third of the animals respond serologically, as measured by CFT. Nevertheless, the majority of cattle are well protected for up to two years (Windsor *et al.*, 1972; Gilbert *et al.*, 1970; Davies, 1968).

During the 1980s and 1990s CBPP control benefited significantly from the Pan-African Rinderpest Campaign (PARC), which promoted combined rinderpest and CBPP vaccination. When the programme came to an end in 1999, many countries also stopped the use of the combined vaccine (PACE, 2003). However, some countries continue to carry out annual CBPP vaccinations using the T1/44 and T1/SR vaccines. These vaccines are not 100% efficacious and confer immunity for a relatively short period of time. Mariner *et al.* (2006) tested the impact of mass immunization on the persistence of infection (herd level prevalence) and found that vaccination reduced the percentage of herds persistently infected by 53% to 81%. Efficacy trials using the T1/44 vaccine strain conducted at 12 to

15 months post vaccination found a protection against macroscopic pathologic lesions of between 66% and 75% (Wesonga and Thiaucourt, 2000; Masiga *et al.*, 1978; Gilbert *et al.*, 1970).

Another trial involving the T1/44 strain in cattle challenged two years post vaccination found a protection of 80% (Windsor *et al.*, 1972). Based on data collected in nine countries in West Africa, the average CBPP vaccine coverage varied from 23% in 1994 to 47% in 1998. Notwithstanding the low efficacy of available vaccines and the low vaccine coverage, vaccination remains one of the control strategies of choice in Africa (Mariner *et al.*, 2006).

#### **2.13.4 Vaccination and treatment**

Although the use of antibiotics is theoretically prohibited, they are widely applied in the field. The consequences of these antibiotic treatments in terms of clinical efficacy, emergence of resistant strains, and persistence of chronic carriers have not been evaluated yet. However, recent work has shown that antibiotic treatment of cattle may greatly reduce the transmission to healthy contacts but this requires treatment of all affected cattle in a group (Huebschle *et al.*, 2004).

Antibiotic treatment of clinical CBPP cases is now standard field practice in many African countries and veterinarians, livestock owners and Community Animal Health Workers attest to its beneficial effects (Amanfu *et al.*, 1998). Effective control of CBPP using a feasible treatment regime can reduce transmission by decreasing the duration of infection and the effective reproductive number. Studies by Mariner *et al.* (2006) revealed that using treatment to reduce the infectious period by 50% resulted in a 64% reduction in mortality

and a reduction in the prevalence of infected herds from 75.4% to 33.2%. The effects of the disease can therefore be reduced by at least half when an appropriate treatment regime is used.

It is intuitively probable that the best approach to the control of CBPP would be to regularly vaccinate cattle in endemically infected areas or those at risk of being infected, while, treating and, if possible, isolating individual animals when they develop clinical disease. In this way, the benefits of both vaccination (achieving high levels of herd immunity) and treatment (enabling animals that would otherwise die or be seriously debilitated to recover) would hopefully act synergistically to reduce losses. In view of this, it seems prudent to enable cattle owners to vaccinate and, where necessary, treat their animals to control CBPP (Yaya *et al.*, 2004).

#### **2.13.5 Stamping out (slaughter of all sick and in-contact cattle)**

This requires full cooperation of cattle owners and an adequate and timely compensation system. This strategy is impractical in developing countries with a pastoral economy. Eradication is difficult to implement and cannot succeed alone as substantial funds along with many countries collaborating together is essential since CBPP is transboundary (Newton, 1992).

#### **2.13.6 Quarantine**

Quarantine of herds during outbreaks in endemic areas is one effective method of controlling the spread of CBPP. The quarantine should be for a minimum of three months and clinical and serological examinations should be performed during this period. All

animals showing positive results should be slaughtered and the remaining animals vaccinated (Radostits *et al.*, 2003).

#### **2.13.7 Combination of slaughter policy and movement control**

This strategy may necessitate compensation as special fund should always be established for this purpose. The disease was eradicated in the United Kingdom in the 19th century, through the slaughter of all animals in the outbreak area, and Southern Africa eradicated the disease in the early 20th century by implementing strict movement control, quarantine and slaughter (Egwu *et al.*, 1996).

#### **2.13.8 Combination of vaccination, slaughter policy and movement control**

Australia succeeded in eradicating CBPP using a combination of the above three control methods. This combination has also been used in many countries in Africa. In the 1960s and 1970s, the countries in East Africa practised all the three methods; these countries were followed by the Central African Republic, Cameroon and Nigeria in 1970s and 1980s, and Guinea (Conakry) in 1992-1993 (Radostits *et al.*, 2003). For combination of these methods to succeed there must be political will by the countries concerned (Newton, 1992).

#### **2.13.9 Surveillance**

Clinical diagnosis, serological surveillance, and post - mortem examination of lesions at abattoirs are essential in the detection of CBPP (Hudson, 1971; Anon, 1993). A country intending to provide evidence of freedom from the disease should form a strong surveillance network. The standard procedures recommended by the OIE for the declaration of freedom from CBPP include, a system of reporting active disease, supported by efficient

laboratory and field services, and an active programme of examination of statistically selected samples from the cattle population, to detect clinical disease or other indications of occurrence of CBPP (Aliyu *et al.*, 2003).

## **2.14 Economic Importance**

The occurrence and endemicity of certain animal diseases, followed by poor nutrition, stand above all other factors in their contribution towards poor productivity and output from the livestock sector (Ikede and Taiwo, 1987). Estimates provided by the Food and Agriculture Organization (FAO/OIE, 1995), indicated that animal diseases cause losses of up to 30% of the annual livestock output in developing countries.

Contagious bovine pleuropneumonia (CBPP) is considered to be a disease of economic significance because of its ability to compromise food security through loss of protein and draft power, reduced output, increased production costs due to costs of disease control, disruption of livestock/product trade, inhibition of sustained investment in livestock production, cause pain and suffering to animals (Ikede and Taiwo, 1987).

Direct production losses were considered as reductions in cattle numbers, beef, milk, and draft power (Ikede and Taiwo, 1987). Indirect losses resulting from the disease include reduced fertility, lost market opportunities through trade bans, quarantine costs and delayed marketing. Annual losses directly or indirectly attributable to CBPP, was estimated to be around US\$ 2 billion (Masiga *et al.*, 1999).

Thomson (2003), has stated that the cost of controlling CBPP using a regional mass vaccination programme in countries of Central, Eastern and Western Africa is quite high (300 million euros) and that even if half of this cost were to be recovered from cattle owners, many governments will still not be able to afford the rest.

Tambi *et al.* (1999) estimated the cost of CBPP control by vaccination in ten African countries during the Pan-African Rinderpes Campaign (PARC) period and found to be an average of 12 euro per head of cattle. The total economic cost of CBPP in the twelve countries was estimated at about 45 million euros. The average economic cost per country was 3.7 million euros. This is close to the US\$ 3.6 million (2.8 million euros) reported by Osiyemi (1981), as the economic cost of CBPP in Nigeria. The estimates for Chad and Mali do not significantly differ from those of Osiyemi. However, estimates for Ethiopia, Kenya and Tanzania are significantly higher while those for Côte d'Ivoire, Ghana, Mauritania and Niger are significantly lower. Egwu *et al.* (1996), reported an economic cost of CBPP in northern Nigeria of US\$ 1.5 million (1.1 million euros), which is similar to the cost obtained for Côte d'Ivoire, Niger and Uganda.

