PREVALENCE AND CO-OCCURRENCE OF *BRUCELLA* SPP AND *COXIELLA BURNETII* IN CATTLE, SHEEP AND GOATS IN MAIGANA AND BIRNIN GWARI AGRO-ECOLOGICAL ZONES OF KADUNA STATE, NIGERIA

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF Ph.D DEGREE IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

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FEBRUARY, 2018

DECLARATION

I declare that the work in this thesis entitled **"Prevalence and co-occurrence of *Brucella* spp. and *Coxiella burnetii* in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State, Nigeria"** has been performed by me in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria Nigeria, under the supervision and guidance of Prof. Junaidu Kabir, Prof. J. U. Umoh and Prof. M. A. Raji. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Shu'aibu Gidado ADAMU _____

_____

Signature Date

CERTIFICATION

This thesis entitled **"Prevalence and co-occurrence of *Brucella* spp. and *Coxiella burnetii* in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna, State, Nigeria"** by Shu'aibu Gidado ADAMU (P13VTPH9011) meets the requirements and regulations governing the award of the degree of Doctor of Philosophy in Veterinary Public Health and Preventive Medicine of Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

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ABSTRACT

A cross sectional study was conducted to determine the seroprevalence of two related reproductive and zoonotic diseases namely brucellosis and Q-fever in cattle, sheep and goats in Maigana and Birnin Gwari Agro-ecological Zone of Kaduna State. A total of 1200 blood samples, consisting of 400 each from cattle, sheep and goats in settled and semi settled production systems were collected and screened for brucellosis using Rose Bengal Plate Test (RBPT) and confirmed with competitive (c) and indirect (i) enzyme immunosorbent assay (ELISA) for *Brucella abortus* and or *B. melitensis* and *Coxiella burnetii* infections. Herds or flock that tested positive with ELISA were retested 6 months later to detect the persistence of the organism using polymerase chain reaction (PCR). Background information on individual animal sampled and the herd was collected using a semi-structured questionnaire and analysed to detect associations and risk factors. The seroprevalence in cattle, sheep and goats were 18.5% (74/400) and 6.8% (27/400), 16.8% (67/400) and 10.8% (43/400), 12.0% (48/400) and 6.0% (24/400) for brucellosis respectively and 6.2% (25/400), 8.0% (32/400) and 8.8% (35/400) for Q fever in cattle, sheep and goats. There was statistically significant association between the species of the animals tested and the rates of infection of brucellosis and Q fever. Bivariate analysis showed female animals of all species tested were more seropositive for antibodies to *Brucella* spp. and *Coxiella burnetii* than males. Breed and age of cattle, sheep and goats were not statistically significant for seropositivity to *Brucella* spp. and *Coxiella burnetii*. Though, there was statistically significant association between the age of goat and the rate of infection with Q fever. Out of 16 (1.3%) that were positive for both brucellosis and Q fever, 2 (0.5%) were cattle, 7 (1.8%) goats and 16 (1.3%) sheep that has multiple

infections/ co-infections of both brucellosis and Q fever. The thirteen LGAs sampled from Kaduna State, brucellosis and Q fever were found spatially distributed in 11 LGAs surveyed. Joint infections were found in 10 LGAs surveyed with the exception of Chikun, Kaduna North and Kaduna South LGAs. Out of the 36 herds that had history of abortion, with 21 (58.3%) was positive for *Brucella* by cELISA and 28 (77.8%) was positive for *C. burnetii* by iELISA. The 36 herds that herd history of abortion were retested after six month later, of the 21 positive from the herd with abortion, 12 (57.1%) was positive with PCR for *Brucella* and 2 (7.1%) was positive with PCR for *C. burnetii* which showed the persistence of *Brucella* and *C. burnetii* infections in the herds. Of the 25 flocks that had history of abortion,5 (20.0) was positive for *C. burnetii*, with 2/10 (20.0%) goats, and 3/10 (30.0%) sheep respectively. The study indicates that the two zoonotic diseases brucellosis and Q-fever exists with high prevalence particularly among female cattle, goats sheep, with goats older than 4 years being a high risk group for brucellosis in the study area. This presents a serious public health problem, calling for greater awareness amongst stakeholders and for coordinated active surveillance for the diseases among diverse cattle, sheep and goats populations in Nigeria. The two organisms were higher in females than in males and in the animals older than 4 years in the three animal species tested. Screening of domestic livestock for brucellosis and Q fever should be performed on regular basis and implementation of strict quarantine control programmes especially in dairy cattle and livestock farms / flocks of shee and goats. This study indicates that *Brucella* specie and *Coxiella burnetii* infections exist with high prevalence mainly among female and older cattle, sheep and goats, the disease can be considered as a potential risk for both susceptible animals and humans in the study area. This presents a serious public health

problem, calling for greater awareness amongst stakeholders and for co-ordinated surveillance for the diseases among cattle, sheep and goats populations in Nigeria.

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LIST OF ABBREVIATION

+ ve	Positive
- ve	Negative
%	Percentage
>	Greater than
<	Less than
χ^2	Chi-square
P<0.05	Probability is less than 5% α-level
P>0.05	Probability is greater than 5% α-level
2-ME	2-Mercaptoethanol
ACERSA	Association pour la certification de la santé animale
AGID	Agar Gel Immunodiffusion
AMOS	*abortus, melitensis, ovis, suis*
APHA	Animal and Plant Health Agency
BAPT	Buffered Antigen Plate test
BCSP	*Brucella* Cell Surface Protein
BCV	*Brucella*-Containing Vacuole
BLM	Balami breed of sheep
Bp	Base Pairs
CAT	Capillary Agglutination Test
CDC	Centers for Disease Control and Prevention
cELISA	Competitive Enzyme Linked Immunosorbent Assay
CFT	Complement Fixation Test

CFSPH	Center for Food Security and Public Health
CFU	Colony Forming Unit
CI	Confidence Interval
DCs	Dendritic Cells
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide Triphosphates
DTT	Dithiothreitol
ECDC	European Centre for Disease Prevention and Control
EDTA	Ethylene Diamine Tetra Acetic Acid
EFSA	European Food Safety Authority
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
ERIC	Enterobacterial Repetitive Intergenic Consensus
FAO	Food and Agriculture Organization
FPSR	False Positive Serological Reactions
FPA	Fluorescence Polarization Assay
Fc	Fragment Crystallizable
FUO	Fever of Unknown Origin
GIS	Geographical Information System
HRP	Horseradish Peroxidase
IDWI	Inverse Distance Weighted Interpolation
iELISA	Indirect Enzyme-Linked Immuno Sorbent Assay
IFA	Immunofluorescence Assay

γ-IFN	Gamma Interferon
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
IS711	Insertion Sequence 711
KDSG	Kaduna State Government
KGR	Kachia Grazing Reserve
LFA	Lateral Flow Assay
LGA	Local Government Area
LPS	Lipopolysaccharide
Mab	Monoclonal Antibody
MACBA	Miyetti Allah Cattle Breeders Association.
MLEE	Multilocus Enzyme Electrophoresis
MLVA	Multiple Locus Variable-number Tandem Repeat Analysis
MLST	Multilocus Sequence Typing
MRT	Milk Ring Test
MSAT	Microtitre Serum Agglutination Test
NASPHV	National Association of State Public Health Veterinarians
NVRI	National Veterinary Research Institute
OD	Optical Density
OIE	Office International des Epizooties
Omp	Outer Membrane Protein

OR	Odds Ratio
PCR	Polymerase Chain Reaction
P_{exp}	Expected Prevalence
PFGE	Pulsed Field Gel Electrophoresis
Q fever	Query Fever
RAPD	Random Amplified Polymorphic Deoxyribonucleic acid
RB51	Rough *Brucella* 51
RER	Rough Endoplasmic Reticulum
Rev.1	Revertant 1 name of *Brucella* vaccine
RIA	Radioimmunoassay
RID	Radial Immunodiffusion
RTq	Real Time Quantitative
REP	Repetitive Extragenic Palindromic
RFLP	Restriction Fragment Length Polymorphism
RBPT	Rose Bengal Plate Test
RLT	Rough Lipopolysaccharide
SAT	Serum Agglutination Test
SG	Sokoto Gudali
SHL	Sahel
SKR	Sokoto Red
S-LPS	Smooth Lipopolysaccharide
SMX	Sulfamethoxazole
SNP	Single Nucleotide Polymorphism

S/P	Serum Percentage	
SPSS	Statistical Package for Social Sciences Software	**UN**
S19	Strain 19	**ITS**
TAE	Tris Acetic acid EDTA	**OF**
TBE	Tris Borate acid EDTA	**ME**
TMP	Trimethoprim	**AS**
TMB	Tetramethylbenzidine	**UR**
USA	United States of America	**EM**
WAD	West African Dwarf	**EN**
WF	White Fulani	**T**
WHO	World Health Organization	^{o}C
YKS	Yankasa	
		Deg
		ree
		Cel
		sius
		µl
		Mic
		rolit
		re
		µm
		Mic

rometer

g	Gravitational Force
h	Hour
kDa	Kilodaltons
min	Minute (s)
mg	Miligrams
ml	Milliliter
O.D	Optical density

CHAPTER ONE

1.0 INTRODUCTION

1.2 Background

Brucellosis is one of the most common zoonotic diseases in the world (Al-Tawfiq and Abukhamsin, 2009). In animals, brucellosis is also called Bang's disease, Enzootic abortion, Epizootic abortion, Slinking of calves, Ram epididymitis orContagious abortion.In humans the disease is calledMalta fever, Undulant fever, Mediterranean fever, Rock fever of Gibraltar or Gastric fever (CFSPH, 2007).Brucellosis is a highly contagious zoonosis caused by*Brucella* species through the ingestion of unpasteurized milk or meat from infected animals or close contact with their secretions. Richey and Harrell (1997) and FAO (2003) described brucellosis as an infectious and contagious bacterial zoonotic disease caused by *Brucella*species. *Brucella melitensis* is considered to have the highest zoonotic potential, followed by *B. abortus*, and *B. suis*(Seleem *et al*., 2010). Brucellosis is considered by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organisation for Animal Health (OIE) as the second most important zoonotic disease in the world, accounting for the annual occurrence of more than 500,000 cases particularly from developing countries (Seleem *et al*., 2010).

Brucellosis is found in all African countries with the probable exception of Madagascar and Mauritius (Thimm, 1982). The infection is mainly transmitted to humans through the ingestion of rawcontaminated milk, through injured or intact skin, mucosa of the respiratory system and conjunctiva (Chukwu, 1987; Seifert, 1996). The disease mainly affects the reproductive organs of infected animals (England *et al*., 2004). Hygromas and

abscesses are the major clinical signs in nomadic or semi-nomadic cattle herds infected with *B. abortus* Biovar types 3(Gur *et al*., 2003). Human brucellosis is a multisystem disease that may present a broad spectrum of clinical manifestations and its complications can affect almost all organs and systems with varying degree (Gur *et al*., 2003). Epidemiological studies of brucellosis were based on serological investigation and these include Serum agglutination test (SAT), complement fixation test (CFT), enzymelinked immunosorbent assays (ELISAs), fluorescence polarisation assay (FPA), Rose Bengal Plate test (RBPT) (Araj, 1999), and isolation of the bacteriafollowed by phenotypic characterization based on physiological and biological properties of the *Brucella*organism. It is usually labourious, time consuming and very hazardous(Lopez-Goni *et al*., 2008; OIE, 2009). The advent of molecular methods, such as deoxyribonucleic acid (DNA) probes andpolymerase chain reaction (PCR) techniques have enhanced diagnostic capacities to a point that identification of causative agents is less obscure (Colmenero *et al*., 2011).

Brucellosis accounts for majority of cases of abortion in livestock (Nicoletti, 1980). Other organisms/diseases that cause abortion in livestock include leptospirosis (Oktay *et al*., 2005), campylobacteriosis (OIE, 2013),*Chlamydophila abortus* (formerly *Chlamydia psittaci*) listeriosis andcoxiellosis(*Coxiella burnetii*) (OIE, 2013)which could occur as individual disease or multiple infections. Among these diseases,coxiellosis (Q fever) remained one of the most under reported diseases in Africa.Coxiellosis (Q Fever) is a highly contagious zoonotic disease caused by *Coxiella burnetii*, an obligate intracellular gram-negative bacterium (Asadi *et al.*, 2013).The Q fever infection has been described in all countries worldwide, except in New Zealand (EFSA, 2010a). A wide variety of animals

can be infected with *C. burnetii*. Ruminants are the most common reservoirs of the organism (Keyvanirad *et al*., 2013). This organism is a small gram-negative obligate intracellular pathogen and has been traditionally placed in the family Rickettsiaceae; however, phylogenetic studies have demonstrated that *C. burnetii* is more closely related to *Legionella*, *Francisella* and *Rickettsiella*(Stein *et al.,* 1993). The organism is characterized by its extremely high infectivity and tenacity in the environment (Maurin and Raoult 1999). This organism is now classified in the family Coxiellaceae and Order Legionellales in the gamma subdivision of Proteobacteria (CFSPH, 2007). Q fever was first described by Edward Holbroock Derrick in Brisbane, Queensland, Australia, during an outbreak of a febrile illness of unknown origin ("Query fever") among abattoir workers (Derrick, 1944). The causative agent of Q fever is classified as a "Category "B" critical biological agent" by the Centres for Disease Control and Prevention and is considered a potential agent of bioterrorism (Alibek, 1999).

Ticks are considered to be the natural primary reservoir of *C. burnetii* while domestic ruminants are considered as the main reservoir for the pathogen which can infect a large variety of hosts, mammals and also birds, fish, reptiles, and arthropods (EFSA, 2010b). Q fever infections remain poorly understood (Pape *et al*., 2009) and their prevalence has been underestimated for many years (Rousset *et al*., 2007a). Infected animals are generally asymptomatic, but shed the organism in urine, faeces, milk, and in very high concentrations in the amniotic fluid during birth or abortion,thereby contaminating the environment (Arricau-Bouvery and Rodolakis, 2005). *Coxiella burnetii* is highly resistant

to environmental conditions and can be resistant to heat, drying, and many common disinfectants.

Ticks are important in transmission, particularly among wildlife, and can also spread infections to domestic ruminants (EFSA, 2010a). There are about 40 species of ticks transmitting the infections including *Rhipicephalus sanguineus* and *Amblyomma vareigatum* (Maurin and Raoult, 1999). Transmission of infection to humans is mainly through the inhalation of contaminated aerosols, but can also occur after consumption of infected raw milk and dairy products (Keyvanirad *et al.,* 2013). Animals may become infected by direct contact with infected animals and contaminated environments and/or from inhalation of aerosolized bacteria. Birth products (including placenta, foetuses, and amniotic and allantoic fluids), excreta, and milk are the most likely sources of infection for humans (Porter *et al.*, 2011).

Reproductive disorders such as abortion, metritis, mastitis, stillbirth, calves weak at birth, and infertility in cattle, sheep and goatsare some of the characteristicsof the disease (Marrie, 2007). The infection can manifest as an acute, chronic or subclinical form (ECDC, 2010). Most cases are asymptomatic; symptomatic infections most commonly present as a non-specific febrile illness that may occur in conjunction with pneumonia or hepatitis (Maurin and Raoult, 1999; CDC, 2011a). Studies of Q fever are based on serological tests with the three most often used test being the indirect immunofluorescence assay (IFA), ELISA and the complement fixation test (CFT). Overall, ELISAs are preferred for practical reasons. Currently, no IFA is commercially available for ruminants (EFSA,

2010a).Isolation of *C. burnetii* is very difficult and dangerous. Recently, PCR has been used to detect *C. burnetii*. PCR is a safe, sensitive and specific method for the detection of *C. burnetii* in different samples (OIE, 2013).

1.2 Statement of the Research Problem

There are reports of abortion, birth of weak animals and other reproductive problems in Nigeria, especially Kaduna State have not been properly investigated (Buhari *et al*., 2015). More often clinical diagnosis of abortion may not identify the causative agents due to lack of facilities for culture and identification of fastidious organisms like *Brucella*and *Coxiella*, particularly in developing countries. Similarly, co-infection in high risk groups such as veterinarians, butchers and animal handlers seems epidemiologically possible but uninvestigated in Nigeria. Six species of *Brucella*are recognized as causes of infection in different animal hosts. *B. melitensis* (small ruminants and camels), *B. abortus* (cattle), *B. suis* (swine), and *B. canis* (dogs) are known to cause human disease. *B. neotomae* (desert wood rats) and *B. ovis* (sheep) are not pathogenic to humans.

Although brucellosis has been eradicated from a number of developed countries, it remains an important cause of economic losses and public health problems in many developing countries (Colmenero *et al*., 1996). In Nigeria, brucellosis has been reported in cattle with prevalence of 15.3% (Hashimu *et al*., 2017), sheep 26.5% (Kaltungo *et al*., 2015), goats 2.5% (Dogo *et al.*, 2016; horses 16.0% (Ardo *et al*., 2016), 9.0% (Tijjani *et al*., 2016), donkeys5.5% (Sadiq *et al.*, 2013),6.7% (Tijjani *et al*., 2017), swine (Maurice *et al.*, 2013), dogs (Cadmus *et al.*, 2011; Momoh *et al*., 2014), human beings 24.1% (Aworh *et*

al.,2013), 11.0% (Adamu *et al*., 2015) and camels 16.5% (Adamu *et al*., 2014b).Investigations confirmed that some infections occur frequently in farmers, when they come in contact with cattle, sheep and goats infected with Q fever (Coleman, 2000).

The risk of Q fever in people who work with domestic animals is related to contact with farm environment rather than any specific animal exposure (Thomas *et al*., 1995). The interest in Q fever is increasing worldwide as indicated by the rising number of reviews published even in countries where its incidence is supposed to be very low (Arricau-Bouvery and Rodolakis., 2005; EFSA, 2010a). Indeed, the disease is considered as a re-emerging zoonosis in many countries. Because of the polymorphism of the clinical picture and because the diagnosis is based exclusively on serology, the prevalence of *C. burnetti* infection among animals is largely unknown (Rey *et al*., 2000). Only limited studies on *C. burnetti* in cattle are available. Addo and Schnurenberger (1977) reported a prevalence of 11.0% in slaughtered cattle from Samaru, Zaria and Kaduna abattoirs and from Bauchi meat processing plant. Adesiyun *et al*. (1984) also reported a prevalence of 59.8% in some dairy cows and their suckling calves in Zaria. Tukur *et al*. (2014) reported evidence of *C. burnetii* infection with a seroprevalence of 14.5% in dairy cows in Kaduna Metropolis. It is likely that co-infection with brucellosis and Q fever could occur in both animals and humans, exacerbating the pathogenesis and outcomes of these zoonotic infections.

1.3 Justification of the Study

Cattle, sheep and goats are the major source of meat supply in Kaduna State and Nigeria as a whole and they play a vital role in the human diet. There is evidence that brucellosis is

endemic and represents a major constraint to livestock production in Nigeria (Ate *et al*., 2007; Bertu *et al*., 2015). Recent studies reported increasing trend in the prevalence of brucellosis (Bertu *et al*., 2015). Like brucellosis, Q fever also cause reproductive disorders such as abortion, stillbirth, mastitis, infertility and weakcalf delivery, leading to serious economic impact on the herd and a reduction in the available source of protein for human consumption (Arricau-Bouvery and Rodolakis, 2005). Losses due to abortion or stillbirths, irregular breeding, loss of milk production and reduced human productivity are some of the economic consequences of the disease (Nicoletti, 1982). The reduced human productivity can hardly be measured in medical care. The economic losses due to abortion can be very high if no control measures are applied (Nicoletti, 1982). Estimates of the costs associated with brucellosis infections remain limited to specific countries. Like brucellosis, coxiellosis also assumes greater public health importance where there is close and frequent association between man and animals, such as in rural areas, where agriculture and animal husbandry are the main sources of income (Ho *et al*., 1995).

The major occupation of the people of Kaduna State is agriculture, mainly food and cash crops and rearing of livestock (KDSG, 2008). Kaduna State has an estimated population of 1,144,000 cattle, 832,000 sheep and 988,000 goats (KDSG, 2008). In view of the relatively smaller population of livestock in relation to the rapidly increasing human population, there is the need to understand and control any factor that will limit the productivity of livestock in the State. Infectious diseases that affect reproduction like brucellosis and Q fever need to be investigated. There is also the need to investigate the current status of brucellosis and Q fever in cattle and small ruminants in Kaduna State in order to devise

appropriate strategies for prevention and control of the diseases. Currently OIE is encouraging syndromic surveillance to reduce the cost of surveillance in livestock (May *et al*., 2009).

Brucellosis in humans represents a major public health hazard, which affects social and economic development in various countries. Animal health workers, butchers, farmers, and those who habitually consume raw milk and come in contact with animals are at high risk of brucellosis (Chukwu, 1987). The dispersal of *C. burnetii* and the associated indirect exposure to the pathogen in community settings has great public health significance because it can lead to multiple infections and clusters of cases (EFSA, 2010a). Direct exposure to domestic ruminant livestock or associated animal products that are the primary reservoirs of *C. burnetii* clearly poses a greater risk of *C. burnetii* infection in comparison to the general population (EFSA, 2010a).

1.4 Aim of the Study

The aim of this study was to determine the epidemiological characteristics and co-occurrence of brucellosis and Q-fever in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological Zones, Kaduna State.

1.5 Objectives of the Study

i. To determine the seroprevalence of *Brucella* spp and *Coxiella burnetii* in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological Zones,Kaduna State.

ii. To determine the occurrence of co-infection in herds and flocks with *B. abortus, B. melitensis, B. ovis* and or *C. burnetti*.

iii. To compare the occurrence of multiple infection with *B. abortus, B. melitensis* and or *C. burnetti* in cattle, sheep and goats.

iv. To detect persistence of single infections of herds and animals with history of abortions or still birth in cattle using PCR.

v. To determine spatial distribution of brucellosis and Q fever in Maigana and Birnin Gwari agro-ecological zones of Kaduna State.

vi. To determine the risk factors associated with brucellosis and Q fever seropositivity in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological Zones, Kaduna State.

1.6 Research Questions

i. What is the seroprevalence of *Brucella* spp and *Coxiella burnetii* in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological Zones, Kaduna State?.

ii. Do *B. abortus, B. melitensis, B. ovis* and or*C. burnetti* occuras co-infection in herds and flocks of cattle, sheep and goats?.

iii. Can *B. abortus, B. melitensis* and or *C. burnetti* can occur as multiple infections in cattle, sheep and goats in the study areas?.

iv. Whether brucellosis and Q fever persist as single infections ofherds and animals with history of abortions or still birth in cattle using PCR?

v. What is the distribution in space of single and multiple infections due to brucellosis and Q fever in Maigana and Birnin Gwari agro-ecological zones of Kaduna State?.

vi. What are the risk factors associated with brucellosis and Q fever seropositivity in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological Zones, Kaduna State?.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. History of Brucellosis

Brucellosis is an ancient and one of the world's most widespread zoonotic diseases affecting both, public health and animal production (Ariza *et al.*, 2007) which is caused by a Gram-negative, facultative intracellular bacterium of the genus *Brucella*. The paleo-pathological evidence form the partial skeleton of the late Pliocene *Australopithecus africanus* suggests that brucellosis occasionally affected our direct ancestors 2.3–2.5 million years ago (D'anastasio *et al.*, 2011). The pathological and molecular findings from the human skeletal and remains of buried cheese from Bahrain (3000-1200 B.C.), Palestine and Jordan (2100–1550 B.C.), Pompeii and Herculaneum (79 A.D.), and Albania (1260–1020 A.D.), also suggested the presence of brucellosis a long time ago (D'anastasio *et al.*, 2011). However, in 1853, Jeffery Allen Marston made the first accurate description of the disease in British army troops serving in Malta during the Crimean war (Evans and Brachman, 2009). In 1887, a British surgeon Captain David Bruce, his wife Mary Elizabeth Steele and the Maltese microbiologist Doctor Giuseppe Caruana-Scicluna discovered "*Micrococcus melitensis"* (*Brucellamelitensis*) from the liver of diseased soldiers in the Mediterranean island of Malta (Wyatt, 2009). After this discovery, a Maltese medical Doctor (known as Temi Zammit) revealed that the causative agent of Malta fever was transmitted from infected goats to humans through contaminated milk

(Wyatt, 2011). In 1897, Danish scientist Bernhard Bang identified "*Bacillus abortus*" (*Brucellaabortus*) in bovine aborted fetuses. In 1914, Jacob Traum isolated *Brucellasuis* from an aborted swine (Pappas *et al*., 2005; Mantur and Amarnath, 2008; Evans and Brachman, 2009). Subsequently, in 1918, Alice Catherine Evans published data on the antigenic relatedness between *B. melitensis* and *B. abortus*. Eventually, in 1920, Louis Meyer and Wilbur Shaw honoured David Bruce by proposing a single new genus named *Brucella*. Later in 1953, van Drimmelen identified *B. ovis* in sheep followed by Stoenner and Lackman in 1957 who identified *B. neotomae* in rodents (Pappas *et al*., 2005).Later Carmichael and Bruner in 1964 also identified *B. canis* in the canines (Pappas *et al*., 2005; Evans and Brachman, 2009). In 1994, Ewalt, Ross and colleagues identified *B. pinnediae* and *B. cetacear* (provisionally) in mink whales, dolphins, porpoises as well as seals (Pappas *et al*., 2005; Evans and Brachman, 2009).

Brucellosis is found in all African countries with the probable exception of Madagascar and Mauritius (Thimm, 1982). In Nigeria, an outbreak of brucellosis was first reported in a government cattle farm located in Zaria in 1934 (Banerjee and Bhalty, 1970). Subsequently, the disease in livestock has been documented by several authors. For example, in 1970, Brucellosis was reported in humans (Banarjee and Bhalty, 1970), in 1971, Esuruoso and Hill also reported the disease in cattle(Esuruoso and Hill, 1971). In 1974, Falade *et al* (1974) reported the disease in sheep and goats, in 1979, Okoh reported the disease in camels (Okoh, 1979) and in 1999 Egbe Nwiyi reported the disease in thesemi-Arid zones of North-Eastern Nigeria (Egbe, Nwiyi *et al.*, 1999).

2.2.History of (Coxiellosis) Q Fever

Q fever was first described in 1935 by Edward Holbrook Derrick in slaughterhouse workers in Brisbane, Queensland, Australia (Derrick, 1937; Honarmand, 2012). Because the disease had an unknown aetiology, it was given the name Q fever (for query). The infection was shown to be transmissible when blood and urine from patients elicited a febrile response after injection into guinea pigs. The infection could be passed to successive animals. Unfortunately, no isolate could be obtained after culture on bacteriological media, and the aetiologic agent was thought to be a virus. The name was chosen over suggestions of "abattoir fever" and "Queensland rickettsial fever," to avoid directing negative connotations at either the cattle industry or the state of Queensland (Marrie, 1990). In 1936, Herald Rea Cox joined Davis at the Rocky Mountain Laboratory to further characterize the "Nine Mile agent." It was called Nine Mile agent due to the proximity of the tick vectors to Nine Mile creek. The two agents were proven to be identical, and the name *Coxiella burnetii* was chosen to honour Cox and Burnet, the two scientists who independently isolated the bacterium. Davis and Cox demonstrated that the aetiological agent was filterable and displayed properties of both viruses and rickettsiae (Honarmand, 2012).In1939, Cox and Bell succeeded in propagating the infectious agent in embryonated eggs (Cox and Bell,1939).

In Montana, a researcher was infected while working with the Nine Mile isolate, and guinea pigs could be infected by injecting a sample of the patient's blood (Dyer, 1938). It was confirmed that the agent causing Qfever and the Nine Mile isolate were the same by demonstrating that guinea pigs previously challenged with the Nine Mile isolate were

resistant to challenge with the Qfever agent (Dyer, 1938).In 1939, Cox and Derrick named the agent of Q fever as *Rickettsia diaporica* and *Rickettsia burnetii*. In 1948, the microorganism was given the name *Coxiella burnetii* in honor of Dr Cox and Dr Burnet, who made important contributions regarding propagation and isolation of this agent (McDade and Joseph, 1990).In Africa, *C. burnetii* was first reported in 1947 in Cameroon, but since then the quantity and quality of epidemiologic research on this pathogen has been limited (Blanc and Maurica, 1947; Dupont *et al*., 1995). In 1977, *Coxiella burnetii* was first reported in Nigeria in slaughtered cattle (Addo and Schnurenberger, 1977). In 1975, the disease was reported in Burkina Faso in human beings (Gidel and Athawet, 1975).

2.3 Aetiology of Brucellosis

*Brucella*spp. are facultative intracellular gram-negative cocco-bacilli, non-spore-forming and non-capsulated. Although *Brucella*spp. are described as non-motile, they carry all the genes except the chemotactic system, necessary to assemble a functional flagellum (Fretin *et al*., 2005; Seleem *et al*., 2010). They belong to the alpha-2 subdivision of the Proteobacteria, along with *Ochrobactrum*, *Rhizobium*, *Rhodobacter*, *Agrobacterium*, *Bartonella*, and *Rickettsia* (Yanagi and Yamasato, 1993).

Currently, there are twelve species described in the genus *Brucella*based on preferential host specific and they include*B. abortus* (cattle), *B. melitensis* (goats), *B. suis* (swine), *B. canis* (dogs), *B. ovis* (sheep), *B. neotomae* (rodents –*Neotoma lepida*), *B. cetacea* (cetacean), *B. pinnipedia* (seal), *B. microti*(rodents –*Microtus arvalis***)**, *B. inopinata*(originally isolated from a human patient, but its preferential host is not known),

B. papionis sp. nov. (baboons) and*B. vulpis* sp. nov (isolated from mandibular lymph nodes of red foxes) (*Vulpes vulpes*)(Scholz *et al*., 2010; O'Callaghan and Whatmore, 2011; De Jong and Tsolis, 2012; Galińska and Zagórski, 2013; Wang *et al.*, 2014; Whatmore *et al*., 2014; Scholz *et al*., 2016). Eight of the species that affect terrestrialanimals are: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae*, and *B. microti*B. *inopinata*(Scholz *et al*., 2008; Seleem *et al*., 2010) and two speceis that affect marine mammals are: *B. ceti* and *B. pinnipedialis* (Foster *et al*., 2007; Seleem *et al*., 2010), while four species that are pathogenic for humans are:*B. melitensis*, *B. abortus*, *B. suis*, and *B. canis*(Wang *et al*., 2014).*Brucellamelitensis* is considered as the most pathogenic specie, followed by *B. suis*, whereas *B. abortus* is the mildest type of brucellosis.

Additionally, *Brucella* isolates from marine mammals can cause human infections (Wang *et al*., 2014). Three of these *Brucella*species can be subdivided into biotypes (Bricker, 2002; Ocampo-Sosa *et al*., 2005)**.** Therefore, three biotypes (1–3) have been identified in *B. melitensis***;** eight biotypes (1–7, 9) in *B. abortus*; and five biotypes (1–5) in *B. suis* (Whatmore, 2009). DNA-DNA hybridization studies demonstrated that there are high degrees of genetic similarity between*Brucella*spp. (Tiller *et al.*, 2010; Wang *et al*., 2014). Brucellosis in sheep and goats (excluding *Brucellaovis* infection) is primarily caused by one of the three biovars of *B. melitensis*. Sporadic infections caused by *B. abortus* or *B. suis* have been observed in sheep and goats, but such cases are rare. Pathologically and epidemiologically, *B. melitensis* infection in sheep and goats is very similar to *B. abortus*infection in cattle (OIE, 2009). Table 2.1 presents the various *Brucella* species and their biovars along with their preferred hosts.

Bacteria of the genus *Brucella* spp. are coccobacilli, Gram-negative, aerobic, non-spore-forming, non-motile and non-capsulated (Bargen *et al*., 2012). Although the organism is able to multiply in life-less media, *Brucella* organisms are better described as facultative intracellular parasites (Moreno and Moriyon, 2002). The *Brucella*e are members of the α–proteobacteria and interestingly (being animal pathogens) have close relationships with soil organisms (e.g. *Ochrobactrum* spp.), with plant symbionts (e.g. *Rhizobium* spp.) and with phytopathogens (e.g. *Agrobacterium* spp.). The species *Ochrobactrumintermedium* is considered to be phylogenetically and taxonomically most closely related to *Brucella* (Scholz *et al.*, 2008). The most important aspect of *Brucella* ecology is their ability to establish an intracellular replicative niche and remain protected from the host immune responses (Bargen *et al*., 2012).

Table 2.1: *Brucella* species and biovars, including 'new' atypical strains, preferential host (s) and their pathogenicity for humans and animals (modified from Godfroid *et al.*, 2004; Sprague *et al.*, 2012).

***Brucella* species**	**Biovars**	**Colony Mophology**	**Prefential Hosts**	**Pathogenicity in Human**
B. abortus	1–6, 9	Smooth	Cattle, elk, camels bison, buffalo,	High
B. melitensis	1–3	Smooth	Sheep, goats, cows, camels	High
	3	Smooth	Nile catfish, dogs	High
B. suis	1	Smooth	Horses	High
	1–3	Smooth	Pigs	High
	2	Smooth	wild boar, hare	Low
	4	Smooth	Caribou, reindeer	High
	5	Smooth	Rodents	Not reported
B. canis	–	Rough	Canines	Moderate
B. ovis	–	Rough	Rams	Not reported
B. neotomae	–	Smooth	Rodents	Moderate
B. ceti	–	Smooth	Seals, dolphins	Yes
B. pinnipedialis	–	Smooth	Seals	Not reported

B. microti	–	Smooth	Soil, vole, fox	Not reported
B. inopinata	–	Smooth	Human	Yes
B. papionis	–	Smooth	Baboons	Not reported
B. vulpis	–	Smooth	red foxes	Not reported

Key: BO: *B. inopinata*-like strain., bvs: biovars.

2.4Aetiology of Coxiellosis (Q Fever)

Coxiella burnetii is a strict, intracellular, pleomorphic, gram–negative coccobacillus bacteriumthat is the causative agent of Q fever in humans and coxiellosis in animals, and isclassified as a Legionellae species which was first observed as a rickettsia-like organism (OIE, 2010). The organism is now classified in the family Coxiellaceae and in the Order Legionellales, in the gamma subdivision of Proteobacteria. *Coxiella burnetii* forms unusual spore-like structures that are highly resistant to environmental conditions (CFSPH, 2007). This organism also has two distinct antigenic phases, phase I and phase II. Phase I, and II cells are morphologically identical, but differ in some biochemical characteristics including their lipopolysaccharide (LPS) composition (CFSPH, 2007).Phase I is the natural phase found in infected animals, arthropods and humans. It is highly infectious and corresponds to the smooth LPS. In contrast, phase II is not very infectious and is obtained only in laboratories after serial passages in cell cultures or embryonated egg cultures. It corresponds to the rough LPS. Compared to phase I, phase II displays a truncated LPS and lacks some protein cell surface determinants (Honarmand, 2012). Genetic variability among different *C. burnetii* strains, as demonstrated by different RFLP-based genomic groups, specific plasmid regions, and LPS variations was tentatively related to virulence. Genomic groups I, II, and III were

associated with animals and ticks isolates, referred to as acute strains, whereas groups IV and V were associated with human Q fever endocarditis isolates, referred to as chronic strains (La Scola *et al*., 1998; Honarmand, 2012).A similar response occurs in humans, and is used to distinguish acute from chronic infections (CFSPH, 2007). In animals, disease due to *Coxiella burnetii* infection is called coxiellosis rather than Q fever (EFSA, 2010a; ECDC, 2010; Asadi *et al*., 2013).

Coxiella burnetii, the most famous species of this genus, causes human Q fever (short for query fever). *C. burnetii* appears to only cause disease in humans and laboratory animals such as rats and guinea pigs. However, it can induce abortion in sheep and goats, and causes reproductive problems in cattle (Thomas *et al*., 2004). La Scola and Raoult (2001) discovered that *C. burnetti* and free-living amoeba *Acanthamoeba castellanii* are able to co-exist in a benign relationship upon infection (La Scola and Raoult (2001). *Coxiella burnetii* organism was able to produce spore-like structures in vacuoles within the amoeba, demonstrating its ability to differentiate it without any interference from other species (La Scola and Raoult, 2001; Jensen *et al*., 2007). The bacterium does not cause any disease in amoeba. Likewise, the amoeba did not utilize the bacterium as food, so it contributes to the survival of *C. burnetti* by supplying an intracellular niche for it to differentiate into a structure similar to a spore and resist harmful environmental conditions (La Scola and Raoult, 2001). Besides surviving in a host cell, *C. burnetti* can adapt to a shared intracellular niche with another parasite such as *Trypanosoma cruzi* without Interfering withthe development of either (Andreoli *et al*., 2006).

2.5 Epidemiology of Brucellosis

Brucellosis is usually caused by *B. abortus*. Non bovine animals, including humans, can also contract the disease and play a role in its persistence and transmission. *Brucellaabortus* has seven recognised biovars, the most reportedare biovars 1–7 and 9. The manifestations of brucellosis in camel, buffalo, bison, yak, elk and several species of African antelope are similar to those of bovine brucellosis and can become epidemiologically important in sustaining the infection in cattle where they share pasture and water holes (CDC, 2011b; Fosgate*et al.,* 2011, Gomo*et al*., 2012). *Brucellamelitensis* is the most virulent species of the *Brucella*genus and has three biovars, with biovars 1 and 3 being the ones isolated most frequently in small ruminants in the Mediterranean, the Middle East and Latin America (Lucero *et al*., 2008; Blasco and Molina-Flores, 2011). Brucellosis is a barrier to trade in animals and animal products and causes significant losses from abortion, as well as being a serious zoonosis (Banai, 2007; Seleem *et al*., 2010). Goats are the classic and natural host of *B. melitensis*and, together with sheep, are its preferred hosts. In pathologicaland epidemiological terms, *B. melitensis*infection in smallruminants is similar to *B. abortus*infection in cattle: themain clinical manifestations of brucellosis in ruminants are abortion and stillbirths, which usually occur in the last trimester following infection and usually onlyonce in the animal's lifetime (Blasco and Molina-Flores, 2011; Díaz–Aparicio, 2013).

2.5.1 Occurrence and distribution of brucellosis in the world

Brucellosis is found worldwide in both animals and humans, except in those countries where brucellosis has been eradicated (Al-Dahouk *et al*., 2013; Scacchia *et al*., 2013). It is well controlled in most developed countries. Clinical disease is still common in the

Middle East, Asia, Africa, South and Central America, the Mediterranean Basin and the Caribbean (CFSPH, 2009; Scacchia *et al*., 2013). *Brucella*species vary in their geographic distribution. *Brucellaabortus* is found worldwide in cattle-raising regions except in Japan, Canada, some European countries, Australia, New Zealand and Israel, where it has been eradicated (CFSPH, 2009). *Brucellamelitensis* occurs in the Middle East and Central Asia, around the Arabian Gulf, in some countries of Central America, Africa and India, (CFSPH, 2009). *Brucella ovis* probably occurs in most sheep-raising regions of the world. It has been reported from North and South America, South Africa, many countries in Europe and in countries with lower levels of economic development (CFSPH, 2009; Hegazy *et al*., 2011).

There is lack of good of substantial informationdata on the occurrence and impact of brucellosis in many developing countries (Perry *et al*., 2002; McDermott and Arimi, 2002). In Jordan for example there is evidence that brucellosis does occur in different animals and humans (Al-Majali *et al*., 2008). Large herd size was reported as one of the major risk factors for the occurrence and higher prevalence of bovine brucellosis compared to small herd size (Tolosa, 2004). Borba *et al*. (2013) reported 11.4% prevalence rate of bovine brucellosis from Maranhão State, Brazil. Also,Ibrahim *et al*. (2010) reported 3.3% prevalence rate of bovine brucellosis from Ethiopia. Prevalence rate as high as 15.33% has been reported fromMymensingh district of Bangladesh (Islam*et al*., 2013).

2.5.2 Occurrence and distribution of brucellosis in Nigeria

Brucellosis has been reported in cattle, sheep, goats, camels, chicken and humans in various parts of Nigeria; thus indicating the magnitude of the disease in the country (Junaidu *et al*., 2008; Junaidu *et al*., 2011; Adamu *et al*., 2014a; Adamu *et al*., 2014b; Adamu *et al*., 2015).The distribution of the disease among humans is not well known but serological evidence has shown that the disease exists. Evidence of the presence of the disease in humans in Nigeria has also been published (Falade, 2002; Adamu*et al*., 2015).Recent studies suggest increasing trend in the prevalence of the disease (Bertu *et al*., 2015) but due to poor disease reporting system in Nigeria, the distribution of the disease was not reported. Subsequently, disease surveillance has not been very effective leading to poor determination of the incidence and the prevalence of brucellosis in the country.

2.5.2.1 Bovine brucellosis

Brucellosis is an endemic disease in Nigeria having been reported sporadically all over the country since 1927, where more of the reports come from established farms than from nomadic herdsmen who control almost 90.0% of the animal population. Because of the poor disease reporting system in Nigeria, the distribution of the disease is not well documented.There was also a reported casehygroma in indigenous cattle in Nigeria (Earshaw and O'Brien, 1929). Numerous serological studies over the years have proved that brucellosis is widespread in Nigerian livestock (Adams and McKay, 1966; Banerjee and Bhatty, 1970; Bale and Kumi-Diaka, 1981; Chukwu, 1987; Gusi *et al*., 2010). Farouk *et al*. (2013) demonstrated *Brucella*antibodies in cattle milk in Jigawa State where they reported the prevalence of 7.4% and 3.4% in marketed milk and in fresh

milk. Adamu *et al*. (2014c) in their studies on serological survey of Brucella antibodies in cattle breeding herds in Yobe State, Northeastern Nigeria reported the prevalence of 23.0%. More recently, Adamu *et al*. (2016) reported the prevalence of 5.4% from cattle in Bauchi State.

2.5.2.2 Ovine brucellosis

Based on serological examination (Okoh, 1980; Falade and Shonekan, 1981; Okewole *et al*., 1988; Brisibe *et al*., 1996) and based on culture and isolation studies (Bale, *et al*., 1982; Ocholi *et al*., 2005) both have confirmed brucellosis in sheep in Nigeria. In a related study conducted in a farm mixed of cattle and sheep, aprevalence of 14.5% was reported by Ocholi *et al*. (2005). The prevalence of 22.3% was reported in sheep from a prison farm in Sokoto State by Junaidu *et al*. (2008), and Tijjani *et al*. (2009) reported the prevalence of 6.0% in abattoir survey in Yobe State. A sero-epidemiological survey of brucellosis in sheep in Plateau State revealed a prevalence rate of 14.5% (Bertu *et al*., 2010) and in North Senatorial District of Kaduna State, the prevalence of 25.6% was recorded (Kaltungo *et al*., 2015).

2.5.2.3 Caprine brucellosis

Caprine brucellosis was first reported by Adams and McKay (1966)and later by Kramer *et al*. (1967). After then there was little information available in Nigeria until in 1974, when Falade reported brucellosis among goats (Falade, 1974). There were other reports of caprine brucellosis from various parts of Nigeria (Falade, 1978; Bale, 1980; Ogundipe *et al*., 1994; Brisibe*et al*., 1996; Tijjani *et al*., 2009)with all revealing prevalence rates

between 2.1–14.5%. A study carried out by Junaidu *et al*. (2008)reported a prevalence of30.7% in goats from a prison farm in Sokoto.In a survey of small ruminants brucellosis in Kaduna North Senatorial District of Kaduna State, 26.2%prevalence was recorded in goats (Kaltungo *et al*., 2013), while Dogo and Maikai (2015) reported a prevalence of 8.2% and 2.5% by RBPT and cELISA in goats in Giwa Local Government Area of Kaduna State, Nigeria.

2.5.2.4 Canine brucellosis

Canine brucellosis in Nigeria was in doubt as there was no antibodies detected during a serological survey by Falade (1977). However, Adesiyun *et al*. (1986) reported serological evidence in the dog with a prevalence rate of 28.6%. With the current increase in importing dogs used for breeding purposes, the spread of brucellosis among dogs in Nigeria is more likely to be increased. Serological study carried out in dogs in Jos North and South Local Government Areas (LGA) of Plateau State revealed aprevalence rates of 32.3%and 29.2% by RBPT and cELISA respectively (Momoh *et al*., 2014).

2.5.2.5 Porcine brucellosis

Swine brucellosis in Nigeria was first reported by Mettam (1947) followed by Adams and McKay (1966). Since then, there has been no adequate information to indicate the prevalence and distribution of infection in pigs with*Brucella* organisms. Later, Bale and

Nuru (1982), reporteda prevalence of 2.7% from piggeries in Northern part of Nigeria based on serology. In a study in anabattoir-based serodiagnosis of swine brucellosis in Makurdi, Benue State. North Central Nigeria, the prevalence rate of 30.6% was recorded (Ngbede *et al.*, 2013).Because of the differences in cultural and religious believes, pig production in Nigeria is limited only to a few parts of Nigeria and this limits the research on the existence of brucellosis in pigs in Nigeria in comparison to other species.

2.5.2.6 Equine brucellosis

Horses have been used inNigeria by the police force for ceremonial parades, by mounted troops and has gained interest in polo games, cultural festivals, racing and security in controlling crowds. Only few reports are available on the presence of brucellosis in horses. Bale and Kwanashie (1984) demonstrated the seroprevalence of brucellosis among horses in Northern Nigeria, which revealed the prevalence rates of 10.0% in Zaria, Kaduna State and 5.3% in Jos, Plateau State. In another study carried out on horses from three different horse stables belonging to Nigerian Army, Nigerian Police Force located in Kaduna and Plateau States, a prevalence of 14.7% was recorded (Ehizibolo *et al*., 2011). Recently, seroprevalence of brucellosis in horse on the Mambilla Plateau of Taraba State, Nigeriashowed prevalence of16% and 6% by RBPT and SAT respectively (Ardo and Abubakar, 2016). Tijjani *et al*. (2016) reported prevalence rates of 9.8% and 9.0% by RBPT and cELISA respectively, in a serological survey for *Brucella*antibodies in horses in Borno State, Nigeria.There are lots of studies on brucellosis done on most of the domestic animalsin Nigeria including horses, but only few studies were done in donkeys. The only study done in Nigeria on the prevalence of

brucellosis in donkeyswas that of Sadiq *et al*. (2013), who reported the prevalences of 5.0% and 3.3% by RBPT and cELISA in Yobe State, Nigeria, while Tijjani *et al.* (2016), reported prevalence of 9.8% and 9.9% by RBPT and cELISA respectively in Borno State, Nigeria.

2.5.2.7 Camel brucellosis

Camel brucellosis was first identified in Nigeria by Okoh *et al*. (1979) when he reported a prevalence of 1.0% using serology. Subsequently, there was a serological survey carried out which revealed a prevalence of 19.1% (Adamu *et al*., 1997) and also a prevalence of 12.5% (Adamu and Ajogi, 1999) in an abattoir survey. In a related study, Junaidu *et al*. (2006a) reported a prevalence of 11.4% in camels at Sokoto abattoir, North Western Nigeria.Aninvestigation of brucellosis in one humped camel (*Camelus dromedarius*) in the Lake Chad area of Borno State recorded a prevalence of 12.1% (Sadiq *et al*., 2008). In a related study by Adamu *et al*. (2009) in a survey of brucellosis using Milk Ring Test and bacteriological culture in camel herds in Yobe State, Nigeria reported a prevalence of 8.3%. Recently, in a study of *Brucella*antibodies in camel (*Camelus dromedarius*) herds in North-Eastern Nigeria, a prevalence of 16.5%was recorded based on cELISA(Gusi *et al*., 2016).

2.5.2.8 Brucellosis in chickens

Almost all the reports of brucellosis in chickens are from Nigeria. Thefew reports onserological evidence of avian brucellosis in Nigeria included those of Bale and Nuru(1982) and Abdu *et al*. (1984). Chickens are kept in most parts of Nigeria due to

their nutritional and economic value (Baba *et al*., 2001; Junaidu *et al*., 2006b). Chickens have been reported to be susceptible to *Brucella*infection (Abdallah *et al*., 1984) causing a decrease in egg production in infected hens with the recovery of *Brucella*from the egg shell, egg yolk and white droppings and internal organs of infected birds (Abdallah *et al*., 1984). A study carried out by Junaidu *et al*. (2006b) where *B. melitensis* and *B. abortus* antigen were used, showed a prevalence rates of 3.0% and 2.6% by RBPT and cELISA of *B. melitensis* in the number of birds sampled in North Western Nigeria. Recently, in a serological survey carried out in local chickens, pigeons and ducks in Nassarawa State, Nigeria Alaga *et al*. (2012) reported prevalence rates of 2.3%, 2.8% and 1.9%by RBPT. In a related study,Adamu *et al*. (2014)in a serological survey of brucellosis in slaughtered local chickens, guinea fowls and ducks in North-Eastern Nigeria recorded the prevalence rates of 1.8%, 7.1% and 5.0% by RBPT respectively.

2.5.2.9 Human brucellosis

Human brucellosis is hardly diagnosed in hospitals in Nigeria.Therefore, levels of human infection may be greater than reported (Njoku 1995; Rajish*et al*., 2003). Thefew reports may be due to some diseases with similar clinical signs that are endemic and hence often misdiagnosed. Diseases like malaria,typhoid fever and pasteurellosis have signs which include acute recurring fever chills, anorexia, headache, fatigue and sweating in the night (Rajish*et al*., 2003). The first case of human brucellosis in Nigeria was reported in 1962 (Collard, 1962) when *Brucella* antibodies were demonstrated in the sera obtained from healthy persons. Most of the brucellosis cases in humans were occupationally related occurring among workers in the livestock industries. Epidemiological studies show

significantlyhigh prevalence of infections among occupationally exposed individuals including herdsmen, abattoir workers, veterinarians, laboratory personnel and those that work in tanneries (Alausa, 1979). A recent seroprevalence study was carried out among abattoir workers in Abuja, Nigeria in which a prevalence of 24.1% was recorded (Aworh *et al*., 2013). Adamu *et al*. (2015) reporteda prevalence of 11.0% among livestock workers at Maiduguri cattle market, Borno State, North Eastern, Nigeria.