In 2003, the Nigeria Animal Diseases Information System (NADIS) under the auspices of Pan-African Control of Epizootics (PACE), classified Nigeria as an endangered zone based on her CBPP status (PACE 2003). The monetary value of these losses was assessed nationally and across the four agro-ecological zones of Nigeria to be NGN 1.307 billion (Alleweldt *et al.*, 2009).

## CHAPTER THREE

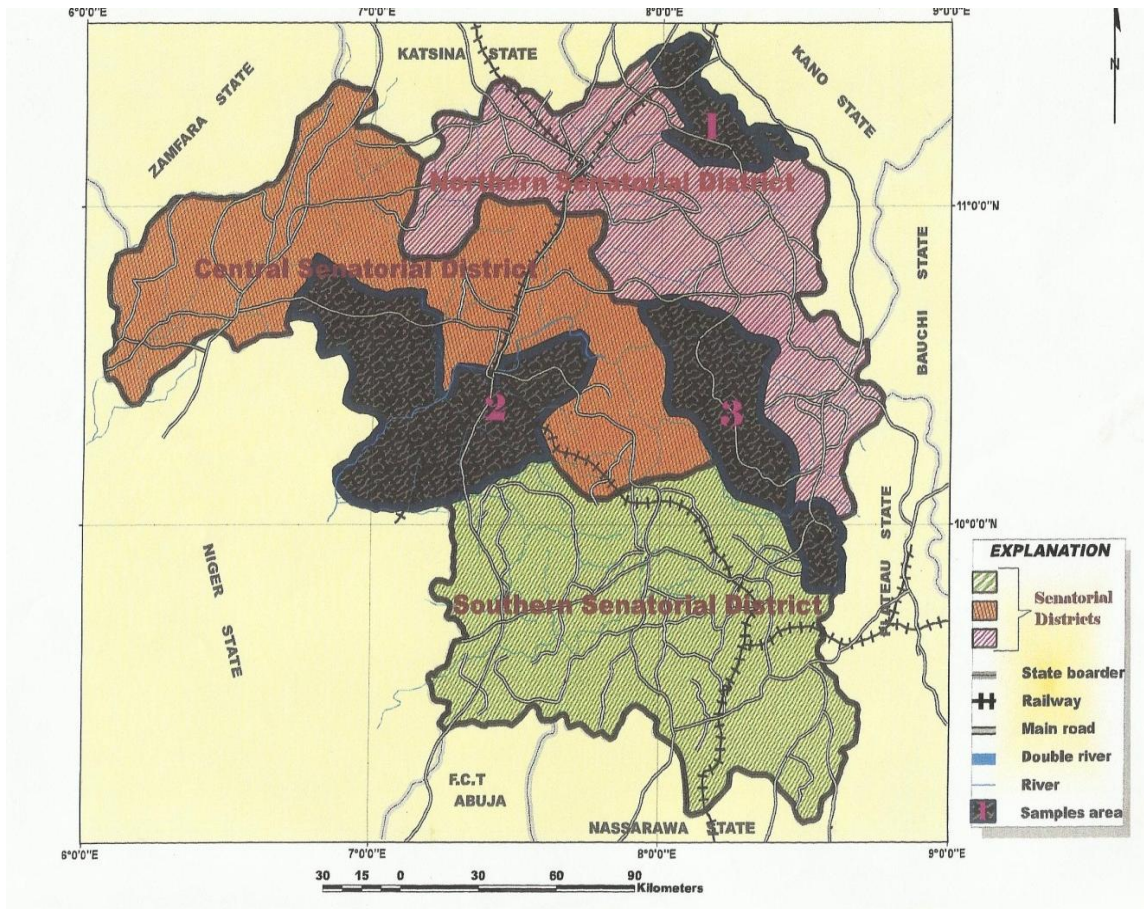
### MATERIALS AND METHODS

#### 3.1 Study Area

The research was carried out in three selected Local Government Areas (LGAs) of Kaduna State, which is located in the North-West geo-political zone of Nigeria. Globally, the State is between Longitude 30'' and 0900 East of the Greenwich Meridian and also between Latitude 0910 and 11°30'' North of the Equator (KDSG, 2011). The State shares border with Niger State to the West, Zamfara, Katsina and Kano States to the North, Bauchi and Plateau States to the East and Abuja, the Federal Capital Territory (FCT) and Nasarawa State, to the South (Fig.3.1).

The State has an area of about 48,473.2 Km<sup>2</sup>, 3 Senatorial Districts and 23 Local Government Areas (LGAs), with a human population of 6.07 million approximately (NPC, 2006). There are two distinct seasons in Kaduna State, namely wet and dry seasons. The wet season spans the period between April/May and September/October with a total amount of annual rainfall varying between 1.00 cm and 1.50 cm, while the dry season spans the period between October/November and March/April. The lowest mean temperature is usually recorded during the harmattan period of between November and February with the range being from 18°C – 23°C. The total annual evaporation transpiration rate varies from 1.49 cm in the Northern part to 1.56 cm in the Southern part of the State (KADP, 2012).

The State is essentially an agrarian state with about 70-75% of the population engaging in farming and it also has potentials for livestock industry (NLR, 1992).



**Fig. 3.1** Map of Kaduna State showing the three Senatorial Districts with Sampled areas (1=Ikara Local Government Area -representing the Northern Senatorial District, 2= Chikun Local Government Area -representing the Central Senatorial District and 3= Kauru Local Government Area -representing the Southern Senatorial District).

**Source:** Department of Cartography; National Geoscience, Kaduna Zonal Office, Barnawa, Kaduna, Kaduna State, Nigeria (Production map from KADP, 2012).

### 3.2 Sample Size

Sample size was determined as described by the method of Mugo, (2008).

$$n = \frac{Z^2 Pq}{d^2}$$

Where:

n= sample size

q = 1-p

P = expected prevalence of 47% (Danbirni *et al.*, 2010)

d = desired absolute precision = 5% or 0.05

Z = appropriate value for the standard normal deviation for the desired confidence = 1.96

Therefore,

$$n = \frac{1.96^2 \times 0.47(1 - 0.47)}{0.05^2} = 382.7$$

This sample size shows a minimum of 382.7cattle heads that can be investigated. However, to increase precision, 900 heads of cattle were bled and sera obtained.

### 3.3 CBPP Agglutination Kit

The Kit (BoviLAT PA6223) was imported from Veterinary Laboratory Agency New Haw, Addlestone Surrey KT 15 3NB United Kingdom and contained the following.

- i. BoviLAT Latex Reagent: 2.0 ml ready-to-use antigen coated latex beads dropper bottle.
- ii. Sodium azide to a final concentration of 0.1%
- iii. Negative Control Serum: Negative bovine serum
- iv. Positive Control: Diluted rabbit antiserum against CPS antigen

- v. Reaction Slides: Black cards with six reaction cells per card
- vi. 20 *ul* Disposable Droppers

### **3.4 Items not supplied with the Kit but used**

- i. Timer: used in monitoring the time for the reactions
- ii. Pipette: aseptic pipettes were obtained from a reliable pharmaceutical shop
- iii. Wooden sticks: Wooden sticks (tooth pick) obtained and sterilised was used for mixing the reagent and sera
- iv. Disposable gloves

### **3.5 Blood Sample Collection**

Kaduna State was considered as the area of study. The 3 Senatorial Districts represent the sample areas. One Local Government Area (LGA) each, was randomly selected to represent each of the senatorial district, namely; Ikara for Northern (1), Chikun and Kauru for Central (2) and Southern (3) senatorial districts respectively. Each of the selected LGAs have 11 political wards. Three political wards were picked in the same manner in each of the LGA. Furthermore, ten herds were randomly identified in each ward selected. Ten heads of cattle were randomly sampled and bled after they were properly restrained and their respective ages, sexes and breeds recorded in each of the herd identified. This gave 100 samples in each ward, and 300 samples from each LGA giving a total of 900 samples in this study. A cattle population of a minimum of 20 heads was considered as a herd in this study (Tambuwal *et al.*, 2011b).

### **3.5.1 Blood sample collection procedure**

Aseptic jugular venupuncture using sterile 18 G hypodermic needle fitted into a 20 ml syringe was made and 10 mls of blood was collected from each animal.

### **3.5.2 Sera collection**

The blood samples collected were kept in a slanting position at ambient temperature for about 6 hours and sera separated from the cellular component. The sera collected were transferred into labelled plain sterile sample bottles and stored at 4<sup>o</sup>C in a cold box.

### **3.6 Sera Analysis (BoviLAT Test Procedures)**

Sera were taken from the cold box and allowed to attained ambient temperature. Twenty microlitre of serum was dropped onto a black reaction card using plastic dropper. This was carefully dispensed to avoid air bubbles. The BoviLAT Latex reagent was shaken well and one drop of the reagent added next to the spot of serum. This was mixed together using a wooden stick (sterile tooth pick) and the mixture spread out inside the reaction cell. A fresh stick was used for each sample. The reaction card was rocked from left to right for three minutes and any agglutination or otherwise was recorded (Plates 2, 3, 4 and 5). A maximum of six reactions were done at a time.

### **3.7 Reaction Process**

The latex cards are coated with capsular polysaccharide (CPS) purified from *Mmm*SC cells. Antibodies recognising the CPS that binded and cross-linked the latex particles causing agglutination at different degrees in the positive cases as follow: i. Positive 3+ (+++), Strong clumping of latex beads, and agglutination beginning within one minute, ii. Positive

2+ (++), Clear agglutination of latex beads, agglutination beginning between one and two minutes and iii. Positive 1+ (+), Fine agglutination of latex beads, agglutination formed between two and three minutes. The sera from animals that might not be suffering from CBPP will not show any reaction, and therefore, it is recorded as negative 0 (-), No agglutination formed within three minutes.

### **3.8 Questionnaire Administration**

Open and close ended structured questionnaire was designed and administered to pastoralists that gave their consent before samples were taken in the herds sampled. In order to test the methodology and the reaction of the respondents to the procedure, a pilot study was carried out to validate and standardize the questionnaire and procedures.

The data collected comprised of information concerning sex, age, and breed, awareness of the existence of CBPP and its associated clinical signs, as well as records of previous and recent suspected outbreaks of the disease in the herds. The questionnaire also assessed the source(s) of vaccines and the vaccination programmes practiced and personnel involved in administering of vaccines. Information was also obtained on the use of antibiotics for the treatment of CBPP. Risk factors which included the use of cattle route(s) and tendency of mixing during grazing and watering, introduction of new animals and quarantine procedures were obtained.

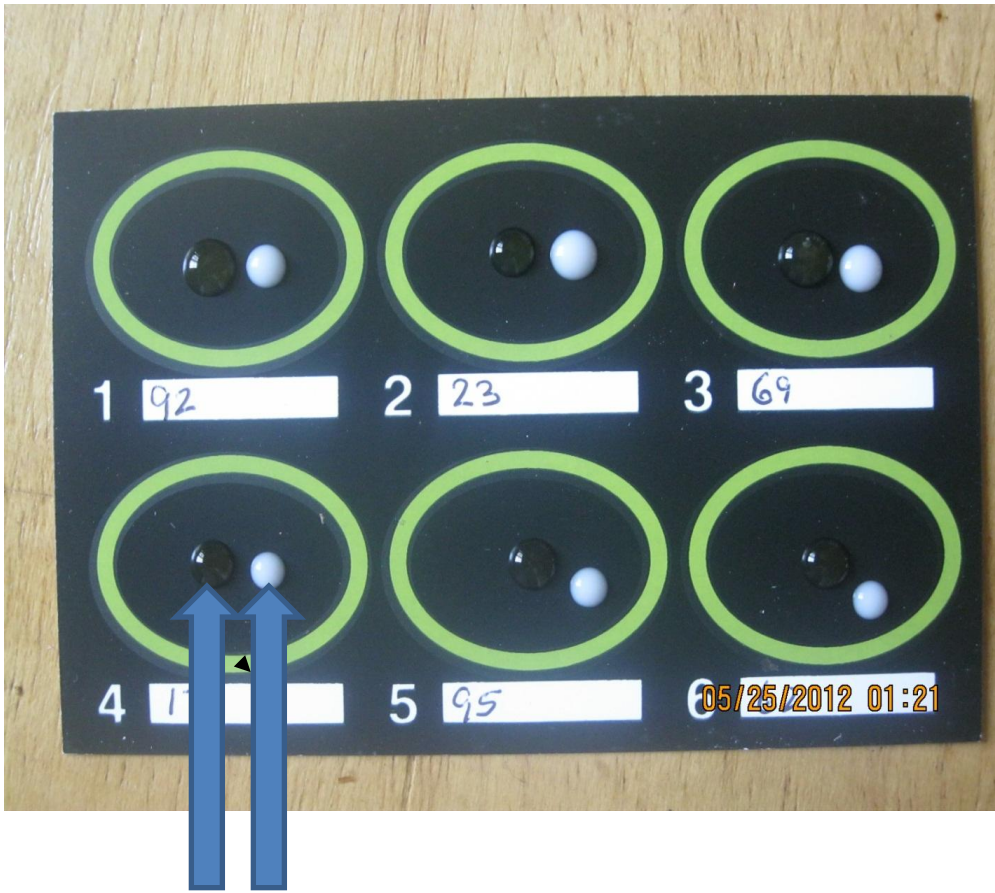
The questionnaire was administered in Hausa language as most of the respondents understood and spoke in Hausa being the common language of communication in the area of the study.