2.5.3Transmission of brucellosis

Brucellosis is transmitted through ingestion of feed, water and grass contaminated by bacteria, aerosol, broken skin, and mucous membrane contact with the contaminated environment, aborted tissue, foetal fluids and foetal placenta (Boukary *et al*., 2010; Olsen and Tatum, 2010).Moreover, transmission within the natural hosts can occur through milk or via semen or genital secretions during mating. Congenital infection during parturition is frequently cleared and only few animals remained infected as adult (Radostits *et al*., 2007). In female animals, the bacterium may localize in the tissues of the udder and then excreted via milk. In male animals, orchitis and epididymitis can lead to temporary or permanent infertility (Corbel, 2006). In cattle *Brucella*is usually transmitted from animal to animal by contact following abortion. Pasture or animal barn may be contaminated and the organisms are probably most frequently acquired by ingestion but inhalation, conjunctival inoculation, skin contamination and udder inoculation from infected milking cups are other possibilities (Godfroid *et al*., 2004; WHO, 2006). The use of pooled colostrum for feeding newborn calves may also transmit infection (Godfroid *et al*., 2004). Transmission of the*Brucella*organism to humans occurs

as a result of consumption of unpasteurized raw milk or other dairy products, especially soft cheese, butter and cream (Boukary *et al*., 2010; Alshaalan *et al*., 2014).Animals may become infected by direct contact with the secretions of infected animals or their products, such as the placenta or aborted materials(Alshaalan *et al*., 2014). The air borne transmission of aerosolized materials through open wounds or mucous membranes either in the animal areas or in the laboratory when dealing with blood and other infected fluid cultures (Pappas *et al*., 2005; Alshaalan *et al*., 2014). It has been found that direct contact with soil, animal feces and dust contaminated with *Brucella*is associated with a higher risk of infection (Pappas *et al*., 2005; Alshaalan *et al*., 2014).

Venereal transmission is not a major route of infection under natural conditions, but artificial insemination with contaminated semen is a potential source of infection (Radostits *et al*., 2010; Chiebao *et al*., 2013). In human's vertical transmission, sexual transmission and breast milk transmission are anecdotally reported routes of infection (Alshaalan *et al*., 2014).*Brucella*can be spread on fomites (Lucero *et al*., 2005). Zoonotic transmission occurs most frequently via unpasteurized milk products in urban settings, while occupational exposure of farmers, veterinarians, butchers and laboratory workers can result from direct contact with infected animals or tissues or fluids associated with abortion (Olsen and Palmer, 2014). Only rare cases of vertical and horizontal transmission between humans have been reported (Çelebi *et al*., 2007; Meltzer *et al*., 2010; Wyatt, 2010) and humans are generally considered to be incidental, or dead-endhosts for *Brucella*species (Meltzer *et al*., 2010). The spillover of *Brucella*e from wildlife to domesticruminants is also possible (Mick *et al*., 2014).

2.5.4 Host range and susceptibility to *Brucella* organism

Animals of all age groups are susceptible to *Brucella*infection but infection persists commonly in sexually mature animals (Ficht, 2003). Young animals tend to be more resistant to *Brucella*infection and frequently eliminate the infection while sexually matured animals are more susceptible (Junaidu *et al*., 2011). In particular, female animals that have reached sexual maturity are most susceptible to infection. It is usually detected in pregnant females through abortions (England *et al*., 2004). The species of *Brucella*and their major hosts are *B. abortus* (cattle), *B. melitensis* (goats, camels and humans), *B suis* (swine), *B. ovis* (sheep),*B. canis* (dogs), *B. neotomae* (rats) (Szulowski and Murat, 2008; Galińska and Zagórski, 2013).*B. ceti* and *B. pinnipedialis* found in sea mammals (whales, seals) (Foster *et al*., 2007; Nymo *et al*., 2011), *B. microti* (common vole) (*Microtus arvalis*) and wild red foxes (*Vulpes vulpes*) (Scholz *et al*., 2008; Scholz *et al*., 2009). While *B. inopinata*was isolated from a breast implant wound of a woman with clinical signs of brucellosis (Scholz *et al*., 2010; Galińska and Zagórski, 2013). *Brucellaabortus* also causes infection in horses and is commonly found in chronic bursal enlargements as a secondary invader rather than a primary pathogen (Radostits *et al*., 2007). Despite their respective host preferences, *B. abortus*and *B. suis* have also been isolated from a great variety of wildlife species, such as bison (*Bison bison*), elk/red deer (*Cervuselaphus*), feral swine and wild boar (*Sus scrofa*), the red fox (*Vulpes vulpes*), the European brown hare (*Lepus europaeus*), African buffalo (*Syncerus caffer*), reindeer (*Rangifer tarandustarandus*) and caribou (*Rangifer tarandus groenlandicus*), chamois (*Rupicapra rupicapra*) and ibex (*Capra ibex*) and wildlife was considered to be a

reservoir for zoonotic brucellosis (Godfroid, 2002). For this reason, wildlife must be considered a potential reservoir for brucellosis in livestock (Godfroid, 2002; Godfroid *et al*., 2013). *Brucellamelitensis* is rarely reported in wildlife. However, sporadic cases have been reported in Europe in chamois (*Rupicapra rupicapra*) and ibex (*Capra ibex*) in the Alps (Godfroid *et al*., 2013).

2.6Epidemiology of Coxiellosis (Q Fever)

Coxiellosis is a zoonotic disease of livestock caused by *Coxiella burnetii*.It is also one of the infectious diseases that has been linked to chronic fatigue syndrome (Angelakis and Raoult, 2010). The sites of chronic infections in sheep and goats are mammary glands and the uterus, and infertility in cattle (To *et al*., 1998). The organism is shed from milk, urine, faeces, or semen from infected animals. At birth, the placenta contains enormous quantities of the organism, which are released into the environment (Tissot-Dupont and Raoult, 2008). The se of infected animals for farming and transportation can help in spreading the organism into the environment and this poses a difficulty in containing the outbreak of coxiellosis (Enserink, 2010). In domestic ruminants, the main clinical signs of coxiellosis include abortion during late pregnancy, stillbirth and decreased neonatal survival (Rodolakis, 2009; EFSA, 2010a; ECDC, 2010).

2.6.1 Occurrence and distribution of coxiellosis (Q fever)

Q fever is a zoonotic disease which is widely distributed throughout the world with the exception of New Zealand.*Coxiella* infection is present in a variety of animals including arthropods, birds, domestic and wild mammals, as well as humans (Rodolakis *etal*.,

2007; EFSA, 2010b). Indeed, the disease is considered as a re-emerging zoonosis in many countries.There were almost eighteen (18) reported cases of Q fever outbreaks from 12 differen countries which involved 2 to 289 people from 1999 to 2004 (Arricau-Bouvery and Rodolakis, 2005). In southern region of the Netherlands, between 2007 and 2009, Q fever became a public health problem with 2,357 human cases (van der Hoek *et al*., 2010; Roest *et al*.,2011).Masala *et al*. (2004) using ELISA, got 47% of goats and 38% of sheep seropositive in Italy. Parisi *et al*. (2006) reported each 21.5% of goats and sheep and 11.6% of cattle for Q feverusing PCR.

In theUSA, studies by McQuiston and Child (2002) reported 41.6% prevalence in goats, 16.5% in sheep and 3.4% in cattle. In addition, Deforge and Cone (2006) in California, reported seroprevalence of 10% in bighorn sheep using complement fixation test.In Asia, To *et al*. (1996) reported a prevalence of 60.4% in cattle with the reproductive disorders. In Southern Sudan, Reinthaler *et al*. (1988) using MAT, reported prevalence of 62.5% in sheep, 53% in goats and 40.4% in cattle. In Zimbabwe, Kelly *et al*. (1993) reportedprevalence of 39.0% in cattle, 10.0% in goats using IFAT. In Chad, Schelling *et al*. (2003) reportedprevalence of 80% in camel, 13% in goats, 11% in sheep and 4% in cattle using ELISA. In Central African Republic, Nakoune *et al*. (2004) reported prevalence of 14.3% in cattle using IFAT. Also Cekani *et al*. (2008) reportedprevalence 9.8% each in sheep and goats, and 7.9% in cattle in Algeria using ELISA.

In Nigeria, Addo and Schnurenberger (1977) reportedprevalence of 11.0% in cattle, 16.5% in sheep and 8.8% in goats, due to *Coxiella burnetii*, in Kaduna and Zaria abattoir using

Capillary Agglutination Test (CAT). Addo (1980) also reported a prevalence of 12.0% in one humped camel (*Camelus dromedarius*) in Kano, Sokoto and Zaria abattoirs using Capillary Agglutination Test (CAT). Tukur *et al*. (2014)reportedprevalence of 14.5% among cattle in Kaduna metropolis using ELISA. In humans, Collard and Udeozo (1959), reported prevalence of 5.4% among pregnant women in Ibadan. In another study Collard (1962), reported a prevalence of 5.5% and 2.7% among Habe and Fulani tribes in Katsina State.

2.6.2 Transmission of coxiellosis (Qfever)

Animals may become infected by direct contact with infected animals and contaminated environment and/or from inhalation of desiccatedparticles contaminated with organism. Birth products (including placenta, foetuses, and amniotic and allantoic fluids), excreta, and milk are the most likely sources of infection (ECDC, 2010; Heydel and Willems, 2011; Porter *et al*., 2011; Reusken *et al*., 2011). The bacteria have also been found in ticks, which may also serve as a source of infection for animals (Toledo *et al*., 2009; Astobiza *et al*., 2011). Vertical and sexual transmission could occur but their importance is not known in animals. Arthropods,principally ticks, may be involved in Q fever transmission. The risk of transmission seems to be linked to wildlife animals.Sexual transmission of Q fever has been reported in mice (Kruszewska and Tylewska-Wierzbanowska, 1992), cattle (Kruszewska and Tylewska-Wierzbanowska, 1997) and humans (Kruszewska *et al*., 1996; Millazzo et al., 2001).

In humans, the primary mode of transmission is inhalation of pathogen-contaminated aerosols from excreta and especially birth products (Kruszewska *et al*., 1996; Millazzo *et al*., 2001). Parturient cats, kittens, dogs and other animals have also been associated with human cases (Marrie *et al*., 1998; Komiya *et al*., 2003). The possibility of acquiring human Q fever from infected dogs has also been reported(McQuiston and Childs, 2002).*Coxiella burnetii* is easily dispersed into the air and airborne transmission of the disease to people living over a mile from the animal sources has been reported (McQuiston and Childs, 2002). As a result, Q fever can occur in persons without occupational risk or other contact with livestock or their environment (Raoult *et al*., 2000; Hackert *et al*., 2012). Transmission may also occur from tick bites, and a number of tick species have been implicated (Tissot-Dupont *et al*., 1999; Herrin *et al*., 2011). Foodborne transmission from drinking contaminated raw milk (Signs *et al*., 2012), exposure during childbirth, through sexual transmission or blood transfusion is possible (Miceli *et al*., 2010). Indirect transmission after exposure to wool, straw, clothing, linens, and other contaminated materials have been documented (Maurin and Raoult, 1999). Person-to-person transmission occurs rarely, though sexual transmission of Q fever from a man with occupationally acquired Q fever to his wife has been reported (Millazzo *et al*., 2001). Sporadic cases of human-to-human transmission following contact with infected pregnant women have been reported and have been suspected to occur by direct aerosol transmission. Nosocomial transmission of Q fever has also been described (Osorio *et al*., 2003).

2.7 Pathogenesis of Brucellosis

The pathogenic potential of *Brucella*spp. is highly dependent on its ability to enter and survive within host cells.The ability of *Brucella*spp. to cause disease requires a few critical steps during infection (Xavier *et al*., 2010). A successful entry of *Brucella*into the host is a crucial step in establishing infection, considering that the digestive tract is the main entrance route of *Brucella*(Paixão *et al*., 2009; Xavier *et al*., 2010).*Brucella*spp. can invade epithelial cells of the host, allowing infection through mucosal surfaces: M cells in the intestine have been identified as a portal of entry for *Brucella*spp. (Paixão *et al*., 2009). Once *Brucella*spp. have invaded, usually through the digestive or respiratory tract, they are capable of surviving intracellularly within phagocytic or non-phagocytic host cells (Carvalho-Neta *et al*., 2008). *Brucella*has the ability to interfere with intracellular trafficking, preventing fusion of the *Brucella*-containing vacuole (BCV)with lysosome markers, and directing the vacuole towards a compartment that has rough endoplasmic reticulum (RER), which is highly permissive to intracellular replication of *Brucella*(Pizarro-Cerdá *et al*., 2000; Poester *et al*., 2013).

The mechanisms that allow host cell invasion by *Brucella*spp. are not completely clear and that specific host receptors that interact with *Brucella*have not yet been identified either(Guzmán-Verri *et al*., 2001; Poester *et al*., 2013). Interestingly, invasion through the digestive tract does not elicit any inflammatory response from the host (Paixão *et al*., 2009). Therefore, *Brucella*spp. invade silently or unnoticed by the innate immune system of the host. In fact, *Brucella*spp. have mechanisms that prevent activation of the host innate immune system (Barquero-Calvo *et al*., 2007; Poester *et al*., 2013). *Brucellaabortus*also induces suppression of the transcription of pro-inflammatory

mediators in trophoblastic cells at very early stages of infection (Carvalho-Neta *et al*., 2008). Trophoblasts are placental cells that are targeted during infection of pregnant cows. After an initial suppression of pro-inflammatory transcripts, *B. abortus* induces expression of pro-inflammatory chemokines by cultured trophoblastic cells, which correlates with the profile of expression observed *in vivo* in the placenta of infected cows (Carvalho-Neta *et al*., 2008).*Brucella*spp. lack classical bacterial virulence factors such as capsule, cytolysins, endotoxin, exotoxins, fimbriae, flagella, plasmids, lysogenic phages, lipopolysaccharide (LPS), and inducers of host cell apoptosis (Moreno and Moriyón, 2006; Poester *et al*., 2013). However, LPS plays an important role in Brucella virulence because it prevents complement-mediated bacterial killing and provides resistance against antimicrobial peptides such as defensins and lactoferrin (Lapaque *et al.*, 2005).

Another important virulence mechanism of *Brucella* is the BvrR/BvrS two-component regulatory system, which is required for modulation of the host cell cytoskeleton upon *Brucella* invasion, and for regulation of the expression of outer membrane proteins, some of which are required for full virulence (López-Goñi *et al*., 2002; Poester *et al*., 2013). Cyclic β-1,2-glucans, which are also part of the outer membrane, are also required for intracellular survival of *Brucella*(Briones *et al*., 2001; Poester *et al*., 2013). The *Brucella*T4SS is required for persistence in mice and induction of the host immune response (Roux *et al*., 2007). It is also essential for elicitation of inflammatory and immune responses during *Brucella*infection in mice (Roux *et al*., 2007), and it is required for micro-granuloma formation during *Brucella*infection (Rolán *et al*., 2009). The T4SS is required for *Brucella*to reach its intracellular replication niche (Celli *et al*., 2003), and

its expression is regulated by the BvrR/BvrS two-component regulatory system (Martínez-Núñez *et al*., 2010). The T4SS delivers *Brucella*effector proteins into the host cell cytosol, but the identities and roles of these effectors have only recently begun to emerge (Marchesini *et al*., 2011).

2.8 Pathogenesis of (Coxiellosis) Q Fever

Following ingestion, *Coxiella burnetii* infects a great number of cell types *in vitro* and *in vivo.* The extra-cellular form of *Coxiella burnetii* attaches itself to a cell membrane and is internalized into the host cells (Porter *et al*., 2011). Phagolysosomes are formed after the fusion of phagosomes with cellular acidic lysosomes. The multiple intracellular phagolysosomes eventually fuse together leading to the formation of a large unique vacuole. *Coxiella burnetii* has adapted to the phagolysosomes of eukaryotic cells and is capable of multiplying in the acidic vacuoles (Hackstadt and Williams, 1981; Porter *et al*., 2011). Multiplication of *C. burnetii* can be stopped by raising the phagolysosomal pH using lysosomotropic agents such as chloroquine (Raoult *et al*., 1990). Though the mechanisms of *C. burnetii* survival in phagolysosomes are still under study. Three proteins that are involved in intracellularsurvival of *C. burnetii* are identified as:a catalase, a superoxide dismutase, and a macrophage infectivity potentiator (Akporiaye and Baca, 1983; Mo *et al.*,1995; Porter *et al*., 2011). Redd and Thompson (1995) found that secretion and export of macrophage infectivity potentiator was triggered by an acid pH *in vitro*. Studies conducted by Zamboni and Rabonovitch (2003) and by Brennan *et al*. (2004) demonstrated that growth of *C. burnetii* was reduced by reactive oxygen intermediates and reactive nitrogen intermediates. Hill and Samuel (2011) analyzed *C.*

*burnetii'*s genome and identified 2 acid phosphatase enzymes. They demonstrated experimentally that both a recombinant acid phosphatase enzyme and *C. burnetii* extracts had a pH dependent acid phosphatase activity. The intracellular cycle of *C. burnetii* leads to the formation of two development stages of the bacterium known as small-cell variant and large-cell variant (Samuel, 2000; Porter *et al*., 2011). Small cell variant is the extracellular form of the bacterium.

Based on the previous studies, small-cell variant are considered to be inactive metabolically and capable of resisting to extreme conditions such as chemical products, desiccation, disinfectants, heat, high or low pH, osmotic pressure and ultra violet rays (Samuel, 2000; Porter *et al*., 2011). Their resistance in the environment (pseudo-spores) would enable the bacteria to survive for long periods of time in the absence of a suitable host. Small-cell variant of *C. burnetii* are reversible (Rousset *et al*., 2007a). Certainly, once inhaled or ingested the small-cell variant attaches itself to a cell membrane and is internalized. After phagolysosomal fusion, the acidity of the newly formed vacuole induces activation of small-cell variant metabolism and its development into large-cell variant. During the morphogenesis from small-cell variant to large-cell variant,no increase in bacterial number is reported (Coleman *et al*., 2004; Porter *et al*., 2011). Large-cell variant is considered to be the metabolically active intracellular form of *C.burnetii*, and they are more pleomorphic than small-cell variant. They have a more dispersed filamentous nucleoid region and their cell wall is thin. They can exceed 1μm in length (McCaul and Williams, 1981; Porter *et al*., 2011).The physical and biological factors responsible for the sporulation-like process remain unknown. Mostnatural

infections by *C. burnetii* are probably due to small-cell variant or pseudo-spores present in the environment (Rousset *et al*., 2007a).*C. burnetii* has developed a cell up take strategy that allows it to invade a broad range of hosts and has developed a unique tactic to multiply in the harsh phagolysosome environment of cells (Rousset *et al*., 2007a).

2.9 Clinical Manifestation of Brucellosis in Animals

The major clinical signs in pregnant animalsare reproductive failure such as abortion and birth of unthrifty newborn in bovine and buffalo cows (Megid *et al*., 2010). Abortion usually occurs from the 5th to the 8th month of gestation. The occurrence of abortion is related to some factors, such as the stage of pregnancy, the number of infecting organisms and the animal resistance (Megid *et al*., 2010). Apart fromabortion, premature stillborn or weak calves may occur. Abortion is often followed by placental retention and metritis, which may cause permanent or transient infertility (Acha and Szyfres, 2003).In bulls,primary clinical signs are decreased libido, infertility, seminal vesiculitis, epididymitis, ampullitis and orchitis is occasionally manifested, and when it occurs it is usually unilateral, but both testicles may be affected (Foster and Ladds, 2007). Infection in males may result in either temporary or permanent infertility, depending on the intensity of the lesions (Xavier *et al*., 2009).Hygroma and abscesses are the major clinical signs in nomadic or semi-nomadic cattle herds infected with *B. abortus* Biovar types 3 (Radostits *et al*., 2004). High concentrations of erythritol are present in female genital tracts and foetal tissues as well, which are also the sites of infection establishment (Radostits *et al*., 2004).

In sheep and goats, *B. melitensis*affects both species, causing abortion in females as the main clinical sign, whereas *B. ovis* affects only sheep and it is the cause of contagious epididymitis of rams (Megid *et al*., 2010).

2.10Clinical Manifestation of Brucellosis in Humans

In humans, brucellosis can be a serious, debilitating and sometimes chronic disease that may affect a variety of organs with signs as joint pain, undulating fever and general body malaise (Bouley *et al*. 2012). Most cases are caused by occupational exposure to infected animals or the ingestion of unpasteurized dairy products (CFSPH, 2009). The incubation period is 1–3 weeks but can be extended to several months. The most common signs of brucellosis are undulant fever in which the temperature can vary from 37ºC in the morning to 40ºC in the afternoon; night sweats with peculiar odour, chills and weakness. Malaise, insomnia, anorexia, headache, fever, arthralgia, constipation, sexual impotence, nervousness and depression are also common (Megid *et al*., 2010;Dean *et al*., 2012). Complications and involvement of internal organs can be diverse, depending on the site of infection, and could include encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, prostatitis, weakness, submaxillary adenopathy, brain abscess and prolonged febrile illness (Tikare *et al*., 2008; Godfroid *et al*., 2011; Olsen and Palmer, 2014).Neurological complications can occur during the onset of illness, convalescence period or even some months after recovery from an acute infection (Tikare *et al*., 2008; Megid *et al*., 2010). Atypical presentations of brucellosis were recorded with peritonitis, pericarditis, pancytopenia and uveitis (Hatipoglu *et al*., 2005). Unusual, spontaneous abortions have been seen in pregnant women infected with *Brucella*in early stages. Cases

of hepatitis, hepatomegaly, splenomegaly, pulmonary involvement, osteoarticular involvement, leucopoenia, anaemia and lymphomonocytosis have been reported (Kokoglu *et al*., 2006; Godfroid *et al*., 2011; Dean *et al*., 2012; Olsen and Palmer, 2014).

2.11 Clinical Manifestation of Coxiellosis in Animals

Many species are susceptible to infection, but most species seem to be infected asymptomatically. Abortion, stillbirth, retained placenta, endometritis, infertility and small or weak offspring can be seen in cattle, goats and sheep (CFSPH, 2007; EFSA, 2010b; Asadi *et al*., 2013). Most abortions occur near term. Several abortions may be followed by uncomplicated recovery, particularly in small ruminants.In other cases, the disease may re-occur yearly. In dogs and cats, infections have been associated with stillbirths and weak offspring. Goats and sheep are the species in which delivery of weak offspring, and early neonatal mortality have been most frequently documented (Khalili and Sakhaee, 2009; Porter *et al*., 2011; Keyvanirad *et al*., 2013). Abortions and perinatal death occur after experimental (intraperitoneal) infection of pregnant mice (CFSPH, 2007). With the exception of reproductive disease, animals are usually asymptomatic. Goats sometimes have a poor appetite and are depressed for 1 to 2 days before an abortion. In addition, *C.burnetii* was found to be significantly associated with placental necrosis and foetal bronchopneumonia (Bildfell *et al*., 2000). Placental retention for 2 to 5 days and agalactia have also been reported. Clinical signs including fever, anorexia, mild coughing, rhinitis and increased respiratory rates occur in experimentally infected sheep but have not been reported in natural infections (CFSPH, 2007). Q fever is therefore considerably underdiagnosed and underreported (Gidding *et al*., 2009).

2.12Clinical Manifestation of Q Fever in Humans

In humans, the infection is mainly asymptomatic and can be acute, chronic or subclinical(Khalili and Sakhaee, 2009; Asadi *et al*., 2013).It is therefore considerably underdiagnosed and underreported (Gidding *et al*., 2009). The incubation period of is 1 to 3 weeks. Size of the inoculum, geographical area, route and time of infection, as well as host factors influence the duration of the incubation period (Marrie *et al*., 1996) and may contribute to the clinical expression of acute or chronic infection (Marrie, 1990).

2.12.1 Acute Q fever

Acute Q fever is characterized by sudden onset of fever with temperature of 104º–105º F, chills, profuse sweating, severe headache with retro-orbital pain, weakness, nausea, vomiting, diarrhea, non-productive cough, and abdominal or chest pain. If untreated, the fever can persist for up to 9 to14 days (Heydel and Willems, 2011). Approximately 30% to 50% of patients develop pneumonia. Acute hepatitis, meningoencephalitis, and myocarditis can also occur. Abnormal liver function and thrombocytopenia are common. Pregnant women are considered to be at increased risk for pre-term delivery or miscarriage although a recently published prospective study of pregnant women from the Netherlands outbreak did not identify an increased risk associated with seropositive status (Quijada *et al*., 2012; van der Hoek *et al*., 2011; Angelakis *et al*., 2013). Majority of people with acute Q fever recover completely (van der Hoek *et al*., 2010; López-Gatius *et al*., 2012).

2.12.2 Chronic Q fever

Chronic Q fever consists in the persistence of infection for more than 6 months. The main clinical manifestation of chronic Q fever is endocarditis in patients with valvulopathies, vascular infections, hepatitis or chronic fatigue syndrome. Moreover, *C. burnetii* infection of pregnant women can provoke placentitis and leads to premature birth, growth restriction, spontaneous abortion or foetal death. Overall, the chronic disease is more likely to develop in immunocompromised individuals (ECDC, 2010; EFSA, 2010a). Chronic Q fever is rare, occurring in fewer than 5% of acute cases. Persons with valvular surgery, valvular prosthesis or aneurisms are at highest risk for development of chronic Q fever. Other risk factors include renal insufficiency, age greater than60 years, pregnancy, and immunosuppression (Kampschreur *et al*., 2012). The prognosis of chronic infections is less favorable than for acute infections (Watanabe and Takahashi, 2008). Antibiotic treatment is less effective and the disease is usually having long mortality rates that can reach up to more than 50% (Watanabe and Takahashi, 2008). Chronic Q fever is rarely reported in children, in contrast with adults with osteomyelitis being one of the most common findings in children with pediatric chronic Q fever (McQuiston, 2009).

2.13 Pathology of Brucellosis

*Brucella*e display strong tissue tropism and replicate within vacuoles of macrophages, dendritic cells (DCs), and placental trophoblasts (Martirosyan and Gorvel, 2013). However, the pathogen has the ability to replicate in a wide variety of mammalian cell types, including microglia, fibroblasts, epithelial cells, and endothelial cells (Martirosyan and Gorvel, 2013; De Figueiredo *et al.*, 2015). There are no pathognomonic signs of brucellosis in animals at individual level; *Brucella*species infected animals generally

develop granulomatous inflammatory lesions which frequently are found in lymphoid tissues and organs such as the reproductive organs, udder and supramammary lymph nodes (Hegazy *et al*., 2004; Martirosyan and Gorvel, 2013). The disease is also characterized by abortion, retained placenta, hygroma of the knee, epididymitis and to a lesser extent, orchitis and infection of the accessory sexglands (Tibary *et al*., 2006). Infected foetal membranes show changes affecting parts or all of the membrane (Adams, 2002). The necrotic cotyledons lose their blood-red appearance becoming thickened and dull-grey in colour (Herenda *et al*., 2002). Pathological changes in bovine foetus infected with *B. abortus*include granulomatous lesions and focal necrosis in several organs, oedema of the sub-cutis and skeletal muscles, sero-haemorrhagic lesions in body cavities, and bronchopneumonia or interstitial pneumonia (Radostits *et al*., 1994). The cotyledons may be red, yellow, normal or necrotic; there may be exudates on the surface (CFSPH, 2009).

2.14 Pathology of (Coxiellosis) Q Fever

The disease takes the form of pneumonia or hepatitis commonly (CDC, 2007). In acute phase, the presence of *C. burnetii* may be demonstrated in the blood, liver, lungs and spleen (Lang, 1990). The aborted fetus may show hepatomegaly. The placenta of aborted sheep is red-brown and opaque with light-brown, soft cotyledons (Anon, 2001). Bronchopneumonia with interstitial pneumonitis is seen, intracellular organisms in monocytes are present and granulomas are found in the bone marrow, liver and spleen. Placental necrosis were also significantly associated with the presence of *C. burnetii* in the trophoblasts (Bildfell *et al*., 2000). As the infection becomes more serious, chronic Q fever

develops, endocarditis, inflammation of the aortic heart valves, has been associated with the chronic complications of the disease. The survival rate is higher for those who do not suffer from chronic Q fever (CDC, 2007).

2.15 Immune Response to *Brucella* Infection

Antibodies of the IgG, IgM, or IgA classes may play a role in protection against brucellosis. Anti-*Brucella*antibodies are proteins that cause agglutination, complement fixation, and precipitation when reacted with their homologous antigens derived from *Brucella*e (Elfaki *et al.*,2015). Most of the reactive antibodies were elicited by *Brucella*lipopolysaccharide (LPS) rather than by cytoplasmic proteins. Indeed, IgM antibodies against *Brucella*LPS were the first to appear following infection and rise gradually during the course of acute infection (Elfaki *et al.*, 2015). In contrast, IgG anti-*Brucella*antibodies appeared later after the onset of infection and were likely provoked by *Brucella*cytoplasmic proteins. On this basis, most of the available serological tests were based on LPS to differentiate between infected and uninfected hosts (Elfaki *et al.*, 2015). Immune response to *B. abortus*will be used as an example because it has been most studied in detail (Nielsen and Yu, 2010; Kaltungo *et al*., 2014a). *Brucella*infection in cattle consists of early IgM isotype which usually appears between 1–2 weeks post-exposure, but may sometimes be delayed. Appearance of this antibody depends on the route of exposure, infecting dose of the bacteria and the immune status of the host animal (Kaltungo *et al*., 2014a).

Innate immunity is composed of non-specific immune response in the early stage of infection before adaptive immunity, mediated by clonal selection of specific lymphocytes (Ko and Splitter, 2003). Thus, the ultimate roles of the innate immune system in *Brucella*infection *in vivo* are to reduce the initial number of infected bacteria without memorization and to provide the environment for generating Th1 immune response in the host (Ko and Splitter, 2003). An important cell-mediated immune mechanism responsible for protection against *Brucella*is the interferon gamma (IFN–γ) up-regulation of macrophages, the main host cellular reservoir for the bacterium (Baldwin *et al*., 1993; Xavier *et al*., 2010). Thus many investigators have considered that Th1 CD4+ T cells are responsible for macrophage activation and attraction of inflammatory effectors cells and therefore they have been suggested to play a major role in acquired cellular resistance (Golding *et al*., 1994; Skendros and Boura, 2013).

The complement system or "complements" helps the ability of antibodies and phagocytic cells to clear pathogens by phagocytosis.Currently three pathways of complement activation mechanism have been elucidated: (a) the classical pathway, mediated by antigen-antibody complexes; (b) the alternative pathway, induced by certain structures on the surface of microorganisms in an antibody-independent manner, and (c) the lectin pathway, activated by the binding of mannose -binding lectin to carbohydrates on microbial surfaces (Ko and Splitter, 2003). In general, smooth strains of *B. abortus* are more resistant than OPS-deficient rough strains to serum bactericidal activity (Corbeil *et al*., 1988) because *B. abortus* LPS obtained from smooth strains does not activate the alternative pathway (Hoffmann and Houle, 1995).

2.16 Immune Response to *Coxiella burnetii* Infection

Cell-mediated immune responses are important in controlling coxiellosis (Q fever), but then the role of humoral responses is not clear.Infection with *C. burnetii* results in humoral and cellular immune responses, both of which are thought to contribute to protection against subsequent infection (Schneeberger *et al*., 2010; Kersh *et al*., 2012). Antibodies are important during the bacteraemic phase of infection in acute coxiellosis (Q fever), but are potentially detrimental during chronic infection, where immune complexes can cause pathology (Raoult *et al*., 1990). Passive transfer of antibody can protect animals against subsequent challenge with *C. burnetii*. No protection was seen if antibody was transferred to severe combined immunodeficiency mice, a strain of mice lacking in lymphocytes and immunoglobulins, showing the importance of cell-mediated response (Zhang *et al*., 2007). Due to the intracellular niche of *C. burnetii*, it has long been thought that cell-mediated immunity must be required for protection against this pathogen. In support of this idea Andoh *et al*. (2007) and Shannon *et al*. (2009) recently found that T cells and interferon-γ are essential for resolution of a primary *C. burnetii* infection. While cell-mediated immunity plays an important role in immunity to *C. burnetii*, passive immunization studies, where serum from vaccinated animals was transferred into naïve animals, clearly demonstrated that antibody alone was capable of providing complete protection in an immunocompetent animal (Zhang *et al*., 2007; Shannon *et al*., 2009).

The development of potential subunit vaccine candidates would benefit from a deeper understanding of the precise mechanisms responsible for antibody-mediated immunity to

C. burnetii. Antibody can provide protection against intracellular pathogens via a number of different mechanisms. These include direct bactericidal activity, complement activation, opsonization, cellular activation via Fc or complement receptors, and antibody-dependent cellular cytotoxicity (Casadevall and Pirofski, 2006).The immune host response to *C. burnetii* infection depends on TLR4. Indeed, *C. burnetii* infection results in the formation of granulomas in spleen and liver, which has been associated with protective immune response (Maurin and Raoult, 1999).

2.17Diagnosis of Brucellosis

Classical tests for the diagnosis of brucellosis include culture and phenotypic characterization which are laborious, time-consuming, pose the risk of infection, and can generate discordant results (Muma *et al.,* 2009). Serological tests are therefore commonly used for *Brucella*diagnosis in cattle and small ruminants, especially at herd and flock level. In cattle, the buffered *Brucella*antigen test, (Rose Bengal test and buffered plate agglutination test), the complement fixation test, the enzyme-linked immunosorbent assay (ELISA) or the fluorescence polarisation assay are suitable tests for screening herds and individual animals (OIE, 2009). In small ruminants, the buffered *Brucella*antigen tests (BBAT) and the complement fixation test (CFT) are usually recommended for screening flocks and individual animals. The serum agglutination test is not considered to be reliable for use in small ruminants (OIE, 2009). The sensitivity of RBPT fulfills the requirements for surveillance of free areas at flock level but it is believed that only the combination of RBPT and CFT or ELISA in infected flocks can obtain accurate individual sensitivity in test-and-slaughter programmes (Munoz *et al*.,

2005). Despite the vigorous attempt for more than one century to come up with a definitive diagnostic technique for brucellosis, diagnosis still relies on the combination of several tests to avoid false negative results (Poester *et al.*, 2010).

Diagnostic tests fall into two categories: (1) direct diagnosis or those that demonstrate the presence of the organisms and (2) indirect diagnosis or those that detect an immune response to its antigens: The isolation of*Brucella*is definitive proof that the animal is infected, but not all infected animals give a positive culture and the methods and facilities that must be employed are not always readily available (WHO, 2006). The laboratory diagnosis of brucellosis relies mainly on serologic and culture methods (Araj, 1999). Although blood and tissue culture remains the "gold standard" for diagnosis, culture has low sensitivity, is time consuming, and is a risk for the laboratory personnel (Bricker, 2002; Navarro*et al*., 2004).

2.17.1*Brucella* culture techniques

Bacterial culture plays an important role in confirming the presence of pathogen and it is essential for antimicrobial susceptibility, biotyping and molecular characterization which provide valuable epidemiological information to know the sources of infection in outbreak scenarios and the strain diversity in endemic regions (Alvarez *et al*., 2011).

2.17.1.1 Blood cultures in brucellosis

In animals with brucellosis, positive blood cultures may be obtained in as low as 6% to as high as 92% of infectedanimals depending on a number of factors that include: (1) stage of the disease i.e. being acute, sub-acute or chronic, (2) prior antimicrobial therapy, (3) the levels of *Brucella*titers, as the higher the bacterial loads the higher the rate of positive blood culture and (4) the microbiological culture technique utilized (Al-Tawfiq and Abukhamsin, 2009). There are several factors that affect growth and detection of *Brucella*species in blood culture.These include: (1) the low level of carbon dioxide released which is the most important limiting factor, (2) the sodium polyanethol sufonate that exerts a harmful effect on the outer membrane of *Brucella*species and (3) the inoculation which is a factor that is inversely proportional to the time of detection.The sensitivity of culturing *Brucella*species from blood varies from 15% to 70% when compared with clinical evidence of infection and positive serologic results depending on the study and the culture method used (Aliskan, 2008). the traditional Ruiz-Castaneda method, (2) the automated systems and (3) the yield-optimizing methods such as the lysis concentration technique. The traditional methods take longer time of incubation of culture from days to weeks is usually needed to improve the yield of organisms (Espinosa *et al.,* 2009). The Bactec 9240 and 9120 automated systems can yield positive cultures in up to 95% of cases within 4 to 7 days (Ozkurt *et al*., 2002). The Bact / Alert automated culture system yield is about 51% within 4 to 5 days, while the broth culture system yield may reach 100% within 5 days (Ozkurt *et al*., 2002).

2.17.1.2 Cultures of bone marrow and other body fluids

Bone marrow aspiration and biopsy are performed selectively for establishing the diagnosis of an infectious illness. In patients with brucellosis, bone marrow biopsies may show specific granulomatous changes and cultures of bone marrow aspirates may become positive thus helping in establishing the diagnosis (Duong *et al.,* 2009). Bone marrow examination and *Brucella*cultures of the marrow aspirate are indicated under the following circumstances: (1) in animals with fever of unknown origin (FUO), particularly in areas that are endemic for brucellosis, (2) in animals strongly suspected to have brucellosis on clinical grounds but have negative serology, (3) in animals having unexplained articular or haematological involvement who live in endemic areas and (4) in areas where advanced facilities such as automated culture systems or molecular techniques such as polymerase chain reaction (PCR) are not readily available. Disadvantages of culture: Isolation of *Brucella*bacteria is time and resource-intensive; it requires level 3 biocontainment facilities and highly skilled technical personnel to handle samples and live bacteria for eventual identification and biotyping (Yu and Nielsen, 2010). Handling all live *Brucella*involves risk of laboratory infection and very strict biosafety rules must be observed.

2.17.2 Serological techniques

In contrast to bacterial culture, serological testing is fast, non-hazardous and more sensitive and therefore preferred in routine clinical practice. However, serological tests can only indirectly prove *Brucella*infections by high or rising titres of specific antibodies. The most commonly used serological tests for the diagnosis of

brucellosis in cattle and small ruminants are: (i) Agglutination Tests, slow tests requiring incubation from 8 to 24 hours: Modified tube (SAT), 2-Mercaptoethanol (2-ME) or dithiothreitol (DTT), are reducing agents), Rivanol test and Milk ring test; (ii) Rapid Agglutination Tests, performed in mins: Rose Bengal test, Modified Rose Bengal test, Buffered plate agglutination test, Card test and Rivanol (precipitation) test; (iii) Precipitation Tests: Agar gel immunodiffusion and Radial immunodiffusion; (iv) Complement Fixation Tests: Warm, Cold, Haemolysis in gel and indirect haemolysis; (v) Primary Binding Assays: Radioimmunoassay, Fluorescence immunoassay, Particle counting fluorescence immunoassay, indirect enzyme immunoassay, Competitive enzyme immunoassay and Fluorescence polarization assay (Nielsen and Yu, 2010)

Following *Brucella*infection, specific immunoglobulin (IgM) antibodies appear within one week, they reach a peak within 3 months and they remain elevated for weeks to months. Other specific immunoglobulin (IgG) antibodies appear within 3 weeks of infection, they reach a peak within 6 to 8 weeks and they remain present (Ozdemir *et al*., 2011). Other specific agglutinating antibodies are detected by Rose Bengal Plate test (RBPT) and modified tube agglutination (SAT) test. Coombs test, Dipstick assay, *Brucella*Capt (BCAP), Lateral flow assay (LFA), Rapid slide agglutination test (RSAT), Complement Fixation Test (CFT), Mercaptoethanol (2-ME)Radioimmunoassay, SAT in the presence of reducing agent and more recently enzyme linked immunosorbent assay (ELISA) have been used for the detection of specific IgG antibodies (Nielsen and Yu, 2010). Coombs test and IgG ELISA are particularly useful in the diagnosis of chronic infection and monitoring of disease relapse. Specific IgM ELISA antibodies are

particularly useful in the diagnosis of acute brucellosis (Pabuccuoglu *et al*., 2011). However, cross-reactions between *Brucella*species and other Gram-negative bacteria such as *Yersinia enterocolitica* O9, *Escherichia coli* O157, *Vibrio cholerae*and *Stenotrophomonas maltophilia* are a major problem (Muñoz *et al*., 2005). False-positive serological results caused by *Y. enterocolitica* O9 may cause up to 15% false positive test results of the cattle herds in regions free from brucellosis, generating considerable additional costs for surveillance programs (Muñoz *et al*., 2005).

2.17.2.1 Acidified antigen tests

There are two commonly used tests, the Buffered Antigen Plate test (BAPT) and the Rose Bengal Plate test (RBPT) or card test (Nielsen *et al*., 1984; Lucero and Bolpe, 1998). The Buffered Antigen Plate Agglutination test (BAPT) has been widely used (Angus and Barton, 1984; Nielsen and Yu, 2010). After thorough mixing of the serum and antigen, agglutination must be visible, 4 min for the RBPT and 8 min for the BPAT. Incubation for extended periods of time may sometimes result in falsereactions, often due to the formation of fibrinclots (Diaz-Aparicio *et al*., 1994; Nielsen and Yu, 2010). Buffered plate agglutination test (BPAT) is a modified agglutination test. It is simple and inexpensive to perform.The test uses antigen at pH of 3.65 and is made from *B. abortus* S119.3 whole cells. The test is liable to false positive results due to vaccinal antibodies and prozoning effect (Nielsen, 2002; Kaltungo *et al*., 2014a). The main advantage of this test is the reduction of non-specifictest reactions).

The RBPT is a rapid, slide-type agglutination assay performed with a stained *B. abortus* suspension at PH of 3.6-3.7 and plain serum. It is simple and ideal for screening test for small laboratories with limited resources (Diaz *et al*., 2011). The drawbacks of RBPT include low sensitivity particularly in chronic cases, relatively low specificity in endemic areas and prozones make strongly positive sera appear negative (Diaz *et al*., 2011). The overall sensitivity is 92.9%. So the use of RBPT should be considered carefully in endemic areas, particularly in individuals exposed to brucellosis and those having history of *Brucella*infection (Ruiz-Mesa *et al*., 2005).The test is based on reactivity of antibodies against smooth lipopolysaccharide (LPS). Due to its high sensitivity, false negative results are rarely encountered. The test may be applied to a serial dilution [1:2 through 1:64] of the serum samples to increase its specificity (Supriya *et al*., 2010; Diaz *et al*., 2011). The present world health organization (WHO) guidelines recommend the confirmation of the RBPT by other assays such as serum agglutination tests (Diaz *et al*., 2011). These tests have been introduced in many countries as the modified screening test followed by confirmatory testing because it is very simple, the consumables are cheap, there is a low equipment requirement and the assay is modified (Nielsen and Ewalt, 2010).Disadvantages of the test include False negative reactions may occur, due to pro-zoning with sera containing very high levels of antibody. In addition, false positive serological reactions (FPSR) may occur due to some cross reacting antibodies and antibodies resulting from *B. abortus*S19 vaccination (OIE, 2009).

2.17.2.2 Agglutination tests

The first agglutination test for the detection of antibody to *Brucella*infection was reported by Wright and Smith over 100 years ago (Pabuccuoglu *et al*., 2011; Kaltungo *et al*., 2014a).

Agglutination tests are generally not used for the diagnosis of infection with *B. ovis* and *B. canis*, rough species of *Brucella*, as the whole cell antigens tend to auto-agglutinate. However, rapid slide agglutination tests have been developed for the serological diagnosis of *B. canis* infection (Reid *et al*., 2009; Nielsen and Yu, 2010) as well as a micro-agglutination test (Kimura, 2008; Nielsen and Yu, 2010). Serum Agglutination Test (SAT) can give a reliable result in bovine brucellosis (Ertek *et al*., 2006). This test is based on the reactivity of antibodies against the smooth lipopolysaccharide of*Brucella*. Excess of antibodies resulting in false negative reaction due to prozone effect can be overcome by applying a serial dilution of 1:2 through 1:64 of the serum samples thus increasing the test specificity (Afify *et al*., 2013). The testis performed at a near neutral pH which makes it more efficient and sensitive in detecting IgM antibody. Hence, it is best used to detect acute infections (Nielsen and Yu, 2010; Kaltungo *et al*., 2014a). It is less effective for IgG, resulting in low assay specificity (Nielsen and Yu, 2010). Due to this fact, the SAT, despite being sensitive, is generally not used as a single test, but rather it is used in combination with other tests (Nielsen and Yu, 2010; Kaltungo *et al*., 2014a).

A modification of this test Microtitre Serum Agglutination Test (MSAT), has involved introduction of ethylene tetra acetic acid (EDTA) (MacMillan and Cockrem, 1985). Basically Serum Agglutination Test (SAT) and Microtitre Serum Agglutination Test (MSAT) have similar principle but differ only in the volume of antigen and antibody

titration which is in milliliter in SAT while in microlitre in MSAT (WHO, 2006). Due to its false positive and false negative results, SAT is only suitable for herd testing, rather than for testing individual animals (Poester *et al*., 2010). The SAT does not detect antibodies to *B. canis* and *B. ovis* because these rough strains of the organism do not have OPS on their surfaces (Poester *et al*., 2010).Tube Agglutination Test measures the total quantity of antibodies and has the disadvantages of reacting with post immunization agglutinins and sometimes those caused by heterospecific antigens (Ertek *et al*., 2006).

2.17.2.3 Complement fixation test

Complement fixation test (CFT) is a prescribed test for international trade, it is a widely used confirmatory test for brucellosis. In spite of the number of reagents required for the test and its technical complications, it is a widely used as confirmatory test for brucellosis (Poester *et al*., 2010). It requires good laboratory facilities and adequately trained staff to accurately titrate and maintain the reagents. There are numerous variations of the CFT in use, but this test is most conveniently carried out in a microtitre format. Either warm or cold fixation may be used for the incubation of serum, antigen and complement. Further it can beconducted either 37°C for 30 min or 4°C for 14–18 hours (OIE, 2009). The basic test consists of *B. abortus* antigen, usually whole cells, incubated with dilutions of heat inactivated (to destroy indigenous complement) serum and a titrated source of complement, usually guinea pig serum (Poester *et al*., 2010). There are two main variations to the CFT. The test has some disadvantages presented for the diagnosis of brucellosis such as complexity and necessity for serum heat inactivation (Nielsen and Duncan, 1990). It is technically challenging because a large number of reagents must be titrated daily, it is also

an expensive test and labour intensive (Nielsen and Yu, 2010). The CFT has been widely used as a confirmatory test in control/eradication programmes (Nielsen *et al*., 1983).

2.17.2.4 Precipitin tests

Precipitin tests were the first tests developed to distinguish *B. abortus*S19 vaccinal antibody from the antibody resulting from infection with pathogenic strains (Diaz *et al*., 1979; Nielsen and Yu, 2010;Poester *et al*., 2010). There are two basic formats: agar gel immunodiffusion (AGID) and radial immunodiffusion (RID) (Nielsen and Yu, 2010). Agar gel immunodiffusion in which soluble antigen(s) and test serum are inserted into adjacent wells, cut in an agar matrix 0.5 to 1.0 cm apart. The reagents then diffuse into the agar for a period of time.This results in the formation of a visible precipitin band where they intersect if the serum contains antibody. As for radial immunodiffusion, the test utilizes antigen placed directly in the agar matrix, pipetting test serum in a well cut in the agar and allowing the serum to diffuse radially to form a precipitin ring if antibody is present in the serum. Both tests use OPS antigens derived from *B. melitensis* or native hapten (Diaz *et al*., 1979; Nielsen and Yu, 2010). Both formats proved to be relatively insensitive with O-polysaccharide (OPS) antigen (Jones *et al*., 1980) while the sensitivity was better with native hapten antigen (Asarta, 1989). The tests are quite labour intensive but provide results not available by any other test procedure at the time. Neither test is currently used extensively. Precipitin tests are widely used for the diagnosis of *B. ovis* infection in sheep using rough lipopolysaccharide (RLPS) or hot saline extracted antigens (OIE, 2009).

2.17.2.5 Reducing agents

Both Dithiothreitol and 2-mercaptoethanol have been used for the serological diagnosis of brucellosis (Klein and Behan, 1981). These reagents reduce disulfide bridges of IgM resulting in production of monomeric molecules with reduced ability to agglutinate. Either reducing agent may be added to serum as a diluent, using dilutions of 1:25 and above. For the diagnosis of brucellosis, reaction at a 1:25 serum dilution is considered 75positive (Poester *et al*., 2010). Care must be taken when using 2-mercaptoethanol as it is toxic and should only be used in a well-ventilated area or a chemical hood (Nielsen and Yu, 2010).

2.17.2.6 Rivanol (precipitation) test

Rivanol Test is aimed at eliminating some non-specific reactions which is based on precipitation of high molecular weight serum glycoprotein from serum solutions; which is mainly IgM, leaving mostly IgG in the serum (Poester*et al*., 2010; Montasser *et al.*, 2011). Acrydine dye such as rivanol (2-ethoxy-6, 9-diaminoacridine lactate) is used to achieve the precipitation process, after which the precipitate is removed by centrifugation. The precipitation tests are usually used as confirmatory tests, because of their laborious protocols (Poester*et al.*, 2010). The test is capable of distinguishing between vaccinated and infected animals, and also chronic carriers (Kaltungo *et al*., 2014a.) Because the protocol is fairly labour-intensive, precipitation tests are generally used as confirmatory tests (Nielsen and Yu, 2010).

2.17.2.7*Primary antigen – antibody binding assays*

2.17.2.7.1 Enzyme linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. Other names, such as enzyme immunoassay (EIA) are also used to describe the same technology. Enzyme-linked immunosorbent assays have been evaluated for many years for their ability to detect serum antibodies to brucellosis in domestic animals (Rahman *et al*., 2011). Enzyme-linked immunosorbent assay has become popular as a modified assay for the diagnosis of brucellosis serologically. It measures IgA, IgG and IgM antibodies and this allows a better interpretation of the clinical situation. The diagnosis of brucellosis is based on the detection of antibodies against the smooth LPS. Detection of IgG antibodies is more sensitive than detection of IgM antibodies for the diagnosis of brucellosis but the specificity is comparable (Araj, 2010; Agasthya *et al*., 2012). Enzyme-linked immunosorbent assay is more sensitive in acute and chronic cases of brucellosis compared to the conventional agglutination methods, and it offers a significant diagnostic advantage in the diagnosis of brucellosis in endemic areas. For case detection and an accurate diagnosis of suspected cases, the combination of ELISA IgM and IgG tests should be used as this combination of laboratory tests has been shown to be the most efficient technique in the diagnosis and detection of brucellosis. Enzyme-linked immunosorbent assay IgM and 2-MET are more promising (Mantur *et al*., 2010; Asaad and Alqahtani, 2012). The assay has been applied in the diagnostic work of neurobrucellosis with varying success. The cut-off values need to be adjusted so as to optimize specificity when used in endemic areas (Araj, 2010). One study showed that the

best cut-off point of ELISA IgG is 53 IU/ml which yields maximum sensitivity and specificity to diagnose acute brucellosis (Dashti *et al*., 2012).

ELISAis an excellent method for screening large populations for *Brucella*antibodies and for differentiation between acute and chronic phases of the disease (Agasthya *et al*., 2012). It is the test of choice for the chronic, local, or complicated cases of brucellosis particularly when other tests are negative while the case is under high clinical suspicion. It can reveal total and individual specific immunoglobulins (IgG, IgA and IgM) within 4-6 hours with high sensitivity and specificity. In addition to the detection of immunoglobulin classes, ELISA can also detect *Brucella*-specific IgG subclasses and other *Brucella*immunoglobulins such as IgE (Araj, 2010). The ELISA is the best for detecting *Brucella*antibody in the cerebrospinal fluid in cases of neurobrucellosis (Araj, 2010). It also has several advantages when compared with other tests. Itdirectly identifies a specific antibody (Rahman *et al*., 2011). It is also more sensitive than other agglutination tests and thus has the potential to detect infected animals (Rahman *et al*., 2011).