One hundred herdsmen/livestock-owners were initially marked for the exercise but only Ninety were involved to correspond with the number of herds sampled in the study. One questionnaire was used in each household where animals are kept in one enclosure or a herd. On arrival at the relevant household, the team was always introduced by the Chief investigator who outlined the project objectives to the household head before requesting for permission to interview which was one on one.

### **3.9 Data Analysis**

The data obtained were presented in Tables and analyzed using Microsoft Excel 2007, GraphPad Prism version 4.0 for Windows and Chi-square ( $X^2$ ). The prevalence of CBPP determined using the formula:-

$$\text{Prevalence} = \frac{\text{Positive samples}}{\text{Total samples analyzed}} \times 100$$

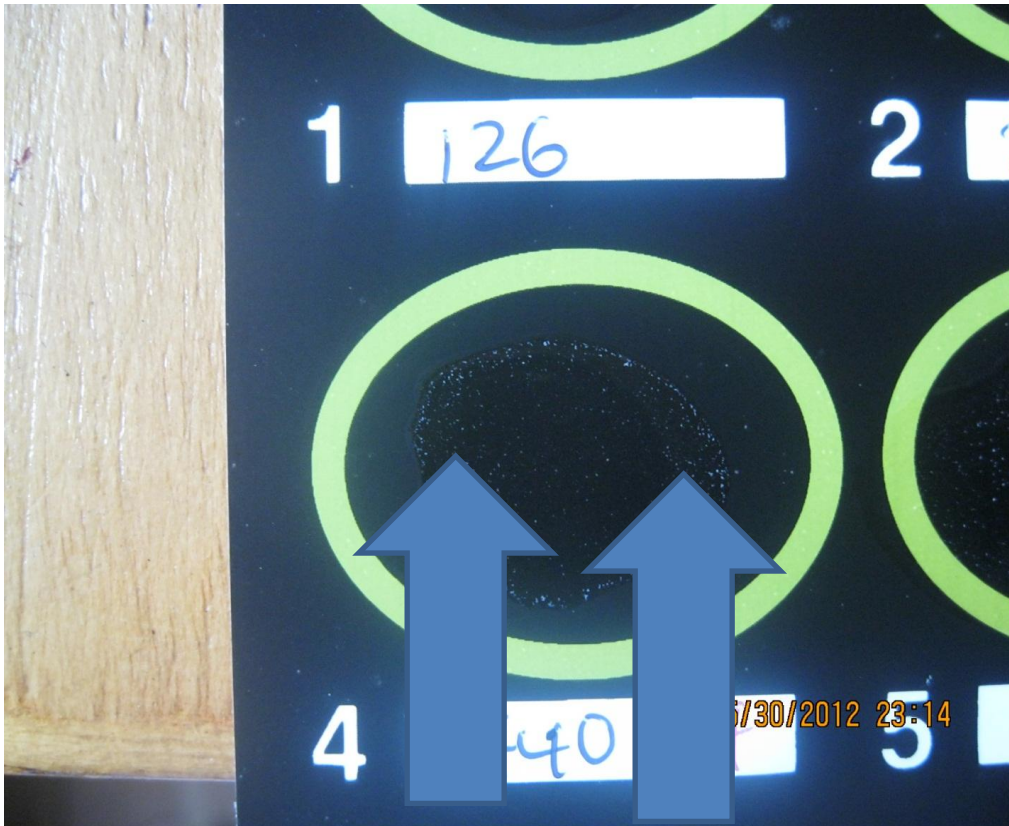


**SERUM REAGENT**

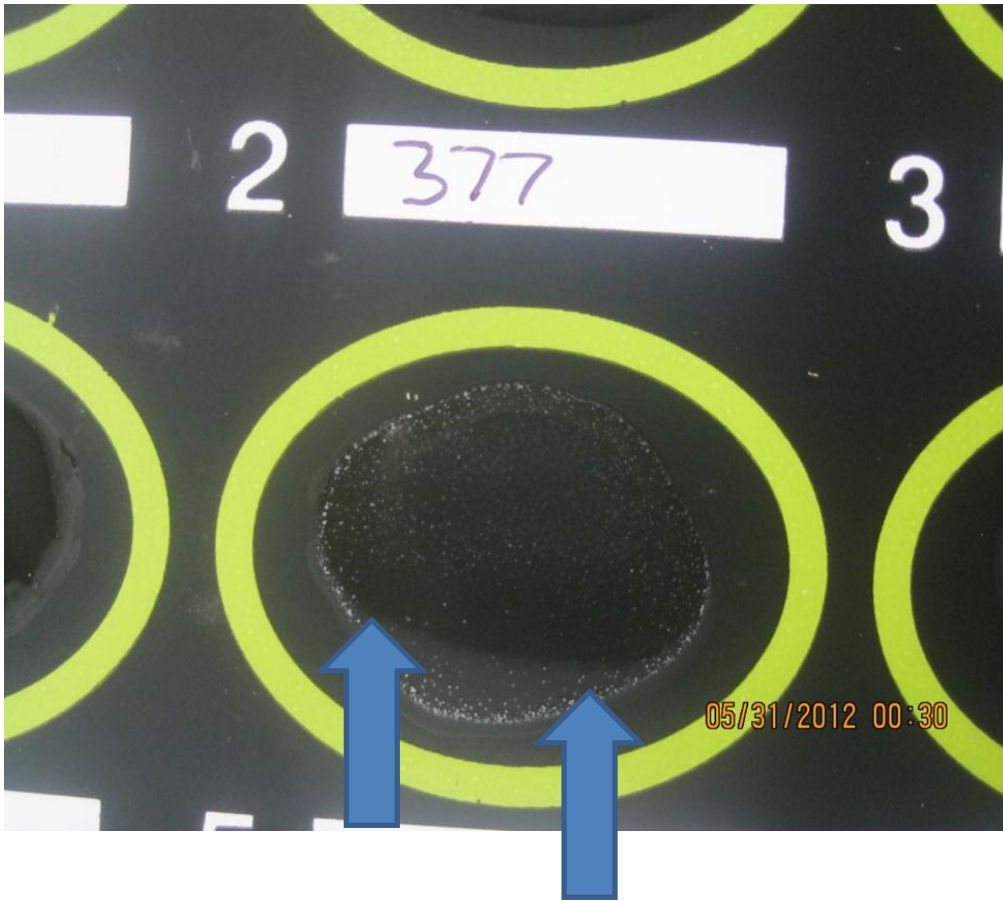
**Plate I:** Reaction beads card coated with casular polysaccharides purified *MmmSC* made up of 6 cells containing spot of serum and reagent next to each other in the cells (arrows), for the Latex Agglutination Test to diagnose CBPP in cattle.