2.17.2.7.2 Competitive enzyme linked immunosorbent assay

The competitive enzyme linked immunosorbent assay (cELISA) is the most complex ELISA and is used to measure the concentration of an antigen (or antibody) in a sample by observing interference in an expected signal output. Hence, it is also referred to as an inhibition ELISA. Competitive enzyme immunoassays (cELISA) were developed in order to overcome some of the problems arising from residual *B. abortus* S19 vaccinal

antibody and from cross reacting antibody (Poester *et al*., 2010). By selecting a monoclonal antibody with slightly higher affinity for the antigen than most of the vaccinal/cross-reacting antibody but with lower affinity than most antibodies arising from infection (Nielsen and Yu, 2010). Reactivity by vaccinal antibody could be eliminated in the majority of cases (Nielsen and Yu, 2010). The specificity of the competitive enzyme immunoassay is very high; however, it is slightly less sensitive than the indirect enzyme immunoassay. This assay is an excellent confirmatory assay for the diagnosis of brucellosis in most mammalian species (Nielsen and Yu, 2010; Poester *et al*., 2010).

The performance of cELISA is comparable to the CFT test.Therefore, it can reasonably be used as a sole test for the diagnosis of brucellosis (Nielsen *et al*.,1995). There is increasing evidence that competitive monoclonal antibody (Mab)-based ELISA (cELISA) may offer distinct advantages over the indirect enzyme linked immunosorbent assay. However, it has been shown that the cELISA eliminates some but not all reactions due to cross-reacting bacteria (Weynants *et al*., 1997).The advantages of cELSA include (1) high specificity, since two antibodies are used the antigen/analyte is specifically captured and detected, (2) suitable for complex samples, since the antigen does not require purification prior to measurement, since both direct and indirect detection methods can be used, (3) this assay is an excellent confirmatory assay for the diagnosis of brucellosis in most mammalian species (Nielsen and Yu, 2010).

2.17.2.7.3 Indirect enzyme linked immunosorbent assay

The indirect enzyme linked immunosorbent assays (iELISA) generally have very high sensitivity but because they are largely unable to distinguish *B. abortus*S19 vaccinal antibody and cross reacting antibody the specificity is low (Nielsen and Yu, 2010). The most commonly used system depends on enzymes conjugates for detection of antibody to smooth lipopolysaccharide preparations which are passively attached to a polystyrene matrix usually in a 96 well format followed by addition of diluted serum or milk (Poester *et al*., 2010). The detection system varies considerably but often a monoclonal antibody specific for an immunoglobulin heavy chain epitope of the test species and conjugated with peroxidase is used (Nielsen and Yu, 2010). This is an OIE prescribed test for international trade (OIE, 2004). One of the advantages of indirect enzyme-linked immunosorbent assay is highly sensitive, simple to perform and there is no requirement for a supply of sheep red blood cells (Nielsen and Gall, 1994; OIE, 2009). Disadvantages ofindirect enzyme-linked immunosorbent assayis problem of differentiating between antibodies resulting from cross-infection and S19 vaccination (OIE, 2009).

2.17.2.7.4 Fluorescence polarization assay

The basis for the fluorescence polarization assay (FPA) is simple. It was initially developed for testing serum. However, the technology has been extended to testing whole blood and milk samples from individual animals. In this test the rate of rotation of a molecule in solution is inversely proportional to its size.This test is based on the rotational differences between a small soluble antigen molecule in solution and the antigen molecule complex with its antibody (Supriya *et al.*, 2010). By attaching a fluorescein molecule to a small molecular weight antigen molecule, the time of rotation

through a given angle can be measured using polarized light (Nielsen and Yu, 2010). The FPA was developed in 1996 and has since been extensively validated (Stournara *et al*., 2007). The FPA is a homogeneous assay, requiring no washing steps or removal of unreacted components (Nielsen and Yu, 2010). Because only 2 reagents, antigen and diluent buffer are required, the test is technically simple and relatively inexpensive. It does require a fluorescence polarization analyzer of which several are available at various costs (Poester *et al*., 2010). The test is a valuable alternative to conventional serological tests (Supriya *et al.,* 2010).

The FPA is very accurate and the sensitivity/specificity can be manipulated by altering the cut-off value between positive and negative reactions to provide a highly sensitive screening test as well as a highly specific confirmatory test (Nielsen and Yu, 2010;Poester *et al*., 2010). The FPA can distinguish vaccinal antibody in most vaccinated animals and it can eliminate reactivity by some cross-reacting antibodies as well (Poester *et al*., 2010). Primary binding assays in general are highly sensitive and specific assays for detection of antibody in various species (Nielsen and Yu, 2010).The main advantages include: Easy data transmission;adjustable sensitivity/specificity values depending on their use indisease control, eradication or surveillance; commercial availability;easily adapted to continuous quality control schemes;Some can distinguish vaccinal antibody;most formats can be used to test multiple species of hosts;some formats are rapid and may be used in the field;some formats are simple to perform;easily automated (Nielsen and Yu, 2010). The main disadvantages include: some commercial kits are very highly priced;some commercial kits are more accurate than others;expensive equipment but may be used for multiple tests;may

be technically challenging due to high dilutions of reagents andmultiple steps of FPA (Nielsen and Yu, 2010).

2.17.2.7.5 Lateral flow assay

The lateral flow assay (LFA) also known as lateral flow immunochromatographic assays, is a simplified enzyme linked immunosorbent assay (ELISA) for the qualitative detection of antigen specific antibodies in milk, serum or whole blood samples (Christopher *et al*., 2010; Yetisen, 2013). The assay is based on the binding of specific antibodies to antigen immobilised on a test strip (cellulose membrane matrix). It allows the detection of specific IgM as well as specific IgG antibodies and that a high sensitivity is assured for all stages of the disease (Nielsen and Yu, 2010). Lateral flow assay basically combines a number of variants such as formats, bio-recognition molecules, labels, detection systems and applications (Sajid *et al*., 2015). Application of the assay does not require specific expertise, equipment or electricity, and test kits may be kept in stock without the need for refrigeration, thus, making the assay a very useful one for poor resource countries including most African countries and migratory herds/flocks (Abdoel *et al*., 2008, Baddour, 2012). However, its interpretation is subjective, depending on the formation of a visible coloured line of reaction and the assay itself tends to be expensive because of the multiple ingredients/components involved (Nielsen and Yu, 2010). Advantages of lateral flow assay include: it requires no equipment for its performance; ease of device preparation; low cost; stability over a wide range of environmental conditions and very long shelf life; simple and user friendly operation; require small sample; most of the

time, allows sample application without pre-treatment; less time of analysis (Sajid *et al.*, 2015).

2.17.2.7.6 Radioimmunoassay

A radioimmunoassay (RIA) has been developed to measure antibodies against *B. abortus* in bovine serum and can be used in the diagnosis of bovine brucellosis (Chappel *et al.*, 1982). The RIA measures the amount of specific antibody of the IgG^1 and IgG^2 subclasses but is insensitive to 1gM, a characteristic which may make it more suitable than the complement fixation test (CFT) or the serum agglutination test (SAT) for distinguishing infected animals from those which have been vaccinated with *B. abortus* strain 19 (Chappel *et al.*, 1982). The RIA is not subject to prozoning or ambiguous reactions, both of which interfere with the interpretation of the CFT (Chappel *et al.*, 1982). The test is very sensitive in-vitro technique used to measure concentrations of antigens by use of antibodies (Nielsen, 2002). Radioisotopes are used as a label to identify one of the reactants (Tizard, 2000). The RIA technique is extremely sensitive and extremely specific, requiring specialized equipment, and it remains the least expensive method to perform such tests. It requires special precautions and licensing, since radioactive substances are used (Yalow and Berson, 1960).

2.17.2.8Milk ring test

The agglutination test has been adapted to test milk for antibody to *Brucella*species.This test is prescribed by the OIE for use only with cow milk. Bulk milk can be screened to detect the presence of infected animals within the herd, which can then be identified by blood testing. This method of screening is extremely effective and is usually the method

of choice in dairy herds (OIE, 2009). The format of the milk ring test (MRT) is a little different in that haematoxylin stained *Brucella*cells are mixed with whole milk or skimmed milk with cream added (Huber and Nicoletti, 1986; Sutra *et al*., 1986). Immunoglobulins present in the milk will, in part, be attached to fat globules via the Fc portion of the fat molecule (Poester*et al*., 2010). The immunoglobulins detected by MRT are IgM and IgA. This test may be applied to individual animals or to pooled milk samples using a larger volume of milk, relative to the pool size (Poester*et al*., 2010; Kaltungo *et al*., 2014a).If antibody to *Brucella*specieis present, antigen will attach to it, resulting in a purple band in the cream layer. If no antibody is present, the fat layer will remain a buff colour and the purple antigen will be evenly distributed throughout the milk (Nielsen and Yu, 2010). The milk ring test is prone to false reactions caused by abnormal milk due to mastitis, presence of colostrum and milk from the late lactation (Kaltungo *et al.*, 2014a).False negatives may also occur in milk with a low concentration of lacteal antibodies or lacking fat-clustering factors (Bercovich, 1998). In spite of these problems, the MRT has been found to be extremely effective, and is usually the method of choice in dairy herds, and may be used as an inexpensive screening test in conjunction with other tests (Corbel, 2006; Kaltungo *et al*., 2014b).Disadvantages are; in large herds (>100 lactating cows), the sensitivity of the test become less reliable, the milk ring test is prone to false reactions caused by abnormal milk such as mastitic milk, colostrum and late lactation cycle milk is the. Still, in spite of its problems, it may be used as an inexpensive screening test in conjunction with other tests (Nielsen and Yu, 2010).

2.17.3 Molecular techniques for typing

Several molecular techniques have been developed and extensively used for typing organisms. Typing techniques have the advantage that, they allow for the investigation of disease outbreaks, give better understanding into the epidemiology of infections and aid in the treatment of infected animals/people (Trindade *et al*., 2003). Typing techniques are evaluated in terms of their performance (discriminatory power, reproducibility, type-ability, and agreement between typing techniques) and convenience in usage (cost and availability of reagents and equipment; rapidity and ease of execution and interpretation of results; and versatility) (Trindade *et al*., 2003).

2.17.3.1 Pulsed field gel electrophoresis

Pulsed field gel electrophoresis (PFGE) is an agarose gel electrophoresis technique used for separating larger pieces of DNA by applying electrical current that periodically changes direction (three directions) in a gel matrix unlike the conventional gel electrophoresis where the current flows only in one direction (Arbeit, 1999; Trindade *et al*., 2003). In PFGE, intact chromosomes are digested using restriction enzymes or restriction endonucleases to generate series of DNA fragments of different sizes also known as restriction fragments length polymorphisms(RFLPs) and patterns specific for a particular species or strain (Shi *et al*., 2010). Pulsed field gel electrophoresis is considered as the 'gold modified' typing method by many researchers for zoonotic and/or foodborne pathogen outbreak investigations and other epidemiological studies (Alonso *et al*., 2005). PFGE has not found widespread use as a routine typing tool for *Brucella*, this likely reflects the very limited diversity identified at sub-species level. The application of this technique has been limited to small studies examining areas such as minor variants of *B.*

ovis, differentiating a vaccine strain from the field isolates and differentiating marine mammal isolates from porpoises, seals and dolphins (Jensen *et al*., 1999; Bourg *et al*., 2007). PFGE is also expensive compared to random amplified polymorphism deoxyribonucleic acid (RAPD), enterobacterial repetitive intergenic consensus (ERIC), repetitive Extragenic palindromic (REP) and plasmid analysis (Alonso *et al*., 2005).

2.17.3.2 Restriction fragment length polymorphism

Restriction Fragment Length Polymorphism (RFLP). One of the first techniques utilized in clinical molecular diagnostics was the detection of genomic changes using Southern blotting and restriction fragment length polymorphism (RFLP). This approach has particularly been applied to various outer membrane protein (*omp*) encoding genes and has become a popular method for differentiation of *Brucella*species and for descriptions of new strains (Vizcaino *et al*., 2000; Bricker, 2002). Much work has focused on the omp2 locus originally characterized and shown to consist of two genes designated *omp*2a and *omp*2b, displaying about 85% sequence identity, and arranged in opposite orientations (Ficht *et al*., 1989). Further studies indicated the presence of species –specific polymorphisms that have been exploited to develop PCR-RFLP approaches (Ficht *et al*., 1996). Cloeckaert *et al*. (1995) used PCR-RFLP to distinguish *Brucella*species with the exception of *B. abortus*biovars 3, 5, 6 and 9 from some *B. melitensis*strains and *B. canis* from *B. suis* biovar 3 and 4. Furthermore, species-specific markers were identified in *omp*25 including a marker for *B. melitensis*in the form of absence of an EcoRv site though *B. suis* biovars 3 and 4, and *B. canis* could still not be distinguished (Cloeckaert *et al*., 1995). Other *omp* genes examined include *omp*31, known to be deleted in *B. abortus* and has marker for *B.*

canis, B. suis biovar2 and (Garci′a-Yoldi *et al.*, 2005). Since RFLP was already a mainstay in molecular analysis when PCR was developed, PCR amplification of a region of DNA followed by RFLP quickly became a widely used approach. In this case, the mutation of interest was known and an enzyme that cuts at the mutation site was used (Megan and Lee-Jun, 2013).

2.17.3.3 Multilocus sequence typing

Multilocus sequence typing (MLST) is a an unambiguous, portable and nucleotide-based technique for typing bacteria using the sequences of internal fragments of (usually) seven house-keeping genes (Spratt, 1999; Urwin and Maiden, 2003). In MLST, different sequences within a bacteria species are assigned as distinct alleles for each house-keeping gene and the alleles at each end of the seven loci define the allelic profile or sequence type for each isolate (Urwin and Maiden, 2003). Approximately 450–500 bp internal fragments of each gene are used and most bacteria have enough variation within the house-keeping genes to provide many alleles per locus thus allowing billions of distinct allelic profiles to be differentiated utilizing the seven house-keeping loci (Enright and Spratt, 1999; Urwin and Maiden, 2003). Multilocus sequence typing (MLST) was developed using the concept of multilocus enzyme electrophoresis (MLEE), but instead it assigns alleles at multiple house-keeping genes directly by DNA sequencing (analyses genes themselves) instead of indirectly through electrophoretic mobility (analyses gene expression) for MLEE (Trindade *et al.*, 2003). The only application of multilocus sequence analysis published to date is the field examined nine discrete genomic loci, equating to 4.396 bp from 160 *Brucella*isolates of all species and biovars known at all time (Whatmore *et al.*, 2007).

The study confirmed the genetic uniformity of *Brucella*with only 1.5% of sites found to be polymorphic across all 160 isolate. In all, 27 distinct sequence types of (ST) were identified concatenated sequence data were used to construct an unrooted neighbor joining tree representing the relationships between STs.These showed that the four previously characterized classical*Brucella*species; *B. abortus,B. melitensis, B. ovis and B. neotomae* corresponded to well separate clusters. The advantages of MLST are that it provides typing data that are unambiguous, portable, more accurate and more discriminatory for most bacteria (Enright and Spratt, 1999; Urwin and Maiden, 2003). These data are readily available, comparable and accessible via the internet in contrast to most typing procedures involving comparison of DNA fragment sizes on a gel (Urwin and Maiden, 2003; Dingle *et al*., 2005). This makes MLST typing data more suitable for global epidemiological studies. Due to the sequence conservation in house-keeping genes, MLST sometimes lacks the discriminatory power to differentiate bacterial strains and thus multi-virulence-locus sequence can be used to solve this problem (Chen *et al*.,2007).

2.17.3.4Random amplified polymorphism deoxyribonucleic acid

Random amplified polymorphism deoxyribonucleic acid (RAPD) is a PCR-based technique in which arbitrary primers (typically 10-mer primers) are used to randomly amplify segments of target DNA under low-stringency PCR condition (Wassenaar and Newell, 2000). This process leads to the amplification of one or more DNA sequences and generates a set of finger printing patterns of different sizes specific to each strain (Trindade *et al.,* 2003). The advantages of RAPD are that, it is relatively cheap, rapid, readily

available, and easy to perform (Shi *et al*., 2010; Rezk *et al*., 2012). In RAPD, the efficiency of amplification, annealing and the length of the product varies with the primed sites, giving rise to both weak and strong amplicon which make interpretation of the results difficult (Wassenaar and Newell, 2000). In addition, RAPD has low reproducibility, average discriminatory power and approximately 80 % type ability (Wassenaar and Newell, 2000). The use of two or more primers improves the discriminatory power of RAPD (Trindade *et al*., 2003).

2.17.3.5Polymerase chain reaction

Polymerase chain reaction (PCR) assays can be applied to detect *Brucella*DNA in pure cultures and in clinical specimens, i.e. serum, whole blood and urine samples, various tissues, cerebrospinal, synovial or pleural fluid, and pus (Debeaumont *et al*., 2005; Queipo-Ortuño *et al*., 2008; Colmenero *et al*., 2010). Several polymerase chain reaction(PCR) based methods have been developed. The best-validated methods are based on the detection of specific sequences of *Brucella*spp., such as the 16S-23S genes, the IS711 insertion sequence or the BCSP31 gene encoding a 31-kDa protein (Baddour and Alkhalifa, 2008). The polymerase chain reaction is a recent and promising technique that allows for rapid and accurate diagnosis of brucellosis without the limitations of conven-tional methodology (Baddour, 2012). This method is more sensitive than blood cultures and more specific than serological tests (Al-Dahouk *et al*., 2013).They identify nucleic acid fragments from thebacteria, thus becoming more useful and practical (Yu and Nielsen, 2010). However, sensitivity, specificity and issues of quality control and quality assurance of the PCR assays vary between laboratories and no modification of

sample preparation, target genes and detection methods have been established yet (Navarro *et al*., 2004; Yu and Nielsen, 2010). The first brucellosis PCR-based test was introduced by Fekete in 1990 (Fekete *et al.*, 1990). They successfully amplified a 635 bp fragment of a 43 kDa outer membrane protein gene from *B. abortus* strain 19. Several studies have reported that PCR is a very useful tool for the rapid diagnosis of acute brucellosis and a good marker for the post treatment follow-up and the early detection of relapses (Mitka *et al*., 2007; Al Ajlan *et al*., 2011). Moreover, many studies have developed PCR-based assays to differentiate 10 *Brucella*species (Gopaul *et al*., 2008; Hinic *et al*., 2008). Up to date, PCR assays have been used in diagnosis of both animal brucellosis and human brucellosis (Yu and Nielsen, 2010). Diagnostic specificity of PCR may reach 100% whilst sensitivity is about 80% (Gemechu *et al*., 2011). Together with serological assays, PCR can be applied for the diagnosis of new cases of brucellosis, asymptomatic but highly exposed individuals as well as relapsed cases regardless of duration or type of the disease without relying on blood cultures, particularly in chronic cases (Nimri, 2003).

The PCR technique has increasingly been used as a supplementary method in *Brucella*diagnosis (Elfaki *et al*., 2005). However the technique has a disadvantage of not being able to identify some species like *B. canis* and *B. neotomae*. In addition, some biovars within a given species gave negative results (Nielsen and Yu, 2010; Scholz and Vergnaud, 2013). The PCR was further enhanced to identify the marine strains like *B. microti* and *B. inopinata*. However, it does not differentiate at the biovar level, or below (Lopez-Goni *et al*., 2008; Huber *et al*., 2009; Mayer-Scholl *et al*., 2010). These tests have

shown promising results regarding their sensitivity, specificity and clinical applicability. They have advantages over the gold modified of culture isolation in that they are easy to perform, require a short period of time and avoid the risk of laboratory acquired infection. Once validated, these diagnostic tests will be the future diagnostic modality of choice (Surucuoglu *et al*., 2009; Solera, 2010; Alshaalan *et al*., 2014).

2.*17.3.6 Multiplex polymerase chain reaction*

Several multiplex Polymerase Chain Reaction (PCRs) have been described for identification of *Brucella*at the species level and partly at the biovar level using different primer combinations. The first multiplex PCR, called AMOS PCR for *Brucella abortus, B. melitensis,B. ovis,* and *B. suis,* was published in 1994. It used five primers to identify *Brucella*at the species level (Bricker and Halling, 1994). Multiplex PCR could detect selected biovars of *Brucella*species; biovars 1, 2, and 4 of *B. abortus*, the 3 biovars of *B. melitensis*; biovars 1 of *B. suis* and *B. ovis*. This assay could not differentiate individual biovars within a species (Yu and Nielsen, 2010). *Brucellasuis* biovars 1, 2, and 3 were identified by a multiplex PCR, which included primers based on sequence variation of the *omp2b* gene. However, the use of multiplex PCR was limited because the *B. suis* biovar 1 pattern produced from animal field isolates of *B. suis* was similar to that of *B. suis* biovars 2 and 3, based on identification by bacteriological methods (Ferrao-Beck *et al*., 2006). A multiplex PCR using 8 multi-locus variable number tandem repeat analysis (MLVA) primers was able to distinguish *B. melitensis* from other *Brucella*species and allowed strain typing (Rees *et al*., 2009). Multiplex PCR was used to identify 7 epidemiologically- linked clusters of *B. melitensis* and the source of a laboratory-acquired infection. The assay was found to be practical for technical and economic reasons.More recently, the novel Bruce-

ladder multiplex PCR assay has been used to identify all *Brucella* species at genus level, including the marine species of *Brucella*, 6 terrestrial species of *Brucella*, and the vaccine strains S19, RB51, and Rev. 1 (Lopez-Goni *et al*., 2008; López-Goñi *et al.,* 2011). Based on the Bruce-ladder PCR, an improved multiplex PCR was developed that differentiates all 9 currently recognized *Brucella* species, including *B. microti*, *B. inopinata*, *B. ceti* and *B. pinnipedialis*. The method was used to identify all known *Brucella* strains and their biotypes in single test (Mayer-Scholl *et al*., 2010).

2.17.3.7Real time polymerase chain reaction

Real-time Polymerase Chain Reaction (PCR) for the rapid detection and differentiation of *Brucella*species in clinical samples has recently been developed, targeting 16S-23S internal transcribed spacer region (ITS) and the genes coding omp25 and omp31 (Kattar *et al*., 2007), BCSP 31 (Colmenero *et al*., 2005; Debeaumont *et al*., 2005; Queipo-Ortuño *et al*., 2008), and IS711 (Queipo-Ortuño *et al*., 2005; Zhang *et al*., 2013). The major advantages of real-time PCR are that it can be performed in a very short time, does not require electrophoretic analysis, and avoids contamination. The samples that can be tested by real–time PCR include *Brucella*isolates (Redkar *et al*., 2001), serum (Queipo-Ortuño *et al*., 2005), blood, and paraffin embedded tissues (Kattar *et al*., 2007; Zhang *et al*., 2013).Detection of *Brucella*at species level using real time PCR assays is also possible from clinical samples of both human and animal origin (Gwida *et al*., 2011; Ali *et al*., 2014). Recently direct molecular typing of *Brucella*organisms from clinical material was also reported using Multiple Locus Variable-number Tandem Repeat Analysis (MLVA–16) (Gopaul *et al*., 2014).

2.17.4 Brucellin skin test

The brucellin allergic skin test can be used as a screening or complementary test in unvaccinated flocks, provided that a purified, lipopolysaccharide (LPS) free, modified antigen preparation is used (OIE, 2009).The test uses a protein antigen derived from *Brucella*(brucellergene or brucellin) (Godfroid *et al*., 2010). Brucellosis is capable of eliciting both cellular and anti-body mediated responses in the host; the brucellin skin test should therefore be considered in cases of false positive serological reactions. The test has a very high specificity such that latently-infected animals devoid of measurable antibody, and unvaccinated animals that are positive reactors to this test, should be regarded as infected (Neilsen and Yu, 2010).The results of this test may aid in the interpretation of serological reactions thought to be false positive serologically due to infection with cross-reacting bacteria, especially in brucellosis-free areas (De Massis *et al*., 2005).However, it would be suitable for the low-cost screening of a cattle population to identify those herds which are infected with brucellosis (MacDiarmid and Hellstrom, 1986).

2.18Diagnosis of Coxiellosis (Q Fever)

Diagnosis of Q fever in ruminants, including differentiating it from other abortive diseases, traditionally has been made on the basis of microscopy on clinical samples, coupled with positive serological results (Lang, 1990; OIE, 2010). At present, direct detection and quantification by PCR are only available in reference laboratories (Sakhaee and Khalili, 2010). Due to the fact that clinical diagnosis of coxiellosis is difficult, serological methods

should be considered as methods of choice for the diagnosis of Q fever on herd level (Sidi-Boumedine *et al*., 2010; Roest *et al*., 2013).

2.18.1 Serology in the diagnosis of coxiellosis (Q fever)

Among the various techniques that can be employed, the three most often used methods are: the complement fixation test (CFT), the enzyme-linked immune sorbent assay (ELISA), and the indirect immuno-fluorescence assay (IFA) (Roest *et al*., 2013).Three older serological tests are no longer used in routine diagnosis these are: the micro-agglutination technique, the capillary agglutination test and the indirect haemolysis test (OIE, 2010). Overall, enzyme-linked immunosorbent assays are preferred for practical reasons. Currently, no indirect immunofluorescence assay is commercially available for ruminants. The production of the commercial antigen for complement fixation test will probably be interrupted in the future. Numerous reports showed a weak sensitivity of complement fixation test compared with other methods (Kittelberger *et al.,* 2009).