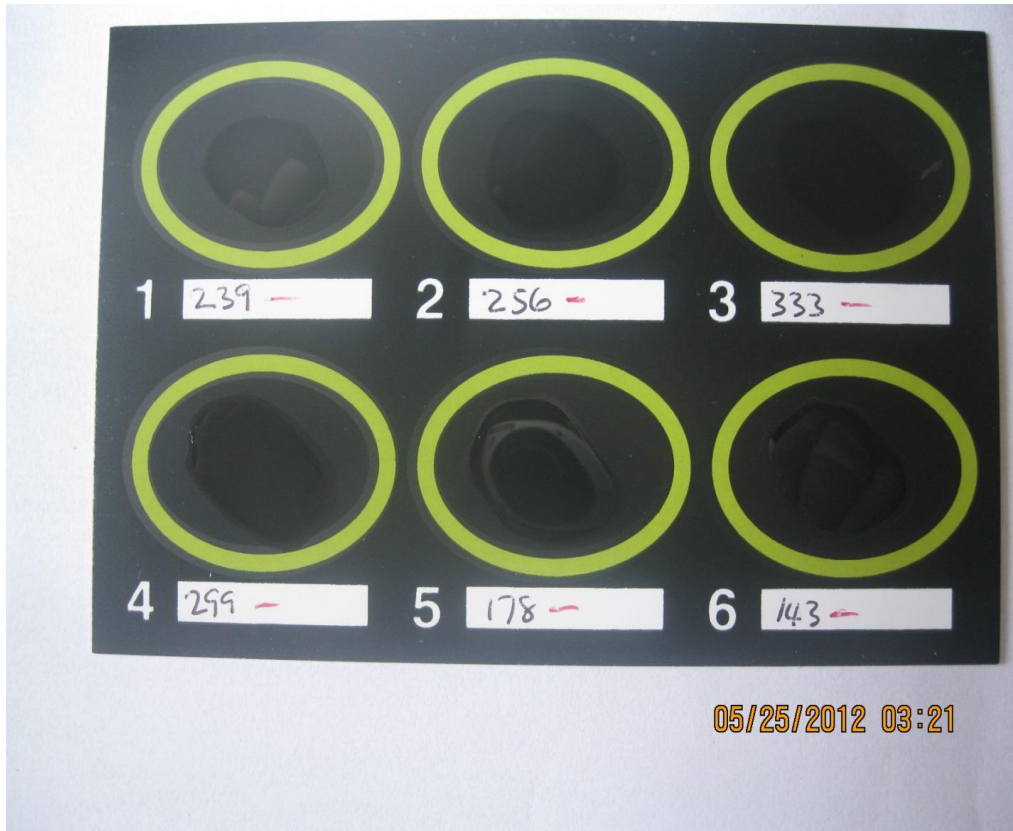
**Plate II:** Strong clumping of latex beads (arrows). Agglutination began within one minute, indicating a positive case Positive 3+(+++).



**Plate III:** Clear agglutination of latex beads (arrows). Agglutination began between one and two minutes, indicating a positive case Positive 2+(++).



**Plate IV:** Fine agglutination of the latex beads (arrows). Agglutination formed between two and three minutes, indicating a positive case Positive 1+(+).



**Plate V:** Negative(-). No agglutination formed in all the cells after three minutes (homogeneous solution), indicating negative cases.

## CHAPTER FOUR

### RESULTS

An overall sero-prevalence of 26.0% for CBPP in the three selected Local Government Areas (LGAs) under the study was achieved. One hundred and thirty eight, 138(46.0%) of the 300 cattle sampled in Kauru LGA were sero-positive, while, 51(17.0%) and 45(15.0%) of the 300 cattle sampled each from Ikara and Chikun LGAs respectively, were similarly sero-positive for CBPP. There is a significant difference in the CBPP sero-prevalence between LGAs based on Chi-square ( $X^2$ ) =93.87,  $P=0.0001$  and  $df=2$ . Kauru LGA have the highest sero-prevalence followed by Ikara LGA and Chikun as indicated in ( $P<0.05$ ) (Table 4.1).

Of the 275 cattle aged range <1-3 years, 47(17.0%) were sero-positive for CBPP, made up of Kauru LGA, 27(31.0%), Ikara LGA, 12(11.8%), and Chikun LGA, 8(9.1%). Similarly of the 375 cattle within the age bracket of 4-6 years old 90 had sero-prevalence of 24% for CBPP as followed, Kauru, 56(47.8%), Ikara 19(17.1%), and Chikun 15(10.2%). The age group >6 years recorded a sero-prevalence of 97(38.8%) for CBPP from the 250 cattle sampled. The sero-prevalence made up of Kauru, Chikun and Ikara LGAs as shown, 55(57.2%), 22(33.3%) and 20(22.7%) respectively. In this age group, Kauru LGA had the highest and Ikara LGA had the lowest. The Chi-square  $X^2=10.61$ ,  $P=0.0027$ , Odd Ratio (OR)=0.5299. The CBPP sero-prevalence in the age of cattle was highest in the cattle age group >6 years old, followed by the cattle age bracket 4-6 years old and the younger ones aged <1-3 years old, indicating significant difference in the CBPP sero-prevalence in age ( $P<0.05$ ) (Table 4.2).

Table 4.3 shows that 196 of the 900 cattle sampled for sero-prevalence for CBPP, were males while, the remaining 704 were females. Of the male cattle, 43(21.9%) were sero-positive for CBPP from Kauru, Chikun and Ikara LGAs having sero-prevalence of 31.4%, 18.8% and 18.3% respectively. Similarly, 191(27.1%) of the 704 female cattle sampled, were sero-positive for CBPP, consisting of 121(49.1%), 35(16.4%) and 35(14.15) from Kauru, Ikara, Chikun respectively. There is no significant difference between sexes in the CBPP sero-prevalence in the cattle sampled as indicated by ( $X^2$ )=2.148, P=0.1424, Odd Ratio (OR)=0.549. There is no association between sexes in the CBPP sero-prevalence in the cattle sampled ( $p>0.05$ ).

With regard to the breeds of cattle sampled during the study, 879(97.7%) were of the White Fulani (Bunaji) breed, while, 14(1.6%) and 7(0.8%) were Red Bororo (Rahaji) and Sokoto Gudali (Bokoloji) breeds respectively. Two hundred and twenty-three (25.4%) of the White Fulani, were sero-positive for CBPP, and were made up of 134(45.9%) from Kauru LGA, 48(16.4%) from Ikara LGA and 41(13.8%) from Chikun LGA. Of the 14 Red Bororo breed of cattle sampled 7(50.0%) were sero-positive. They are, 3(75.0%) of the 4 sampled from Chikun and 4(57.2%), of the 7 sampled from Kauru. Similarly, 7 Sokoto Gudali were sampled, 2(40.0%) from the 5 sampled in Ikara were sero-positive, while, 1 each from Chikun and Kauru were sero-positive to CBPP respectively. Based on ( $X^2$ )=5.718, P-value=0.0572 and df=2. It shows that there no significant difference in the sero-prevalence in the breeds of cattle sampled ( $p>0.05$ ), (Table 4.4).

**Table 4.1** Sero-prevalence of CBPP in Cattle by Local Government Area in the Three Selected LGAs of Kaduna State, Nigeria.

LGA	No. Sampled	No. Positive	% Positive
Ikara	300	51	17.0
Chikun	300	45	15.0
Kauru	300	138	46.0
Total	900	234	26.0

P-value=0.0001,  $X^2=93.87$  df=2

**Table 4.2:** CBPP Sero-prevalence by Age in Cattle in the Three Selected Local Government Areas of Kaduna state, Nigeria.

AGE	LGA						Total sampled	Total positive (%)
	Ikara		Chikun		Kauru			
	Sampled	Positive (%)	Sampled	Positive (%)	Sampled	Positive (%)		
<1-3 years	101	12(11.8)	87	8(9.1)	87	27(34.0)	275	47(17.0)
4-6 years	171	19(17.1)	147	15(10.2)	117	56(47.8)	375	90(24.0)
>6 years	88	20(22.7)	66	22(33.3)	96	55(57.2)	250	97(38.8)
Total	300	41(17.0)	300	45(15.0)	300	138(46.0)	900	234(26.0)

P-value = 0.0027,  $X^2 = 9.010$ , df = 1, OR = 0.5299

**Table 4.3:** Sero-prevalence of CBPP by Sex in Cattle in the Three Selected Local Government Areas of Kaduna State, Nigeria

LGA	SEX			
	Male		Female	
	Sampled	Positive (%)	Sampled	Positive (%)
Ikara	87	16(18.3)	213	35(16.4)
Chikun	55	10(18.8)	245	35(14.1)
Kauru	54	17(31.4)	246	121(49.1)
Total	196	43(21.9)	704	191(27.1)

P-value=0.1428,  $X^2=2.148$ , df=1, OR=0.7549

**Table 4.4:** Sero-prevalence of CBPP in Cattle by Breed in the Three Selected Local Government Area of Kaduna State, Nigeria

LGA	BREED					
	White Fulani (Bunaji)		Red Bororo (Rahaji)		Sokoto Gudali (Bokoloji)	
	Sampled	Positive	Sampled	Positive	Sampled	Positive
Ikara	292	48(16.4%)	3	0(%)	5	2(40%)
Chikun	295	41(13.8%)	4	3(75.0%)	1	1(100%)
Kauru	292	134(45.8%)	7	4(57.1%)	1	1(100%)
Total	879	223(25.3%)	14	7(50.0%)	7	4(57.0%)

P-value = 0.00573,  $X^2 = 5.718$ , df = 2

Of the 90 respondents, 11(12.2%) were literate, made up of 5(5.6%) from Chikun LGA and 3(3.3%) each from Ikara and Kauru LGAs respectively. Furthermore, 80(88.9%) of the respondents were aware of the existence of CBPP in cattle with 28(93.3%), 27(90.0%) and 25(83.3%), from Chikun, Ikara and Kauru respectively. Sixty-six (73.3%) of the respondents observed that the disease was very much in their communities. These include; 25(83.3%), 22(73.3%) and, 19(63.3%), from Ikara, Kauru and Chikun respectively. Twenty five (83.3%) of the respondents reported experiencing the disease in their herds, made up of 12(40.0%) in Kauru LGA, 8(26.7%) in Chikun LGA and 5(16.7%) in Ikara LGA. Of the 90 respondents 17(18.9%) indicated experiencing losses through outbreaks of CBPP in their herds as followed; 8(26.7%), 5(16.7%) and 4(13.3%) from Kauru, Chikun and Ikara LGAs, respectively (Table 4.5).

Awareness to CBPP vaccine was 80(88.9%), and that, 67 (74.4%) of them were in the habit of vaccinating their cattle against the disease, while, 20 (22.2%) do not vaccinate at all. Furthermore, 33 (36.7%) of the respondents believed that vaccinated animals could be protected for 6 to 12 months. Respondents that reported vaccinating their cattle after 12 months since the last vaccination were 34(37.8%).

The respondents reported that they never vaccinated sick animals. Among the respondents, 18(20.1%) reported vaccinating only healthy cattle and 26 (28.9%) reported vaccinating their cattle on annual basis, while, 41 (45.6%) of them reported vaccinating occasionally (Table 4.6).

Table 4.7 showed 46(51.1%) of the 90 respondents obtained the CBPP vaccines from the National Veterinary Research Institute (NVRI), Vom, while, 21 (23.3%) obtained it from the open market. Furthermore, in the administration of CBPP vaccines, 14(15.6%) of the 90 respondents indicated that Veterinarians administered, while, 18(20.0%) each, administered by Animal Health workers and drug vendors, and 40 (44.4%), is done by herdsman and cattle owners themselves.

**Table 4.5** Literacy and Awareness Levels of Respondents with regard to CBPP in the Three Selected Local Government Areas of Kaduna State, Nigeria.

LGA	Respondents	Literate (Read/Write English)	Awareness of CBPP	Knowledge of CBPP in the Community	Recent experience CBPP outbreaks	Reported losses
Ikara	30	3(10.0%)	27(90.0%)	25(83.3%)	5(16.7%)	4(13.3%)
Chikun	30	5(16.7%)	28(93.3%)	19(63.3%)	8(26.7%)	5(16.7%)
Kauru	30	3(10.0%)	25(83.3%)	22(73.3%)	12(40.0%)	8(26.7%)
Total	90	11(12.2%)	80(88.9%)	66(73.3%)	25(27.8%)	17(18.9%)

**Table 4.6:** CBPP Vaccination Records by Livestock Owners/ Herdsmen (Respondents) in the Three Local Government Areas of Kaduna

State, Nigeria.

LGA Respondent	Do vaccinate their cattle	Do not vaccinate	Vaccinate 6-12mo ago	Vaccinate over 12mo ago	Vaccinate only sick ones	Vaccinate only healthy ones	Vaccinate all in herd	Vaccinate annually	Vaccinate occassionally
Ikara 30	22(73.3%)	8(26.7%)	14(46.7%)	8(26.7%)	0	5(16.7%)	17(56.7%)	9(30.0%)	13(43.3%)
Chikun 30	25(83.3%)	5(16.7%)	14(46.7%)	11(36.7%)	0	6(20.0%)	19(63.3%)	9(30.0%)	16(53.3%)
Kauru 30	20(66.7%)	10(33.3%)	5(16.7%)	15(50.0%)	0	7(23.3%)	13(43.3%)	8(26.7%)	12(40.0%)
<b>Total 90</b>	<b>67(74.4%)</b>	<b>23(25.6%)</b>	<b>33(36.7%)</b>	<b>34(37.8%)</b>	<b>0</b>	<b>18(20.0%)</b>	<b>47(54.4%)</b>	<b>26(28.9%)</b>	<b>41(45.5%)</b>

**Table 4.7** Sources and Personnel administering CBPP Vaccines in Cattle in the Three Selected LGAs of Kaduna State, Nigeria.