2.18.1.1 Complement fixation test

Complement fixation test(CFT) detects complement-fixing antibodies present in the serum. In veterinary practice, complement fixation test was the method of reference for serological diagnosis according to OIE (AFSSA, 2004). Complement fixation test usually utilizes phase II antigens only (AFSSA, 2004; OIE, 2010). Complement fixation test is capable of detecting approximately 65% of infected subjects during the second week after initial clinical signs and 90% during the fourth week (OIE, 2005). Complement fixation test is specific but less sensitive and more laborious than indirect

immunofluorescence assay or enzyme-linked immunosorbent assay (Kittelberger *et al*., 2009; Rousset *et al*., 2009). Due to its low sensitivity, Rousset *et al*. (2007b)advised not to use complement fixation test for serological screening in animals.

2.18.1.2 Indirect enzyme-linked immunosorbent assay

The indirect enzyme-linked immunosorbent assay (iELISA) is also a method for the diagnosis of coxiellosis (Q fever). This technique has a high sensitivity and a good specificity, it is easy to perform (Kittelberger *et al*., 2009; Rousset *et al*., 2009). Indirect enzyme-linked immunosorbent assay is preferred to indirect immunofluorescence assay and complement fixation test, particularly for veterinary diagnosis, because it is convenient for large-scale screening and, as it is a reliable technique for demonstrating *C. burnetii* antibody in various animal species (Jaspers *et al*., 1994; OIE, 2010).The enzyme-linked immunosorbent assay kits are available commercially and are frequently coated by phase I and phase II antigens, and these are of two origins: antigens of the American Nine Mile strain of *C. burnetii* isolated from an endogenous tick, and or antigens of a strain originating from infected European domestic ruminants. Enzyme-linked immunosorbent assay tests can be automated which facilitates large scale studies (Rousset *et al*., 2007b).

2.18.1.3Indirect immuno-fluorescence assay

In human medicine, indirect immuno-fluorescence assay (IFA) is commonly considered the reference diagnostic test in Q fever and is the most frequently used worldwide (AFSSA, 2004). It is accurate, highly sensitive and specific (Fournier *et al*., 1996). The

study by Rousset *et al*. (2007b) on goats demonstrated an overall good agreement between immuno-fluorescence assay and enzyme-linked immunosorbent assay results. Immuno-fluorescence assay is capable of detecting the two antigenic variants of *C. burnetii* (phase I and phase II). The two forms of the infection, acute and chronic, have different serological profiles: during acute infection, IgG antibodies are elevated against phase II only whereas during chronic infection, high levels of IgG antibodies to both phase I and II of the bacteria were observed (Tissot-Dupont *et al*., 1994).

2.18.2Bacterial culture

In vitro cell culture of the bacteria is the gold modified for diagnosis of bacterial infections. *C. burnetii* can be cultured efficiently in the yolk sac of chicken embryos but also on diverse cellular specimens, such as human embryonic lung fibroblasts, mosquito cells, green monkey kidney (Vero) cells, tick tissue cultures, (Baca and Paretsky, 1983). However, technically, culture of *C. burnetii* remains a difficult process and sensitivity of this diagnostic method is low. Recently, *C. burnetii*was grown outside a host cell in a cell-free laboratory medium (Omsland *et al*., 2009). This medium has a composition that strictly corresponds to the organism's metabolic requirements in the phagolysosome(*in vivoCoxiell*a is strictly intracellular). Culture is generally avoided because isolation of *C. burnetii* is difficult, hazardous, and time-consuming and requires a biosafety Level 3 laboratory (Field*et al*., 2000; Masala*et al*., 2004). As a result culture is rarely performed, especially in veterinary medicine (Fournier *et al*., 1998; Masala*et al*., 2004). Moreover, epidemiological studies based on bacterial isolation are not practical.

2.18.3 Coloration and direct visualization

Direct visualization of *C. burnetii* on smears or frozen tissue is a method of coxiellosis (Q fever) diagnosis. Smears are taken from the placenta of aborted ruminants, from the fetus stomach content or from other body tissues. *C. burnetii* does not stain reliably with Gram stain, and Giemsa stain is preferentially used (Rousset *et al*., 2007b).The sensitivity and specificityof direct visualization by bacterioscopic examination is poor, due to possible confusion with other pathogens such as*Chlamydophila abortus*,*Brucella*spp., or *Chlamydia* spp. (Guatteo *et al*., 2006).Control positive slides of *C. burnetii*, *Chlamydophila abortus* and *Brucella*must be used for comparison during diagnosis.

2.18.4 Immunohistochemistry

Immunohistochemistry (IHC) has been performed for the diagnosis of chronic cases of Q fever. It can be utilized for detection of *C. burnetii* in tissues fixed in paraffin or acetone smears (Raoult *et al*., 1994). An avidin-biotin-peroxidase complex immunohistochemistry staining method for diagnosis and routine screening of ovine and caprine placental tissues after abortion have been developed (Dilbeck and McElwain, 1994). This technique is rapid and does not necessitate live bacteria or fresh tissues for diagnosis. Furthermore, it renders retrospective studies on stored samples possible. However, immunohistochemistry is not useful for large scale epidemiological studies. No specific antibodies for immunochemistry are commercially available (OIE, 2010)

2.18.5 Polymerase chain reaction

Polymerase chain reaction (PCR) methods have been used successfully to detect *C. burnetii* DNA in cell cultures, biopsy samples, blood samples, arthropods, serum samples and other biological samples. The polymerase chain reaction methods of *C. burnetii* detection are generally performed for the health investigations of ruminant herds or flocks prone to abortions (Ughetto *et al*., 2009; Sidi-Boumedine *et al*., 2010). Polymerase chain reaction offers substantial benefits for the identification of *C. burnetii* compared to other laboratory techniques, especially in the early clinical stages of the illness (Frangoulidis *et al*., 2009). Its sensitivity and specificity are high. However, the usefulness of conventional PCR is limited by its inability to quantify the bacteria present. The development of Real Time Quantitative polymerase chain reaction (RTq PCR) not only renders PCR a rapid diagnostic tool but also provides quantifiable information. RTq PCR can be automated and thus can be used in large scale studies. The qualities of PCR make it very useful for early diagnosis of infection during the period when antibodies are not yet present (Fournier and Raoult, 2003). Several primers are available for diagnosis (Schneeberger *et al*., 2010). A primer originating from the frequently repeated DNA sequence IS1111 (7 to 120 copies per genome) is commonly used and has proven to allow very sensitive testing (Frangoulidis *et al*., 2009; Schneeberger *et al*., 2010). In a study by Schneeberger *et al*. (2010), *C. burnetii* DNA was detected in 98% of seronegative acute Q fever patients and in 90% of anti-phase 2 IgM seropositive patients.

PCR became progressively negative as the serological response developed (Schneeberger *et al*., 2010). Hou *et al*. (2011) noticed a significant association between the absence of IgM antibodies and a positive PCR result. Negative serology was most frequent in Q fever

cases where sampling had been performed within the two weeks of illness. In such cases, the authors recommend routine PCR testing as well as serology. When PCR was included in the diagnostic procedure primarily based on serology, a marked increase of sensitivity was observed (78% versus 29% when serology was used as unique diagnostic method) (Hou *et al*., 2011). Polymerase chain reaction and Real Time Quantitative Polymerase chain reaction are considered methods of choice for the DNA detection in diverse samples (Beaudeau *et al*., 2006). However, PCR and RTq PCR are incapable of distinguishing bacterial DNA from live and/or dead bacteria (Kramer *et al*., 2009). The advantage is that sampled tissues can be frozen, put into formalin or fixed with paraffin (Fournier and Raoult, 2003). The disadvantage is that interpretation of positive results in fresh samples can be difficult (Watanabe and Takahashi, 2008). Polymerase chain reaction kits are gradually becoming available (Arricau-Bouvery and Rodokalis, 2005). Analysis of samples by PCR should be performed on sampling day to minimize the risk of obtaining false-negative results. Indeed, the study by Guatteo *et al*. (2007) reported that only two thirds of milk and one half of vaginal mucus samples that were initially positive on the day of sampling remained positive after storage at -20°C during three days. Samples found to be persistently positive had a significantly higher estimated initial bacterial titre than those which became negative after storage.Currently, PCR has become the tool of choice for Q fever diagnosis (OIE, 2010).

2.18.6 Skin test

A skin test method was proposed to investigate the cellular response and to improve detection of infected cows at herd level (Guatteo *et al*., 2008a). The skin test consists in

an intradermal injection of extremely diluted inactivated vaccine (Coxevac®, CEVA-Santé animale, France). The diluted vaccine induces an antigenic reaction. If the animal has previously been infected by Q fever a nodule of variable size will appear at the site of injection. This test could easily be applied by veterinarians practicing in the rural areas.

2.19Treatment of Brucellosis

Treatment of brucellosis in animals is rarely recommended among domestic food animals. Treatment is not an option given disease eradication goals.Thus, infected animals are culled (Glynn and Lynn, 2008). The administration of oxytetracycline concurrently with vaccination may reduce the antibody response (Radostits *et al*., 1994).Treatment of dogs with *B canis* infections is also not recommended, and no identified regimen has been established (Glynn and Lynn, 2008). Oral administration of enrofloxacin (5 mg/kg, PO, q 12 h for 30 days) and additional regimens including various combinations of orally or parenterally administered gentamicin, ciprofloxacin, and doxycycline is recommended in breeding facilities where *B canis* has become established (Wanke *et al*., 2006; Glynn and Lynn, 2008) Single antimicrobial regimens have not been proven effective. Disadvantages of treatment include the expense of the antimicrobials, the lengthy treatment period with potential for multiple required courses, declining owner compliance, uncertain results, and ongoing public health risks (Hollett, 2006; Glynn and Lynn, 2008)

In human, the essential element in the treatment of all forms of human brucellosis is the administration of effective antibiotics for an adequate length of time. Management of brucellosis in humans relies on adherence to the following criteria:Using an antibiotic that

has the ability to act intracellularly and in acidic media, combination therapy antimicrobials for a prolonged duration depending to the system involved (Alshaalan *et al*., 2014).There is a limited number of antibiotics that can be used for the treatment of *Brucella*infections including doxycycline, rifampicin, trimethoprim sulfamethoxazole, streptomycin, gentamicin and ciprofloxacin (Alshaalan *et al*., 2014).A common combination for children yielding successful results is as follows:Rifampicin and Trimethoprim/sulfamethoxazole (TMP/SMX) for children below 8 years of age.Doxycycline and TMP/SMXor rifampicin for children older than 8 years of age. This combination has been shown to have the highest success rate and should be used in children above 8 years to avoid the staining of the teeth in younger children.In cases of pregnancy, positive results have been achieved with the use of TMP/SMZ or rifampicin. Caution is needed with the use of rifampicin as *Brucella*strains seem to acquire resistance to this antibiotic in a one-step fashion, and this may then offer cross-resistance to TMP/SMZ (Sandalakis *et al*., 2012).In serious infections, such as neurobrucellosis and endocarditis, three to five drugs need to be used for a longer period of time, usually for three to 12 months, gentamicin for 7 days or streptomycin for 14 days can be used for patients requiring hospitalization (Alshaalan *et al*., 2014). The combination of doxycycline with streptomycin is currently the best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis (Seleem *et al*., 2009).

2.20Treatment of (Coxiellosis) Q Fever

In animals, more research is needed to determine the effectiveness of treating entire herds or individual animals to prevent or control shedding of *C. burnetii* and to prevent adverse pregnancy events associated with coxiellosis. Use of tetracycline in feed during pregnancy cannot be recommended as a herd-level control measure for *C. burnetii*due to low bioavailability of the drug with oral administration (Reinbold *et al*., 2010). Based upon current data, antibiotic usage in the absence of ongoing abortions is not warranted. If abortion is ongoing, parenteral treatment of individual animals with long-acting oxytetracycline at 20 mg/kg given 20 days apart is helpful in late gestation to prevent adverse pregnancy. Parenteral treatment of individual animals does not appear to reduce shedding of the organism in birth fluids, though it may prevent abortions (Stuen and Longbottom, 2011; Astobiza *et al*., 2013).

In humans, doxycycline has the highest therapeutic efficacy against *C. burnetii* and is the treatment of choice for acute Q fever in adults, children over 8 years, and in children of all ages with severe illness (CDC, 2011a; Anderson *et al*., 2013).Oral doxycycline should be given for 14 to 21 days at a dosage of 100 mg twice daily to adults and 2.2 mg/kg body weight twice daily to children under 45 kg (100 lbs). When tetracyclines are contraindicated for children under 8 years of age, pregnant women, and those allergic to it, trimethoprim/sulfamethoxazole, cephalosporins, fluoroquinolones or macrolides may be used (Anderson *et al*., 2013). The benefits of using doxycycline outweigh the potential risk of staining of permanent teeth in severely ill or hospitalized children under 8 years with acute Q fever (Tissot-Dupont and Raoult, 2007; CDC, 2011a). Treatment should be given within the first 3 days of illness for maximum efficacy and should not be delayed pending

the results of laboratory tests or withheld because of the initial negative laboratory result in the first week of illness (Anderson *et al*., 2013). Treatment with IFNγ has proven effective in certain cases (Morisawa *et al*., 2001; Maltezou and Raoult, 2002).

In chronic Q fever, patients should be referred to an infectious disease specialist for management.These patients require long-term antibiotic therapy using a combination of doxycycline and hydroxychloroquine, periodic diagnostics, and long-term monitoring (Fennolar *et al*., 2001).The recommended dosage for adults is doxycycline 100 mg every 12 hours and hydroxychloroquine 200 mg every 8 hours for duration of 18 to 36 months. The dosage of hydroxychloroquine is adjusted three months after initiation of treatment to obtain a plasmatic level of 1 ± 0.2 mg/l (Maurin *et al*., 1992). An ophthalmologic follow-up is recommended every 6 months to detect accumulation of hydroxychloroquine in the retina, during the treatment. Rolain *et al*. (2003) advised a plasmatic concentration of 5 mg/l of doxycycline for effective treatment. Bacterial resistance to doxycycline was observed in a patient with endocarditis (Rolain *et al*., 2005). Thus, serological testing on a regular basis to evaluate response to treatment is high. In infected pregnant women, administration of long-term cotrimoxazole (320 mg trimethoprim and 1600 mg sulfamethoxazole for at least 5 weeks), a bacteriostatic antibiotic, is recommended (Carcopino *et al*., 2007). After delivery, a daily treatment with doxycycline 100 mg every 12 hours and hydroxychloroquine 200 mg for minimum of 1 year is advised for chronically infected women. Long-term cotrimoxazole therapy significantly reduced the number of obstetric complications, the frequency of placentitis, and the development of a chronic serological profile (Carcopino *et al*., 2007).

2.21 Prevention, Control and Eradication of Brucellosis

2.21.1 Prevention of brucellosis

Brucellosis may be prevented via vaccination of domestic livestock, serologic testing, quarantine of herds, and slaughter of infected animals. Vaccination is effective for cattle (*B. abortus* S19 and RB51), sheep, and goats (*B. melitensis* Rev-1) but requires a sustained vaccination programme over several year.These vaccines are recommended by the Office International des Epizooties (OIE) for use in the control of brucellosis in livestock (Garin-Bastuji and Blasco, 2008). These are live attenuated vaccines that are also known to cause disease in humans during the process of preparation or by accidental inoculation and that inadvertent exposure requires careful follow-up (Godfroid *et al*, 2005; WHO, 2006). No vaccine is available for protecting swine against *B. suis*(Young, 1995).It is nearly always more economical and practical to prevent diseases than to attempt to control or eliminate them (WHO, 2006). The measures of prevention include: (i) careful selection of replacement animals. These, whether purchased or produced from existing stock, should originate from *Brucella*-free herds or flocks, (ii) isolation of purchased replacements for at least 30 days. In addition a serological test prior to commingling is necessary; (iii) prevention of contacts and commingling with herds or flocks of unknown status or those with brucellosis; (iv) herds and flocks should be included in surveillance measures such as periodic milk ring tests in cattle (at least four times per year), and testing of slaughtered animals with simple screening serological procedures such as the RBPT; v) proper disposal (burial or burning) of placentas and non-viable foetuses. Disinfection of contaminated areas should be performed thoroughly

(WHO, 2006). Prevention of animal brucellosis depends on the establishment of an efficient national surveillance programme and the presence of an active reporting system (Anon, 1993).

2.21.1.1 Vaccination in brucellosis

There is general agreement that the most successful method for prevention and control of brucellosis in animals is through vaccination (WHO, 2006). Up to date, the most commonly used animal vaccines are: (1) *B. abortus* S19, (2) *B. melitensis* Rev.1 and (3) *B. abortus* RB51 (Hotez *et al*., 2012).Scientists are now considering recombinant strains, DNA vaccines and submit vaccines as well as inducing new attenuated strains from isolations. Control of brucellosis in humans relies on control of the disease in animals. While several animal vaccines are available, there is no effective and safe vaccine for prevention of brucellosis in humans (He and Xiang, 2010; Oliveira *et al*., 2011). The use of vaccine provides increased resistance against field strain infection and reduces incidence of abortions and disease (Schurig *et al*., 2002).

2.21.1.1.1 *Brucellaabortus strain 19 (S19) vaccine*

Vaccination with S19 vaccine for *B. abortus* provides long life immunity against field strain of *Brucella*following natural infection in cattle, it is the most commonly used vaccine for the prevention of brucellosis in cattle (Nicoletti, 1990). The strain was isolated in 1923 from milk of Jersey cow, which was left at room temperature in the laboratory for almost a year and was found to have become attenuated. This strain was found to be capable of inducing protective immunity in cattle though the effectiveness

may vary depending on the age at vaccination time, route and dose of administration and also the prevalence of the disease in vaccinated herds (Nicoletti, 1990). This vaccine is an attenuated organism of smooth morphology which does not normally grow in the presence of erythritol. The strain is of low virulence such that vaccination with 40–120 x 10^9 CFU (classical dose)of living *B. abortus*gives good protection. Though, it has some disadvantages such as:(i) induction of persistent antibody response due to the presence of LPS with an O–chain on S19 that cannot be differentiated serologically from that due to natural infection and its excretion in milk which can serve as a source of infection to man, (ii) induction of abortion in pregnant cows (Plommet, 1991).Vaccination enhances the resistance of the animals in infected herds, and reduced doses of *B. abortus*;S19 live vaccine is given to adults with systematic administration to heifers 4–12 months old (Chukwu, 1987).

2.21.1.1.2 Brucellaabortus RB51 vaccine

The vaccines strains RB51 or 45/20 provides excellent immunity and recommended for the control of brucellosis in cattle and gives lifelong immunity (Stevens *et al*.,1995; OIE, 2004). The ″R″ refers to rough, ″B″ refers to *Brucella*, while ″51″ refers to an internal laboratory nomenclature which was used when the vaccine was derived. The vaccine was selected by growth of *B. abortus* strain 2308 in the presence of rifampicin (Schurig *et al*., 1991). RB51 strain is devoid of the O–chain, its roughness being very stable after multiple passages in-*vitro* and in-*vivo* through various animals' species (Colby, 1997). This vaccine strain has been reported effective as S19 vaccine in protecting against *B. abortus* in cattle without inducing anti–O chain antibodies as detected serologically

(Palmer *et al*., 1997). This vaccine can also be effective for the prophylaxis against *B. melitensis* infection in goats (Suarez *et al.*, 1998). One of the distinct advantages of this RB51 vaccine is the fact that vaccination does not result in "false positive" test results or "false positive reactors." Vaccination of animals usually results in elimination of clinical disease and the reduction in numbers of organisms excreted by animals which become infected (WHO, 2006).

2.21.1.1.3BrucellamelitensisRev.1 vaccine

Brucellamelitensis Rev.1 vaccine is used in sheep for the immunization against infection by *B. melitensis*. The live attenuated *B. melitensis*Rev.1 strain is presently recognized as the best vaccine available for the prophylaxis against brucellosis in small ruminants (Blasco, 1997). Immunization is the most appropriate first step in countries where *B. melitensis* is endemic, either as preliminary approach to eradication or as sole eradication measures. Immunization is usually restricted to lambs and kids at the aged of 3–8 months, a reduced dose (10^3–10^6 CFU) has been used subcutaneously in the field trials and reported as an effective and safe method of controlling brucellosis in both pregnant and non-pregnant small ruminant (Delgado *et al*., 1995; Uysal, 1995).

2.21.2 Control of brucellosis

Eradication of brucellosis by test-and-slaughter is unfeasible in developing countries because of limited resources to compensate farmers whose animals are slaughtered during such screening programmes (Godfroid *et al*., 2011). However, a mass vaccination strategy (avoiding pregnant animals in mid-gestation) may be applied to control

brucellosis in developing countries. The aim of an animal control programme is to reduce the impact of a disease on human health and the economic consequences (WHO, 2006).The main components of brucellosis control and eradication strategies include: (1) improving quality of the veterinary services and establishment of diagnostic laboratories that adopt international standard, (2) adoption of appropriate control and eradication programmes (Donev, 2010), (3) elimination of the pathogen at its animal source by hygiene measures such as careful herd management and health education, vaccination of animals in addition to testing and slaughtering of infected animals (Donev, 2010), (4) active and continuous surveillance and identification of animals and herds at high risk (Russo *et al*., 2009; Donev, 2010; Hotez *et al*., 2012), (5) vaccination of young female animals is the most practical, economic and effective control program as mass vaccination has been adopted by many countries, (6) testing and slaughtering of seropositive adult animals (Donev, 2010) (7) health education and application of strict hygiene measures to personnel in direct contact with animals, (8) prompt diagnosis and appropriate treatment of infected humans to prevent chronic disease and further complications (Donev, 2010), (9) coordination and cooperation between public health officials, veterinary officers and various governmental sectors as expanded regional and even international cooperation is needed for optimal global and animal control, with restriction of animal transportation across open borders (Russo *et al*., 2009; Donev, 2010). The successful elimination of brucellosis in some countries is a consequence of well-conducted comprehensive control and eradication programmes, with screening and confirmatory tests in addition to prompt removal of reactors, in addition to bio security and vaccination (Songer and Post, 2005). Control and prevention schemes require

effective collaboration between all sections of the community; control programmes must be properly planned, coordinated and resourced(WHO, 2006).

In human the control of brucellosis depends on minimizing/controlling disease burden in animals and reducing animal to human transmission (Zinsstag *et al*., 2007; Rubach *et al*., 2013). The consumption of non-pasteurized dairy products from *Brucella*-infected animals is the most frequent route of human infection in general. So, pasteurization of milk will reduce *Brucella*transmission to humans (Rubach *et al*., 2013). The risk of infection among occupationally exposed group of people can be reduced through personal hygiene measures and adoption of safe working practices, including use of protective clothing, disinfection of protective clothing, and disinfection of potentially infected utensils and premises. To avoid aerosol transmission, eating and smoking must be forbidden in the abattoirs/heavily contaminated environments while handling animals and inhalation of dust or aerosols derived from dried excreta or tissues released at abortion, parturition or slaughter should be prevented by the use of suitable respirators (Islam *et al*., 2013). However, these measures may be unfeasible in low and middle-income countries due to resource limitations and nomadic animal husbandry practices (Corbel, 2006). In this situation, steps should be taken to reduce the impact of the disease by educating the population regarding the disease and its risk factors (Corbel, 2006; Islam *et al*., 2013).

2.21.2.1 Test and isolation/slaughter

There are no pathognomonic signs of brucellosis in animals at individual level; the occurrence of abortion storms in naive herds/flocks is usually a strong indicator of

infection. Therefore, serological (and sometimes allergic) tests are the usual method of identifying possible infected animals. Bacteriological procedures are useful for confirming test results and for epidemiological studies (WHO, 2006). The isolation of test-positive animals is essential, especially during and after parturition (WHO, 2006). Serological tests are the usual method of identifying possible infected animals (Nicoletti, 1993). Repeated herd or flock tests are necessary to further reduce the incidence of brucellosis and to confirm elimination (WHO, 2006).

2.21.2.2 Hygiene

The goal in the application of hygiene methods to the control of brucellosis is reduction of exposure of susceptible animals to those that are infected, or to their discharges and tissues (WHO, 2006). Factors such as the methods of animal husbandry (e.g. commingling of herds or flocks), patterns of commerce, prevalence of clinical signs, type of facilities, and degree of dedication of the owners of animals, will alsodetermine success (WHO, 2006).

Since most brucellosis contamination occurs following an abortion or delivery by an infected female, and then the long inter-calving might contribute to a lower incidence of brucellosis in nomadic or extensively raised camels (FAO, 2003). Those handling foetal membranes, fluids, placentas, and aborted foetuses should use appropriate hygienic practices; hand gloves, masks, protective eye wear should be worn when handling infected animals; these are classical procedure in disease control (Songer and Post, 2005).

2.21.2.3 Control of animal movements, animal/herd identification

The aim of surveillance and control programs is the reduction of infection in the animal populations to reduce the effect of the disease on animal health and production, thus minimizing its impact on human health (Melzer *et al.*, 2007). Animals should be individually identified by brand, tattoo or ear tag. Unauthorized sale or movement of animals from an infected area to other areas should be forbidden (WHO, 2006). Movement of infected animals must be prohibited and import permissions should be given only to certified brucellosis-free farms or areas (OIE, 2009). The control of animal movements between herds, and especially from farms or regions with a high prevalence of disease is a basic principle of animal disease control and is a necessary and highly effective measure (EC, 2009). The application of pre-movement testing reduces the risk of spread of brucellosis between herds/holdings or within the herd and provides additional assurance for the purchaser in this regard. It also serves indirectly as an additional assessment of the brucellosis status of the herd and region of origin (EC, 2009). This particularly applies to the movement of animals into and between herds/holdings in areas or regions of high prevalence. Likewise, the movement of animals from unrestricted herds in these regions to herds outside these regions poses a relatively high risk of spread (EC, 2009).Test and slaughter is also unlikely to be successful in cattle if the remainder of the herd is unvaccinated, especially in large populations (WHO, 2006).