LGA	Respondents	Sources of Vaccines		Personnel Administering			
		Veterinary Research Institute No. (%)	Open Market No. (%)	Veterinarians No. (%)	Animals Health Workers No. (%)	Drug Vendors No. (%)	Herdsmen No. (%)
Ikara	30	18 (60.0)	4(13.3)	5(16.7)	7(23.3)	6(20.0)	12(40.0)
Chikun	30	16 (53.3)	9(30.0)	6(20.0)	6(20.0)	5(16.7)	13(43.3)
Kauru	30	12 (40.0)	8(26.7)	3(10.0)	5(16.7)	7(23.3)	15(50.0)
Total	90	46(51.1)	21(23.3)	14(15.6)	8(20.0)	18(20.0)	40(44.4)

## CHAPTER FIVE

### DISCUSSION

Contagious bovine pleuropneumonia (CBPP) is an endemic disease in Nigeria since it was first reported in 1924 (Foluso, 2004). This is due to the transhumance and nomadic nature of cattle rearing and failure of control measures in Nigeria (Egwu *et al.*, 1996). Latex Agglutination Test (BoviLAT PA6223) for CBPP is an easy and fast diagnostic technique to perform in the field (Ayling *et al.*, 1999b). The result of this study shows an overall sero-prevalence of 26.0%. This is higher than that of Nawathe (1992), and Aliyu *et al.*, (2000), who reported prevalence of 0.52% and 0.29%, in Borno State and 5 States in Northern part of Nigeria respectively. Also, higher than the report of Adamu and Aliyu (2006), of 0.33% in an epidemiological study in Borno State. The higher sero-prevalence in this study could be as a result of the diminishing control measures due to incomplete and irregular vaccination programmes over the years, as well as steady illegal introduction of infected cattle into the areas (particularly through transhumance and nomadism) that were initially thought to be free of the disease (Aliyu *et al.*, 2000). It could also be as a result of epidemiological trend of the disease with the presence of carriers in some herds which might not have been detected clinically and hence the maintenance of the disease and gradual spread of the disease (Egwu *et al.*, 1996).

The result of this study showed Kuru LGA has a higher sero-prevalence of 46.0% than Ikara and Chikun LGAs with 17.0% and 15.0% respectively. This might be as a result of the strategic location of Kuru LGA, where a major cattle route, from the North-west passes through to the southern part. Also, the presence of two grazing reserves, Libere and

Laduga located in Kajuru and Zango Kataf LGAs which share borders with Kauru LGA, harbour large population of cattle. The movement of these cattle in and out of Kauru could enhance easy spread of the disease. Furthermore, an outbreak of the disease in a herd of cattle in Igabi LGA, that shares border with Kauru LGA, was previously reported by Danbirni *et al.* (2010), might also have contributed to the higher sero-prevalence. The disease have been shown to be presence in older animals than younger ones which is in agreement with the findings of Boelert (2005), that explain the fact that age is a measure of exposure and chances of being infected. Thus, younger animals have a lower risk of being sero-positive than older ones.

The result of this study shows that sex is not a factor in the epidemiology of CBPP, indicating uniform susceptibility. The result also revealed that infection among breeds studied is uniform hence not a factor in the spread of the disease as shown by Santini *et al.*, (1992), that ruminants of *Bos* genus are generally susceptible to CBPP.

The study discovered synonyms to CBPP such as; bazana, ottu, ciwon-huhu and rangaza. The study also revealed a high level of illiteracy in which only 12.2% are literate among the pastoralists. This is in agreement with the report of Tahir (2001), that pastoralists and their childred have little or no access to formal education. Awareness of pastoralists to CBPP is shown as 88.9% in this study. This is higher than the report of Tambuwal *et al.* (2011b) of 65.0%. The higher value might be attributed to the frequent outbreaks experienced as a result of the increasing spread of the disease. Suspected outbreaks observed in this study was 38.9%. This is higher than 5.8%, reported by Tambuwal *et al* (2011b). This higher value could be due to failure of control measures as seen in the low vaccine coverage,

uncontrol cattle movement and transhumance activities. The study revealed that 76.0% of outbreaks were treated using antibiotics (mostly the tetracyclines L.A). Huebschle *et al.* (2004) reported 69.0% of the farmers in Ethiopia admitted treating CBPP cases with antibiotics. The increasing use of antibiotics by herdsmen is possibly an attempt to alleviate the suffering of the affected cattle as reported by Mariner *et al.*, (2006). Huebschle *et al.* (2004), Yaya *et al.* (2004) and Ayling *et al.* (2005) have confirmed some beneficial effects on the use of antibiotics though complete elimination of the causative organism from the affected cattle may not be achieved, thereby creating a potential pool of disease carrier.

The study also, established the knowledge of the communities on CBPP and its recognition to be 73.3%. This is higher than the 22.3% reported by Tambuwal *et al.*, (2011a). The increase in the ability to recognise the disease might be due to the increase in the endemicity of the disease with new cases being recorded regularly, as result of frequent outbreaks. The vaccination coverage of 36.7% obtained in this study is slightly higher than the 32.5% reported by Tambuwal *et al.* (2011b) and 14.2% by Aliyu, *et al.* (2000). This higher vaccine coverage is possibly due to the increased awareness of the disease and hence the desire for protection of cattle herds.

The study also revealed that 51.1% of the pastoralists obtained their vaccines from NVRI and 23.3% from open market. The personnel administering of the vaccines, where, 15.6% Veterinarians, 20.0% Animal Health workers, 20.0% drug vendors and the herdsmen 44.4%. Vaccination by unqualified personnel lead to low vaccination coverage, which

might have also contributed to the spread of the disease in the area of study, as clearly observed by Adekunle *et al.*, (2002), Ameh *et al.*, (1998) and Halle *et al.*, (1998).

## CHAPTER SIX

### CONCLUSIONS AND RECOMMENDATIONS

#### 6.2 Conclusions

- Contagious bovine pleuropneumonia (CBPP) sero-prevalence in cattle in the study area was 26.0%, which is higher than those reported by earlier workers like Nawathe (1992) and Aliyu *et al.* (2000) of 0.51% and 0.29% respectively.
- Bovi-LAT Kit provides a fast and easy to perform diagnostic technique in the field, therefore, it is good for early detection of cattle with CBPP and, this will be useful in planning control measures in the study area.
- The study similarly found a high level of illiteracy among the pastoralists and it may be one of the reasons that could have contributed to the low vaccination coverage against CBPP in the study area.
- The study also found that the pastoralists were exposing their cattle to many risks of contacting the disease by way of grazing and watering their animals at communal grazing lands as well as addition of new animals to their herds without the necessary quarantine procedures.
- The sero-prevalence obtained in this study may not be the true reflection of the CBPP infection, because BoviLAT Latex Agglutination is not capable of differentiating antibodies from natural infection and recently vaccinated animals with CBPP vaccine. It is one of drawback in this method.