2.21.3 Eradication of brucellosis

Eradication means the elimination of a pathogenic agent from a country or a zone (WHO, 2006). A highly organized effort is needed to reach eradication in either a territory and in

a population (WHO, 2006). Eradication is conceptually very different from control, no matter how well planned and implemented the control programme is (WHO, 2006). Despite tremendous efforts and financial investments, many countries have yet to eradicate this disease (Bikas *et al*., 2003). On a long-term basis, eradication programmes in general are more economically advantageous compared to control programmes (WHO, 2006). Eradication of brucellosis in small ruminants can be achieved mainly by depopulation of infected flocks (Minas, 2006). In case that *B. melitensis* infection is endemic or its prevalence is high in an area, the first step for its eradication must be the control of the disease by immunization of the susceptible animals (Minas, 2006). In fact, an eradication programme involves the mobilization of an amount of resources (financial and human) that may not be available or whose returns for the investment may require a time span longer than any decision-making authority can afford (WHO, 2006). Some of the developed countries have successfully eradicated animal brucellosis by combined vaccination and testand- slaughter programs (Pappas *et al*., 2006), along with effective disease surveillance and animal movement control (Godfroid *et al*., 2013).

2.22Public Health Education

The World Health Organization (WHO) recommended that public health education focusing on occupational exposure and consumption of raw milk and other dairy products should be an important part of any brucellosis control programme (Corbel, 2006; Musallam *et al.,* 2015). From a public health point of view, the main sources of brucellosis are either food-related or contact with infected animals either in an occupational or recreational contact (WHO, 2006). Person-to-person transmission is not a significant

problem except through blood or organ transfer which should be subject to proper control (WHO, 2006). Active cooperation between health services and veterinary services should be promoted (Meky *et al*., 2007). Food safety is one of the principal pillars on which protection of human health resides (WHO, 2006). Human brucellosis has serious public health consequences in endemic areas (Corbel, 2006). Public health education should be included among the essential activities to be performed within the framework of brucellosis control programmes or even as an independent activity. The key objectives are to enable individuals to define their own problems and needs; to understand what can be done to deal with these problems using their own resources and external support and to decide on appropriate action (WHO, 2006).

2.23 Economic Importance of Brucellosis

Brucellosis is one of the most frequent bacterial zoonotic diseases in low earning countries, where control programmes have not been established. It is a numerous burden disease with economic impacts attributable to human being, livestock and wildlife diseases. The epidemiology and economic impact of brucellosis vary by geographical location and livestock system (McDermott *et al*., 2013). It is widely distributed in low-income countries of the world with high endemicity in Mediterranean, Middle East, Latin America, South Asia and sub-Saharan Africa. It is a major cause of direct economic loss and impediment to trade and exportation (Lopez-Goni *et al*., 2008). Economic losses through abortion, deaths of young animals due to stillbirths, decreased milk production in dairy cows, increased cost of animals'replacement and lowered sale value of infected animals, temporary infertility due to metritis and weak offspring and sterility are

consequences of the brucellosis (Radostits *et al*., 2007). The reduced human productivity can hardly be measured in medical care (Nicoletti, 1982). There are also indirect economic losses due to enforcement of control and eradication measures, these include cost of vaccines and vaccination, logistics and personnel cost. There are also losses due to compensation paid for slaughtered animals infected with brucellosis (FAO, 2004).Although estimates of the costs associated with brucellosis infections remain limited to specific countries, all data suggest that worldwide economic losses due to brucellosis are extensive not only in animal production (reduced milk, abortion and delayed conception), but also in public health (cost of treatment and productivity loss). The economic losses due to brucellosis in Nigerian were estimated at US$575,605 per year or US$3.16 per bovine (Ajogi *et al*., 1998), also Ajogi and Akinwumi (2001) estimated losses in Wase and Wawa-Zange cattle reserves at ₦ 9.38 million and ₦ 3.38 million per annum respectively. Recent estimates of losses in meat and milk production as a result of brucellosis are $800 million annually in the USA (Richey and Harrell, 2008). Although brucellosis eradication programs can be very expensive, they are estimated to save $7 for each $1 spent on eradication. The USA national brucellosis eradication program, while costing $3.5 billion between 1934 and 1997, the cost of reduced milk production and abortion in 1952 alone was $400 million (Acha and Szyfres, 2003; Sriranganathan *et al*., 2009).

2.24 Economic Importance of Coxiellosis (Q Fever)

Coxiellosis has an economic importance where there is close and frequent association between man and animals, such as in rural areas, where agriculture and animal husbandry

are the main sources of income. Moreover, *C. burnetii* infection in animals reared for consumption has been associated with abortions, especially in sheep and goats, and infertility in cattle and have an economic impact on production (Ho *et al.*, 1995). In an economic evaluation of the National Q-fever Management Programme in Australia, increased vaccine uptake from 65 to 100% among meat industry workers, and from zero to 20% in agricultural industry workers resulted in an incremental cost per life year gained of $ 20,002 and $ 24,950 respectively, and a cost per quality adjusted life year (QALY) of $ 6,294 and $ 7,984. This included some indirect costs in the form of work-cover payments resulting in cost savings for both the industry groups (Kermode *et al.*, 2003).

2.25Public Health Importance of Brucellosis

Brucellosis is an infectious disease of domestic and wild animals with serious zoonotic implication in humans (Acha and Szyfres, 2003). It is the second most important zoonotic disease in the world after Rabies (FAO, 2003), and has been reported in most of the developing countries. The disease is an important public health problem in many parts of the world (Acha and Szyfres, 2003; Cadmus *et al.*, 2010). Four out of the ten known *Brucella*species can infect humans and the most pathogenic and invasive species for humans is *B. melitensis* followed in descending order by *B. suis*, *B. abortus*and *B. canis* (Seleem *et al.*, 2010). Livestock health affects human health because brucellosis can be transmitted to humans. Individuals most at risk include farmers and ranchers, veterinarians, artificial inseminators, meat inspectors, slaughterhouse workers, laboratory technicians, and sportsmen and hunters (Godfroid *et al.*, 2005). The Centre for Disease

Control and Prevention lists *Brucella*as a category B bioterrorism agent, the Center also classifies *B. abortus, B. melitensis* and *B. suis* as "agents of mass destruction" (CDC, 2002; Elzer, 2002).

2.26Public Health Importance of Coxiellosis (Q Fever)

Q fever is an important occupational hazard, especially for those in close contact with farm animals such as veterinarians, dairymen, abattoir workers and animal transport workers, as well as laboratory personnel who handle infected animals or cultures of the pathogen. *Coxiella* organisms could potentially be used in biological weapons (Spencer and Wilcox, 1993). In most countries, Q fever activity is still significantly associated with livestock and the meat industry. The incidence of chronic Q fever is unknown in most part of the world, and only five deaths attributed to Q fever were reported between 1982 and 1994 (Maurin and Raoult, 1999). The seroprevalence of Q fever has been found to be three times higher in HIV-positive patients than in blood donors (Raoult *et al*., 1993). In most European countries, California (USA), and Australia (Garner *et al*., 1997), the disease most often affects the active population between 30 to 60 years of age and more frequently in men. A sex ratio of 1:1 was reported in Nova Scotia, Canada (Marrie and Pollak, 1995). Mammals also shed *C. burnetii* in milk and consumption of raw milk and milk products could be a source of infection to humans and newborn animals (Gikas *et al*., 1994). Humans may acquire Q fever from infected domestic poultry by consumption of raw eggs or inhalation of infected fomites. Anti *C. burnetii* antibodies have been found in snakes and tortoises in India (Yadav and Sethi, 1980), but *C. burnetii* has not been isolated from these animals. *C. burnetii* may persist without symptoms in humans throughout life. An occupational risk of

zoonotic infections was reported in Dutch forestry workers and muskrat catchers (Moll-van-Charante *et al*., 1998). However, conditions like pregnancy, a cardiac valvular abnormality, a vascular aneurysm or prosthesis, hemodialysis and immunodeficiency, including AIDS may promote reactivation of dormant *C. burnetii* (Maurin and Raoult, 1999).

2.27Prevention and Control of Coxiellosis (Q Fever)

Sanitary and prophylactic measures should be applied at herd and human level, in order to limit disease transmission, in the case of a Q fever outbreak. Human and animal infections must be diagnosed early and treated immediately to prevent the development of chronic infections and secondary complications. Q fever being a zoonosis, prophylaxis at herd level is fundamental (Porter *et al.*, 2011).

2.27.1 Prevention of coxiellosis (Q fever)

For most effective prevention, the Q fever microbe should be eliminated from animals. Eradications programs, however, are not yet available because Q fever spreads so effectively among animals. So far, research on vaccination programs for animals has not had practical success (Böttcher *et al*., 2011). Although human and animal vaccines for *C. burnetii* have been developed, none are commercially available for use in Nigeria. Also, lack of knowledge on shedding patterns among ruminants makes definitive determination of Q fever shedding status difficult,therefore, prevention efforts must focus on minimizing contact with animals that may be shedding *C. burnetii* in body secretions and excretions (Horigan *et al.*, 2011). Although it may not be practical or possible to

eliminate the risk of Q fever in a typical farm setting, the risk for spread can be decreased by 1) proper sanitation–good hygiene, especially when working with parturient animals; 2) segregated kidding/lambing areas; 3) removal of risk material from birthing areas (birthing products/fluids, contaminated bedding, manure); 4) good manure management; 5) control of ticks on livestock; and 6) restriction of moving peri-parturient animals (close to birthing or giving birth within the past two weeks) off the farm (OIE, 2010; Horigan *et al.*, 2011).

At human level, prevention of exposure to animals or wearing gloves and masks during manipulation of animals or their litter is advised (Whitney *et al*., 2009; Porter *et al*., 2011). Post exposure prophylaxis guidelines for the general population have been established in the USA (Moodie *et al*., 2008). Identification of an antibiotic capable of severely diminishing or completely stopping shedding would radically modify the management of *Coxiella* infection at a herd level. Pasteurization of all milk products should be performed if ingestion is proven an effective route of infection and after determination of the minimal infecting dose of bacteria (Porter *et al*., 2011).

2.27.2 Control of coxiellosis (Q fever)

Measures for the control of coxiellosis (Q fever) in animal should be implemented, particularly for domestic ruminants. Only a combination of measures is expected to be effective (OIE, 2010). Long-term options include preventive vaccination, manure management, changes to farm characteristics, wool shearing management, a segregated kidding area, removal of risk material, control of other animal reservoirs and tick control. The culling of pregnant animals, a temporary breeding ban, stamping out, identifying and

culling shedders and controlling animal movements are considered as suitable options in the case of human outbreaks (Kersh *et al*., 2010). Adding 0.5% of Calcium Cyanamid to contaminated dung is an effective disinfectant (Arricau-Bouvery *et al*., 2001). High risk material (aborted fetuses, contaminated bedding and placenta) should be buried deep with lime or incinerated. Contaminatedmaterials should immediately dealt with using hypochlorite 500 ppm, phenol based solutions, or 5% peroxide (Porter *et al*., 2011). In the United Kingdom, Health Protection Agency guidelines suggest the use of 2% formaldehyde, 1% Lysol, 5% hydrogen peroxide, 70% ethanol, or 5% chloroform for decontamination of surfaces (Porter *et al*.,2011). Animal infection is thought to occur primarily through exposure to contaminated environments. Given the ubiquitous nature of *C. burnetii* and its persistence in the environment, complete eradication of the bacteria from an infected farm would be nearly impossible (NASPHV, 2013). Nevertheless, transmission can be reduced with good hygiene and other management practices that reduceenvironmental load, such as immediately removing and disposing of aborted fetuses, dead newborns, and placentas. To control spread of coxiellosis between premises, producers should refrain from moving or selling animals, particularly periparturient animals, while abortions are ongoing (FDA, 2013;NASPHV, 2013).

2.27.2.1 Vaccination in coxiellosis

Vaccines are not available for domesticated ruminants in Nigeria. Vaccines may prevent infections in calves, decrease shedding of organisms and improve fertility in infected

animals. Vaccines does not completely prevent infection, but reduces the rates of abortion, which limit the spread of the organism. In Slovakia a wide programme of cattle vaccination has significantly reduced the local incidence of coxiellosis (Kovacova and Kazar, 2002), they do not eliminate shedding of the organism (Porter *et al*., 2011). In animals the vaccines that are considered the most effective composed of inactivated whole phase 1 bacteria (OIE, 2005). Indeed, in goats, the inactivated phase I vaccine (Coxevac®, CEVA-Santé animale, Libourne, France) protects efficiently against abortion and has been shown to prevent bacterial shedding in vaginal mucus, feces and particularly in milk. However, vaccination proved more effective in nulliparous animals than in parous animals. Furthermore, vaccination did not clear infection in previously infected goats (Hogerwerf *et al*., 2011; Porter *et al*., 2011) and cattle (Guatteo *et al.*, 2008b; Porter *et al*., 2011). The major problem associated to vaccination with inactivated phase 1 vaccine is the impossibility of distinguishing vaccinated and naturally infected animals using ELISA methods (Porter *et al*., 2011). At herd level, the effectiveness of a vaccination program could be evaluated by monitoring bacterial shedding in BTM (Porter *et al*., 2011).In humans, vaccination against Q fever could be effective in certain areas, but only possible for individuals that have not previously been in contact with *Coxiella burnetii* to prevent serious reactions (OIE, 2005; Rousset *et al*., 2007a).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Maigana and Birnin Gwari Agro-ecological zones of Kaduna State. Kaduna State is located in the centre of Northern Nigeria, specifically North West Geo-political Zone of Nigeria (KDSG, 2008). Kaduna State occupies a land area of about 48,473.2 square kilometers and has Kaduna as its capital (KDSG, 2008). The State is located between longitude 6^{o} 20' and 9^{o} E and Latitude 9^{o} 10' and 11^{o} 30N and it is located at an elevation of 704 meters above sea level (KDSG, 2008). Kaduna State has 23 Local Government Areas, and 47 Development Areas (Fig 3.1). The State shares boundaries with Niger State to the West, Zamfara, Katsina and Kano States to the North, Bauchi and Plateau States to the East and Federal Capital Territory (FCT) Abuja and Nassarawa State to the South. The State has distinct wet and dry seasons and is within the Northern Guinea Savannah zone and part of the Sudan Savannah zone of Nigeria with daily temperatures ranging from 36.0°C and a relative humidity of 12–72% along with the mean annual rainfall of 1,524mm. Kaduna State has a population of 6,113,503 persons,comprising 3,090,438 males and 3,023,065 females (NPC, 2006) and an estimated cattle population of 3.1 million (KDSG, 2008). Majority of the population consists of small scale farmers. Thus, agriculture is the major occupation of the communities in the State with about 80% of the people engaged actively in livestock and crop farming (KDSG, 2008).

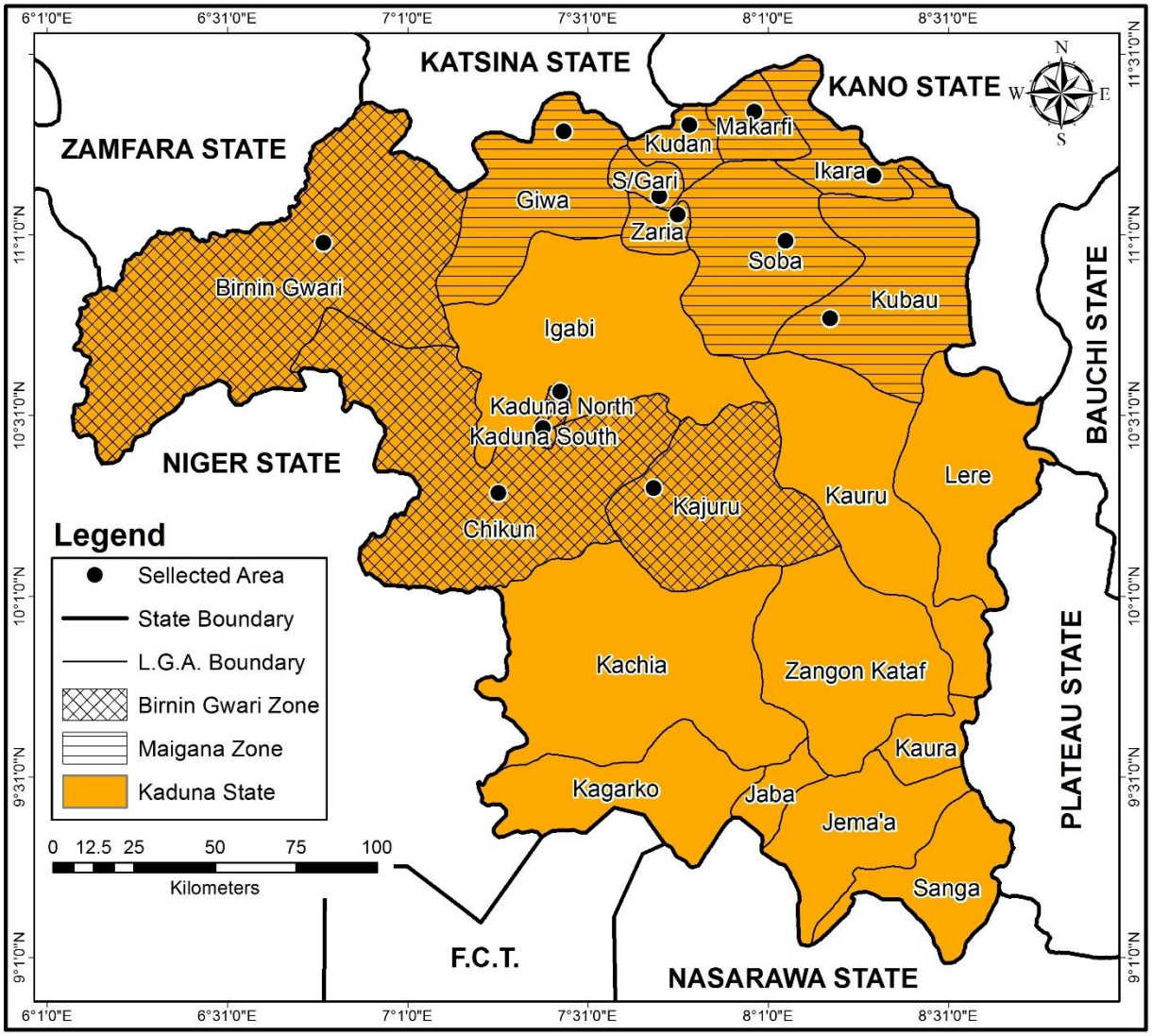


Figure 3.1 Map of Kaduna Stateshowing sampling area

Source: Adapted and modified from the administrative map of Kaduna State

3.2 Study Design

A cross-sectional study consisting of a questionnaire-basedsurvey and two serological surveys for detecting *Brucella* and *Coxiella burnetii* infectionswas carried out between May, 2015 and March, 2016 in Maigana and Birnin Gwari Agro-ecological zones of Kaduna State (Fig 3.1). The study population consisted of herds of cattle and flocks of sheep and goats.

3.3 Sampling

Stratified random sampling technique was used to select herds and flocks from each zone with the wards forming the first strata and herds the second strata. In each stratum, simple random sampling was used proportionate to size.All the Local Government Areas (L.G.As) from Birnin Gwari and all the L.G.Asfrom Maigana agro-ecological zones were sampled. Similarly, within each selected LGA, three wards were randomly selectedfrom which three herds were also selected randomly.Target populations were cattle, sheep and goats from settled and semi-settled Fulani herds/flocks. A list compiled together with the Miyetti Allah Cattle Breeders Association was used as sampling frame for the selection of herds and flocks. A Herd in this study is considered to bea group of aminimum of 5 heads of cattle kept together for a specific purpose, while a flock was defined asa group of a minimum of 10 heads of sheep or goatskept together for a specific purpose.

3.3.1 Inclusion criteria

i. Only settled and semi-settled herds/flocks raised extensively were included.

ii. Herds that hada minimum of5 cattle and flock that had a minimum of 10 sheep and or goats only were included.

iii. Cattle herds/flocks within Birnin Gwari and Maigana agro-ecological zones only were included.

iv. Only herds/flocks whose owners consented were studied.

v. Only animals that could be restrained by the owners were sampled.

vi. Only cattle older than 12 months, sheep and goats that were older than 6 months were included in the study.

3.3.2 Sample size determination

The sample size for this study was determined using the following formula, with an expected disease prevalence of 20.6% for brucellosis (Mai *et al*., 2012), and 14.5% for Q fever (Tukur *et al*., 2014), accepted absolute error of 5%, and a confidence level of 95% by using a simple random sampling design (Thrusfield, 2005):

$$n = \frac{1.96^2 P_{exp} \ (1 - P_{exp})}{d^2}$$

$$n = \frac{1.96^2 \text{ x } 0.206 \ (1 - 0.206)}{(0.05)^2}$$

n = 251.23

$$n = \frac{1.96^2 \text{ x } 0.145 \ (1 - 0.145)}{(0.05)^2}$$

Prevalence of 20.6% = for brucellosis

n = 190.50

Prevalence of 14.5% for Q fever

d = 0.05

where n = required sample size, P_{exp} = expected prevalence, d = desired absolute precision.

According to the formula, a minimum of 251 samples was required for brucellosis and 190 samples for Q fever. In this study, 400 each of cattle, goats and sheep were randomly selected from the herds/flocks.

3.4Questionnaire Design and Data Collection

A semi-structured questionnaire was developed and pretested for use (Appendix I) to collect information about each sampling unit (herd or flock). One questionnaire was administeredto each sampling unit. The questionnaire was designed to comprise open ended questions to facilitate ease of data processing, minimize variation, and improve precision of responses (Thrusfield, 2005). The questionnaire was administered by the researcher or trained assistants using the native language, Hausa and Fulfulde dialects. Astandardized translation template was developed together by the researcher and his assistants in order to avoid variation in meanings of terms and administrator variations.Important herd and animal level data recorded included herd/flock location, herd/flock size, herd medical history, availability of Veterinary Services, herd owner education level and type of labour employed. Other information sought included presence of other animals on the farm (camel, horses, donkeys and dogs), history of abortion and vaccination and disposal of aborted foetus and afterbirth (Appendix I).

3.5Sample Collection and Handling

Five milliliters of blood sample was collected aseptically from the jugular vein of each animaland divided into two equal parts and placedinto clean EDTA and plain vacutainer tubes (Becton Dickson, UK) respectively. Each sample was labelled using codes describing the specific animal and herd/flock, with unique identification number and information aboutspecies, sex, age, breeds, and location were recorded in a form (Appendix II) for data analysis, and missing information was recorded as "unknown". The ages of the animals sampled were verified using the cornual and dental markers (Pace and Wakeman, 2003). Breed of the animal was recorded based on physical characters of each animal (Bourn *et al*., 1994; Felius *et al*., 2011).The samples were transported on ice inside coolers to the laboratory. The plain vacutainer tubescontaining the clotted blood were set tilted on a table at a room temperature to allow clotting. The clotted blood in the tubes was centrifuged (at 3000 G for 5 min) to obtain clear serum. The harvested sera were stored at -20^{o} C pending testing for the evidence of infection with *Brucella* and *C. burnetii*. The whole blood in EDTA bottles was stored at 4 ^{o}C for DNA extraction for PCR.

3.6Serological Tests

Serological tests were conducted using Rose Bengal Plate Test (RBPT) and competitive enzyme-linked immunosorbent assay (cELISA) for brucellosis, and indirect enzyme linked immunosorbent assay(iELISA) for Q Fever in theDepartment of Veterinary Public Health and Preventive Medicine Bacterial Research Laboratory, Ahmadu Bello University Zaria, Nigeria. The RBPTantigen and cELISA kits were obtained from Animal and Plant Health Agency, (APHA) New Haw, Addlestone, Surrey, KT15 3NB,

United Kingdom, while iELISA ID Screen® Q Fever Indirect Multi-species kits were supplied by IDvet, Innovative Diagnostics, Montpellier, France.

3.6.1 Rose bengal plate test (RBPT) for brucellosis

The RBPTwas done using the commercial RBPT in accordance with the method described by Alton *et al*. (1988)and results were interpreted in accordance with the manufacturer's instructions (Animal and Plant Health Agency (APHA).The method was done as follows:

Both the antigen and sera were kept for 30 min at room temperature ($21^{o}C \pm 5^{o}C$) before the beginning of the test and then shaken gently to re-suspend any bacterial sediment. All sera samples were screened using RBPT antigen.Briefly, 30 μL of plain serum were dispensed on a white glossy ceramic tile and mixed with an equal volume of RBPT antigenusing sterile applicator stick. The mixture on the tile was then rocked gently at room temperature for 4 min, and any visible agglutination and/or the appearance of a typical rim was taken as a positive result and negative if there was no agglutination(Alton *et al*., 1988).