### **6.3 Recommendations**

- ❖ BoviLAT seems to have good promise due to its simple, fast and easy to perform for the diagnosis of CBPP in the field, therefore, it should be encouraged.
- ❖ BoviLAT to be compared with the OIE recommended test procedures e.g ELISA and PCR for adoption as a routine test for CBPP epidemiology on a wider scale.
- ❖ The disease, CBPP, has been found to be very much with cattle in the study area and as such, efforts should be made by Governments (LGAs, State and Federal) to intensify vaccination of cattle against the disease.
- ❖ To conduct more extensive study on the disease with a view to having a full picture of the actual status of the disease in the study area, the state and the nation as a whole.
- ❖ The control of cattle movement in grazing reserves and check-mating of unauthorized vaccination by drug vendors and pastoralists can reduce the spread of CBPP in the study area.
- ❖ Local Government authorities to have cold chain facilities for storage of vaccines to enhance good source and availability to farmers at short distances within the Local Government Area.

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## APPENDICES

**Appendix I:** List of *Mycoplasma spp.* along with hosts and disease conditions they cause  
Source: Songer and Post, (2005).

Species	Host(s)	Disease(s)
<i>M. agalactiae</i>	Goats, sheep	contagious agalactia
<i>M. alkalescens</i>	Cattle	arthritis, mastitis
<i>M. bovis genitalium</i>	Cattle	infertility, mastitis
<i>M. bovis</i>	Cattle	arthritis, mastitis, pneumonia, abortion, abscesses, otitis media, genital infections
<i>M. bovoculi</i>	Cattle	conjunctivitis
<i>M. californicum</i>	Cattle	mastitis
<i>M. canadense</i>	Cattle	abortions, mastitis
<i>M. capricolum ssp. Capricolum</i>	Goats, sheep	mastitis, septicemia, polyarthritis, pneumonia
<i>M. capricolum ssp. capripneumoniae</i>	Goat	contagious caprine pleuropneumonia
<i>M. conjunctivae</i>	Sheep, goats	infectious keratoconjunctivitis
<i>M. cynos</i>	Dogs	pneumonia
<i>M. dispar</i>	Cattle	bronchiolitis
<i>M. equigenitalium</i>	Horses	abortion, pneumonia
<i>M. felis</i>	Cats	conjunctivitis, pneumonia
<i>M. gallisepticum</i>	Chickens, turkeys	airsacculitis, sinusitis
<i>M. gatae</i>	Cats	chronic arthritis, tenosynovitis

<i>M. hyopneumoniae</i>	Pigs	enzootic pneumonia
<i>M. hyorhinis</i>	Pigs	polyarthritis, polyserositis
<i>M. hyosynoviae</i>	Pigs	polyarthritis
<i>M. lowae</i>	Turkeys	embryo mortality
<i>M. meleagridis</i>	Turkeys	airsacculitis, skeletal normalities, pleuropneumonia
<i>M. mycoides ssp capri</i>	Goats	arthritis, mastitis, pneumonia, septicemia
<i>M. mycoides ssp mycoides</i> (Small colony type)	Cattle, domestic, water buffalo	contagious bovine pleuropneumonia
<i>M. mycoides ssp mycoides</i>  (Large colony type)	Goats/sheep	mastitis, septicemia, polyarthritis, pneumonia
<i>M. ovipneumoniae</i>	Goats, sheep	pleuropneumonia
<i>M. pulmonis</i>	Lab. rats, mice	murine respiratory mycoplasmosis
<i>M. synoviae</i>	Chicken, turkeys	infectious synovitic

**Appendix II:** List of *Mycoplasma mycoides* clusters. Source: Taylor *et al.*, 1992

Species	Main disease (s)	Main (and other) hosts
<i>M. mycoides</i> subsp. <i>mycoides</i> SC variant	CBPP	Cattle (goats, sheep, buffalo)
<i>M. mycoides</i> subsp. <i>mycoides</i> LC variant	Caprine pneumonia, contagious agalactiae	Goats (sheep, cattle)
<i>M. mycoides</i> subsp. <i>capri</i>	Caprine pneumonia	Goats (sheep) but rare
<i>M. capricolum</i> subsp. <i>capricolum</i>	Caprine pneumonia, contagious agalactiae, arthritis	Goats (sheep, cattle)
<i>M. capricolum</i> subsp. <i>capripneumonia</i>	Contagious caprine pleuropneumonia,CCPP	Goats (sheep)
<i>M. bovine</i> group 7 (Bg7)	Arthritis, also mastitis, calf pneumonia	Cattle



**Appendix IV: Department of Veterinary Medicine, Faculty of Veterinary Medicine,  
Ahmadu Bello University, Zaria.**

**QUESTIONNAIRE ON SEROPREVALENCE OF CONTAGIOUS BOVINE  
PLEUROPNEUMONIA IN KADUNA STATE, NIGERIA**

**A. RESPONDANT BIODATA**

1. Name of L.G.A. ....
2. Name of Ward .....
3. Age of herdsman.....
4. Level of Education-----
5. Other sources of income.....

**B. KNOWLEDGE OF THE DISEASE**

1. What species of animals are present on the livestock holding (herd): Cattle [ ] Sheep [ ]  
Goat [ ]
2. Are you aware of the disease CBPP? YES [ ] or NO [ ]
3. If yes, is the disease known to the community? YES [ ] or NO [ ]
4. Does it have a local name? YES [ ] or NO [ ]
5. If yes what is the local name? [ ]
6. Have you had outbreak of CBPP in your herd? YES [ ] or NO [ ]
7. If yes, what age groups were affected? 0-1year [ ] 2-3 years [ ] 4years and above [ ] All ages[ ]
8. Have you lost animals from this disease? YES [ ] or NO [ ]
9. List three signs of CBPP you observed: a. b. c.
10. What action did you take on the diseased animals? Treated [ ] Sold [ ]
11. If treated, with what? Antibiotics [ ] Local herbs [ ]

12. Who treated? Vet. Doctor  Drug vendor  Self

13. What was the response to the treatment? Recovered  No response

### C. VACCINATION HISTORY

1. Are you aware of CBPP vaccine? YES  or NO

2. If yes do you vaccinate your cattle against CBPP? YES  or NO

3. If yes, how many times in a year? Once  Twice  Not every year

4. When did you vaccinate your cattle last? 6 mon ago  a yr ago  2 yr ago  3 yr and Above

5. What groups of your cattle were vaccinated? Only sick ones  healthy ones only  All

6. Where did you obtain the vaccine? Research/Vet. Centres  Open market

7. Who conducted the vaccination? Vet. Dr.  Drug vendors  Self

### D. RISK FACTORS

1. Mixing at:      Grazing YES  NO  Drinking point (water) YES  NO

Market YES  NO  Vaccination Camp YES  NO

Major stock route YES  NO

2. Introducing new animals into the herd? YES  NO

3. If yes, from where? Bought from open market  borrowed bull for breeding

4. Are you aware of quarantine procedure? YES  NO

5. Were these new animals quarantined before introduction into the herd? YES  NO

6. Do nomadic herds normally pass through this area? YES  or NO

8. Do your animals mix with such nomadic herds? YES  NO

7. If yes, how often? Occasionally  Frequently

THANK YOU