3.6.2 Competitive enzyme linked immunosorbent assay (cELISA) for brucellosis

The competitive enzyme linked immunosorbent assay (cELISA) was donein accordance with the manufacturer's instructions (Animal and Plant Health Agency, (APHA) New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom). The reagents in the kit were reconstituted as directed by the manufacturers. Samples, reagents and plate(s) were brought to room temperature ($21^{o}C \pm 5^{o}C$) prior to starting the test. Plates were pre-

coated with *B. melitensis* LPS antigen. Each plate had 96 wells of 8 rows and 12 columns each. Twenty microlitre (20µl) of serum sample were added into each of the 80 wells, columns 11 and 12 were left for controls.Twenty microlitre (20µl) of the positive control were added into wells F11, F12, G11, G12, H11 and H12. Similarly, twenty microlitre (20µl) of the negative control werepipetted into wells A11, A12, B11, B12, C11 and C12. The remaining wells acted as conjugate control and have no serum added. One hundred microlitre (100µl) of the prepared conjugate solution was immediately dispensed into all wells. This gave a final serum dilution of 1/6. The plate was then shaken vigorously (on the microtitre plate shaker) for 2 minutes (min) in order to mix the serum and conjugate solution. The plate was then covered with afoil and incubated at room temperature($21^oC \pm 5^oC$) for 30 min on a rotary shaker at 160revs/min. The content of the plate was shaken out and rinsed 5 times with washing solution, then thoroughly dried by tapping on absorbent paper towel. Also 100µl of substrate solution were added to each well and incubated for 10 minat room temperature ($21^oC \pm 5^oC$). The reaction was slowed down by adding 100µl of stopping solution to all wells. The condensation was removed from the bottom of the plate with absorbent paper towel. The optical density (O.D) of the controls and samples were measured at 450nm in a microplate photometer.

Plate acceptance criteria (validation)

The results were considered valid if the following apply;

i. The mean O.D of the 6 negative control wells is greater than 0.700(the optimal mean negative O.D is 1.000)

ii. The mean O.D of the 6 positive control wells is less than 0.100.

iii. The mean O.D of the 4 conjugate control wells is greater than 0.700 (the optimal mean conjugate control O.D is 1).

iv. The binding ratio is greater than 10

$$\text{Binding Ratio} = \frac{\text{Mean of 6 negative control wells}}{\text{Mean of 6 positive control wells}}$$

Interpretation of test results

The lack of colour development indicated that the sample tested was positive. A positive/negative cut-off point was calculated as 60% of the mean of the optical density (O.D) of the 4 conjugate control wells. Any test sample given an O.D equal to or below this value was regarded as being positive.

3.6.3 Indirect multispecies enzyme linked immunosorbent assay for Q fever

Serum samples were checked for the presence of antibodies against *C. burnetii*using commercial iELISA in accordance with the manufacturer's instructions. The reagents in the kit were reconstituted as directed by the manufacturers. Samples, reagents and plate (s) were brought to room temperature ($21^{o}C\pm 5^{o}C$) and all reagents were homogenized by vortexing before starting the test. The micro-wells were coated with *C. burnetii* phases I and II strain, isolated in France from an aborted bovine placenta.Each plate had 96 wells of 8 rows and 12 columns each.In order to avoid differences in incubation time between specimens, a 96 well plate containing the test samples and control specimens were prepared before transferring them into an iELISA microtitre plate using multichannel pipette.Ninety microlitre (90µl) ofdilution buffer 2 were added into each micro-well,

10µl of the negative control was added into wells A1 and B1. Ten microlitre (10µl) of the positive control was added into wells C1 and D1. Ten microlitre (10µl) of each sample was immediately dispensed into the remaining wells. The plate was then incubated at 21^{o}C for 45 min.After incubation, the contents of the wells (plate) were shaken out and washed 3 times with approximately 300µl of the wash solution. The microtitre plates were tapped againsta clean absorbent tissue paper in order to remove all the contents of the plates. The drying of the wells were avoided between washings. After washing, an anti-multi-species horseradish peroxidase (HRP) conjugate was added to the wells and incubated for 30 min at room temperature. After washing the plates with wash solution to eliminate conjugate, the substrate solution tetramethylbenzidine (TMB) was added to each well. The plates were incubated for 15 min at room temperature. The stop solution was added to stop the reaction, then the optical density (O.D) of the microtitre plate was read at an absorbance of 450 nm using ELISA reader.

Plate acceptance criteria (validation)

The test is valid if:

i. The mean value of the Positive Control O.D. (O.Dpc) is greater than 0.350. ($O.D_{PC} > 0.350$)

ii. The ratio of the mean O.D. values of the Positive and Negative Controls ($O.D_{PC}$ and $O.D_{NC}$) is greater than 3. ($O.D_{PC} / O.D_{NC} > 3$)

$$\text{Mean O. D}_{\text{NC}} = \frac{0.120 + 0.106}{2} = 0.113$$

$$\text{Mean O. D}_{\text{PC}} = \frac{1.824 + 2.186}{2} = 2.005$$

$$\text{Ratio of mean O. D of PC and NC} = \frac{2.005}{0.113} = 17.7, \quad 17.7 > 3\text{(validated)}$$

Interpretation of test results

For each sample, the S/P percentage (S/P%) was calculated following the manufacturer's formula:

$$S/P = \frac{O.D_{sample} - O.D_{NC}}{O.D_{PC} - O.D_{NC}} \text{ X } 100$$

Result	**Status**
S/P ≤ 40%	Negative
40 % < S/P ≤ 50 %	Doubtful
50 % < S/P ≤ 80 %	Positive
S/P > 80 %	Strong positive

All the doubtful results were classified as negative, for the purpose of analysis.

3.7Detection of Persistence of *Brucella* and *Coxiella burnetii* in Seropositive Cattle Herds

The 36 cattle herds consisting of those with history of abortion that were seropositive for *Brucella abortus* and *Coxiella burnetii*were visited after 6 months to detect persistence of infection in the herd. In each seropositive herd 2-3 boold sample were randomly collected from cattle depending on the size of the herd. The blood samples were pooled into one to represent each herd.

3.7.1 Detection of *Brucella* spp using polymerase chain reaction

The presumptive*Brucella* spp. positive samples were further studied usingPCR. The reference strains, *B. abortus* KGR1, JPL1 and vaccine strain S19 with batch No. 03/2016 used as positive control were kindly provided by Bacterial Research Laboratory of National Veterinary Research Institute Vom, Plateau State Nigeria.

3.7.1.1Extraction of genomic DNA fromBrucella

Genomic DNA was extracted from whole blood using the Quick- g DNA^{TM} miniprep kit, (Zymo Research, South Africa) following the manufacturer's instructions.A modification of the method described by Miller *et al*. (1988) was used.Briefly, 100 µl of whole blood samples in EDTA tube were transferred into a micro-centrifuge tube.Then, 95 µl of 2X Digestion Buffer and 5 µl of Proteinase K solution were added into it. The tubes were inverted three times to mix the contents and then incubated at 55°C for 20 min. After incubation,700 µl of Genomic Lysis Buffer were added to the tubes and mixed thoroughly by vortexing to re-suspend the pellet.The mixture was transferred to a Zymo-Spin ^{TM}IIC column in a collection tube and then centrifuged at 10,000 x g for 1 minute. Then 200 µl of DNA Pre-Wash Buffer was added to the spun column in a new collection tube and centrifuged at 10,000 x g for 1 minute. About 400 µlof g-DNA wash buffer was added to spun column, then centrifuged at 10,000 x g for 1 minute. The spun column was transferred to a clean micro-centrifuge tubes, then ≥ 50 µl of DNA Elution buffer was added to the spun column and incubated at 25 °C for 2–5 min, then centrifuged at 10,000 x g for 30 seconds to elute the DNA. The concentration and purity of the DNA were then determined spectrophotometrically by reading absorbance at 260 and 280 nm. The eluted DNA was used immediately or stored at <- 20 °C for future use.

3.7.1.2 Primers used in PCR for the detection of B. abortus, B. melitensis and B. ovis

The selection of DNA sequences to design the Polymerase chain reaction primers was based on species specific and /strain specific genetic differences (López-Goñi *et al*., 2008) (Table 3.1).

3.7.1.3Brucella DNA amplification

The primer pairs used are provided in Table 3.1. Polymerase Chain Reaction was realized in a volume of 50 µL, consisting of 22.5 µL of ultrapure water, 5 µL of reaction buffer 10 X (500 nM KCL; 15 nM MgCl2, 100 nM TrisHCL, pH 9.0), 8.0 µL of dNTP mixture (200 nM of each nucleotide [dCTP, dATP, dGTP, dTTP]), 1.5 µL of MgCl2 (50 nM), 5 µL of the primer JPF, F1 and (10 pmol/ µL), 5 µL of the primer JPR, F1 and (10 pmol/ µL), 0.5 µL of Taq DNA polymerase (5 units per µL). Amplification of target DNA sequence was done using an initial denaturation of 94ºC for 5 min, and 40 cycles of 94ºC for 1 minute (denaturation), 60 ºC for 1 minute (annealing) and 72ºC for 1 minute (extension) with a final extension of 72ºC for 10 min (Leal-Klevezas *et al*., 1995). Finally, all amplification products were analyzed using electrophoresis with 1.5% agarose gel containing ethidium bromide at 0.5µg/mL and subsequent observation using an ultraviolet transilluminator.

Table 3.1: Oligonucleotide primer sequences used for the identification of *B. abortus*, *B. melitensis* and *B. ovis*

***Brucella* Species**	**Primer sequence (5' – 3')**	**Product size (bp)**	**Reference**
B. abortus	F (5'- GCGCTCAGGCTGCCGACGCAA -3') R (3'- ACCAGCCATTGCGGTCGGTAA -5')	193	Baily *et al.*,1992
B. melitensis	F (5'-CATGCGCTATGTCTGGTTAC-3') R (3'-AGTGTTTCGGCTCAGAATAATC-5')	252	Redkar *et al*., 2001
B. ovis	F (5′-GCCTACGCTGAAACTTGCTTTTG-3′) R (3′-ATCCCCCCATCACCATAACCGAAG-5′)	228	Xavier *et al*., 2010

3.7.2 Detection of *Coxiella burnetii*using PCR

The presumptive *Coxiellaburnetii* positive samples were further studied using PCR.The reference strain, *C. burnetii* with Code/Batch: ADN Cb NM/2015 07P6, catalogue reference S 00413 wasused as positive control. The strain was kindly provided by the National Reference Laboratory for Q fever OIE Reference Laboratory for Q fever. ANSES Sophia-Antipolis Laboratory, France.

3.7.2.1 Extraction of genomic DNA fromCoxiella burnetii

Genomic DNA was extracted from the whole blood of suspected animals using the Quick-g DNA ^{TM} miniprep kit, (Zymo Research, South Africa) following the manufacturer's instructions. A modification of the method described by Miller *et al*. (1988) was used. Briefly, 100 µl of whole blood sample in EDTA tube were transferred into a micro-centrifuge tube then 95 µl of 2X Digestion Buffer and 5 µl of Proteinase K solution was added. The tube was inverted three times to mixed the contents and then incubated at 55 °C for 20 min, 700 µl of Genomic Lysis Buffer was added to the tube and mixed thoroughly

by vortexing to re-suspend the pellet. The mixture was transferred to a Zymo-Spin ™ IIC column in a collection tube and then centrifuged at 10,000 x g for 1 minute. Then 200 µl of DNA Pre-Wash Buffer was added to the (spin) spun column in a new collection tube and centrifuged at 10,000 x g for 1 minute. About 400 µl of g-DNA wash buffer was added to spun column, then centrifuged at 10,000 x g for 1 minute. The spun column was transferred to a clean micro-centrifuge tube, then ≥ 50 µl of DNA Elusion buffer was added to the spun column and incubated at 25 ℃ for 2–5 min, then centrifuged at 10,000 x g for 30 seconds to elute the DNA. The concentration and purity of the DNA was then determined spectrophotometrically by readings absorbance at 260 and 280 nm. The eluted DNA was used immediately or stored at < - 20 ℃ for future use.

3.7.2.2 Primers used in PCR for the detection of Coxiella burnetii

The selection of DNA sequences to design the Polymerase chain reaction primers for the detection of *Coxiella burnetii* was based on specie specific (López-Goñi *et al*., 2008) (Table 3.2).

3.7.2.3Coxiella burnetii DNA amplification

Amplification of the repetitive transposon-like region of *C. burnetii*was employed using 3 pairs of primers targeting the sequence (Table 3.2). Each 25 µL reaction mixture was made of 5 µL of the PCR buffer, 0.2 µL of the taq polymerase, 1 µL of each of the four primers (10 pm/µL), IS111F1, IS111R1, IS111F2 and IS111R2, sterile distilled water, and 2 µL of DNA. The IS111F1 and IS111R1 primers, which were designed to amplify a 485-bp fragment of the *htp*AB-associated repetitive element, were used for the first amplification,

and reamplification was performed using the IS111F2 and IS111R2 primers, which amplify a 260-bp fragment (Seshadri *et al*., 2003).We used TaqMan Universal PCR Master Mix (UMM 2×) following the amplification protocol: Following an initial denaturation step at 95 ^{o}C for 8 min, the rapid PCR program was made of 35 cycles of denaturation at 95 ^{o}C for 15sec, annealing at 52 ^{o}C for 5sec, and extension at 72 ^{o}C for 18s. Reamplification or second round PCR was performed using 35 cycles of denaturation at 95 ^{o}C for 15sec, annealing at 48 ^{o}C for 5sec, and extension at 72 ^{o}C for 18sec. The amplification was completed by holding for 10 min at 68 ^{o}C to allow complete extension of the PCR products.

Table 3.2: Oligonucleotide primer sequences used for the amplification of *C. burnetii*

Primer	**Primer sequence (5' – 3')**	**Product size (bp)**	**Reference**
IS111 F1	(5'-TACTGGGTGTTGATATTGC-3'),	485	Panning *et al*., 2008
IS111 R1	(3'- CCGTTTCATCCGCGGTG-5')		
IS111F2	(5'-GTAAAGTGATCTACACGA-3')	260	Fenollar *et al*., 2004
IS111R2	(5'- TTAACAGCGCTTGAACGT-3')		
CoxP4	(5'-TTAAGGTGGGCTGCGTGGTGATGG-3')	448	Seshadri *et al*., 2003
CoxM9	(3'-GCTTCGTCCCGGTTCAACAATTGC-5')		

3.8Electrophoresis 1.5% Agarose Gel Analysis

Immediately after PCR amplification, 1.5g of agarose powder was weighed out, then 100mls of 1X TBE (Tris Borate acid EDTA), was added and heated in a microwave oven at maximum heat for 3 min. The solution was cooled under tap wateror swirled gently at room temperature and then 5 µl of ethidium bromide was added. The cooled gel was poured into gel-casting tray with attached combs, placed at room temperature until full polymerization took place (15 min). The combs was removed and gel tray was carefully placed in electrophoresis tank, totally submerging in buffer solution (TBE). The samples were loaded into the wells together with the controls. The positive and negative terminals of electrophoresis tank were then connected to power source and run at 100Vfor 20 min.

3.9 Assessment of Spatial Distribution of *Brucella*spp. and *Coxiella burnetii* Infections

Each herd was localized on map of the area using points. The inverse distance weighted interpolation (IDWI) of ArcMap® technology was used to determine the spatial

distribution of *Brucella* and *Coxiella burnetii* infected herds and flocks in Maigana and Birnin Gwari agro-ecological zones of Kaduna State. Using the geographical information system (GIS) data of the 110 cattle herds and the *Brucella* and *Coxiella burnetii* antibodies seropositivity per herd, a *Brucella* and *Coxiella burnetii* antibodies spatial distribution map was created to display the spatial distribution of *Brucella* and *Coxiella burnetii* antibodiesinfection across the entire Maigana and Birnin Gwari agro-ecological zones of Kaduna State. Closely related locations such as in the same village were assigned a common point due to low level of resolution.

3.10 Statistical Analysis

Herds and individual animal-derived data were stored in Microsoft Excel. Data generated were analysed using Statistical Package for Social Sciences (SPSS) version 20.0 statistical software (SPSS Inc., Chicago, IL, USA).Statistical methods used included descriptive statisticsto determine frequencies and percentages. Prevalence was calculated using number of positive divided by the total number of samples testedand expressed as a percentage. Relationship between demographic, management and other variables with disease positivity was determined using Chi-square (χ^2) and Fisher's Exact Test to test for association.Strength of association was calculated using Odds Ratio (OR)at 95% Confidence Interval (CI).

CHAPTER FOUR

4.0 RESULTS

4.1 Seroprevalence of Brucellosis in Cattle, Sheep and Goats in Maigana and Birnin Gwari Agro-ecological Zones ofKaduna State Based on Sex Using RBPT

A total of 1200 sera samples fromherds and flocks ofcattle, sheep and goatsin Birnin Gwari and Maigana agro-ecological zones were tested for *Brucella*antibodies usingrose bengal plate test (RBPT). Of these, 189 revealed *Brucella* antibodies in their sera, giving an overall seroprevalence of 15.8%. The highest proportion of brucellosis positive sera was recorded in cattle, with aseroprevalenceof 74 (18.5%), followed by sheep 67 (16.8%) and the least was observed in goats 48 (12.0 %). On the basis of sex distribution in cattle, the seroprevalence rate of 18 (12.9%) was observed out of the 139 males tested, while out of the 261 femalestested, 56 (21.5%) tested positive. In sheep, the seroprevalence rate of 16 (11.3%) wereobserved out of 141 males tested, while of the 259 females, 51 (19.7%)

werepositive. In goats, 10 (6.8%) were positiveout of the 147 males tested, and 31 (12.5%) out of the 253 femalestested were positive(Table 4.1).

4.2Seroprevalence of Brucellosis in Cattle, Sheep and Goats in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State Based on Sex UsingcELISA

A total of 1200 sera samples from herds and flocks of cattle, sheep and goatsin Birnin Gwari and Maigana Agro-ecological zones were tested for *Brucella*antibodies using cELISA. Overall 94 (7.8%) showed *Brucella* antibodies in their sera, with thehighest seroprevalence rate of 43 (10.8%) recorded in sheep sampledfollowed, by seroprevalence of 27 (6.8%)observed in cattle sampled withthe least seroprevalence rate of 24 (6.0%) observed in goats. There was statistically significant association in the seroprevalence rates between the species tested (P<0.05), (OR=0.882, 95% CI= 0.500–1.556). By sex, female cattle showed the highest seroprevalence of 7.3% while the least seroprevalencewas observed in males5.8%.There was no statistically significant association in the seroprevalence rates between the sex of cattle tested (P>0.05), (OR=0.778, 95% CI= 0.331–1.825). In sheep, the seroprevalence 9.9% was recorded, while a seroprevalence of11.2% was recorded in femalessampled. There was no statisticallysignificant association in the seroprevalence rates between the sex of sheep screened (P>0.05), (OR= 0.874, 95% CI= 0.446–1.715). Of the 253 female goats tested 18 (7.1%) was seropositive, while 8(4.1%) of the 147 male goats were seropositive. There was no statistically significant association in the seroprevalence rates between the sex of sheep screened (P>0.05), (OR= 0.556, 95% CI=0.215–1.433) (Table 4.2).

Table 4.1*Brucella* infection seroprevalence rates in cattle, sheep and goatsbased on sex in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on sex using RBPT

Species	Sex	Number Examined	RBPT +ve No. (%)	RBPT –ve No. (%)
Cattle	Male	139	18 (12.9)	121 (87.1)
	Female	261	56 (21.5)	205 (78.5)
	Sub total	**400**	**74 (18.5)**	**326 (81.5)**
Sheep	Male	141	16 (11.3)	125 (88.7)
	Female	259	51 (19.7)	208 (80.3)
	Sub total	**400**	**67 (16.8)**	**333 (83.2)**
Goats	Male	147	10 (6.8)	137 (93.2)

	Female	253	38 (15.0)	215 (84.9)
	Sub total	**400**	**48 (12.0)**	**352 (88.0)**
	Grand Total	**1200**	**189 (15.8)**	**1011 (84.2)**

Table 4.2 Seroprevalence of brucellosis in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on sex using cELISA

Species	Sex	Number Examined	Number Positive (%)	Number Negative (%)	Chi-square	OR	95% CI lower	upper	P value
Cattle	Male	139	8 (5.8)	131 (94.2)	0.335	0.778	0.331	1.825	0.563
	Female	261	19 (7.3)	242 (92.7)		1*			
	Sub total	**400**	**27 (6.8)**	**373 (93.2)**					
Sheep	Male	141	14 (9.9)	127 (90.1)	0.153	0.874	0.446	1.715	0.696
	Female	259	29 (11.2)	220 (88.8)		1*			
	Sub total	**400**	**43 (10.8)**	**357 (89.2)**					
Goats	Male	147	6 (4.1)	141 (95.9)	1.516	0.556	0.215	1.433	0.218
	Female	253	18 (7.1)	235 (92.9)		1*			
	Sub total	**400**	**24 (6.0)**	**376 (94.0)**					
	Grand Total	**1200**	**94 (7.8)**	**1106 (92.2)**	**7.226**	**0.882 1***	**0.500**	**1.556**	**0.027**

1* = Reference

4.3 Seroprevalence of Brucellosis in Cattle, Sheep and Goats in Maigana and Birnin Gwari Agro-ecological Zones ofKaduna State Based on Age Using RBPT

Based on age the seroprevalence of brucellosis in cattle byRBPT,the highest seroprevalence was recorded amongcattle older than 4 years with 43 (19.0%) out of 226 samples tested,while the least seroprevalence was recorded among cattle less than 4 years with 31 (17.8%) out of 174 samples tested.In sheep, the highest seroprevalencewas observed in the age bracket of 2 to 4 years, with 48 (17.9%) out of 268 samples tested and was followed by the sheep less than2 years old, with seroprevalenceof 2 (20.0%) out of 10 samples tested. The least was observed in the sheep older than 4 years, with theseroprevalence of 17 (13.9%) out of 122 samples tested. The age distribution of *Brucella* antibodies in goats sampled showed that the highest seroprevalence (13.4%) was recorded in goats 2 to 4 years, followed by the those less than 2 years old (9.8%) and the least was was recorded in goats older than 4 years (9.5%)(Table 4.3).

4.4 Seroprevalence of Brucellosis in Cattle, Sheep and Goats in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State Based on Age Using cELISA

Table 4.4showed the age distribution of *Brucella* antibodies in cattle, sheep and goats sampled using competitive enzyme linked immunosorbent assay (cELISA). In cattle, the age distribution of brucellosis is highest in the cattle older than 4 years with the seroprevalenceof 16 (7.1%) out of 226 samples tested, while the least was observed in cattle less than 4 years with the seroprevalence of 11 (6.3%) out of 174 samples tested. There was no statistically significant difference in the seroprevalence rates between the age of cattle screened (P>0.05), (OR= 0.886, 95% CI= 0.400–1.960). In sheep the seroprevalence of 11.5% was recorded in sheep older than 4 years and the seroprevalence

of 10.8% was recorded in the age bracket of 2 to 4 years, while there was no recorded seroprevalence in sheep less than 2 years. There was no statistically significant difference in the seroprevalence rates among different age group of sheep tested (P>0.05), (OR= 1.068, 95% CI= 0.543–2.103). In goats, the age distribution of *Brucella* antibodies was highest 6.3%in the age bracket of 2 to 4 years followed by those older than 4 years. 6.3% and least was in goats less than 2 years old with the seroprevalence of 3.9%. There was no statistically significant difference in the seroprevalence rates among different age groups of goats tested (P>0.05), (OR= 1.002, 95% CI= 0.380–2.644).

Table 4.3 *Brucella* infection seroprevalence rates in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on age using RBPT.

Species	Age (years)	Number Examined	RBPT +ve No. (%)	RBPT –ve No. (%)
Cattle	< 4 Years	174	31 (17.8)	143 (82.2)
	> 4 Years	226	43 (19.0)	183 (81.0)
	Total	**400**	**74 (18.5)**	**326 (81.5)**
Sheep	< 2 Years	10	2 (20.0)	8 (80.0)
	2–4 Years	268	48 (17.9)	220 (82.1)
	> 4 Years	122	17 (13.9)	105 (86.1)
	Total	**400**	**67 (16.8)**	**333 (83.2)**
Goats	< 2 Years	51	5 (9.8)	46 (90.2)
	2–4 Years	254	34 (13.4)	220 (86.6)
	> 4 Years	95	9 (9.5)	86 (90.5)
	Total	**400**	**48 (12.0)**	**352 (88.0)**

Table 4.4 Seroprevalence of brucellosis in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on age using cELISA

Species	Age (Years)	Number Examined	Number Positive (%)	Number Negative (%)	Chi-square	OR	95% CI lower	upper	P value
Cattle	≤ 4 Years	174	11 (6.5)	163 (93.72)	0.090	0.886	0.400	1.960	0.765
	≥ 4 Years	226	16 (7.1)	210 (92.9)		1*			
	Sub total	**400**	**27 (6.8)**	**373 (93.2)**					
Sheep	≤ 2 Years	10	0 (00)	10 (100)	1.273	>999	00000000		0.529
	2 – 4 Years	268	29 (10.8)	239 (89.2)		1.068	0.543	0.543	
	≥ 4 Years	122	14 (11.5)	108 (88.5)		1*			
	Sub total	**400**	**43 (10.8)**	**357 (89.2)**					
Goats	≤ 2 Years	51	2 (3.9)	49 (96.1)	0.448	1.652	0.321	8.496	0.799
	2 – 4 Years	253	16 (6.3)	238 (97.3)		1.002	0.380	2.644	
	≥ 4 Years	95	6 (6.3)	89 (97.3)		1*			
	Total	**400**	**24 (6.0)**	**376 (94.0)**					

1*	=	Reference

4.5 Seroprevalence of Brucellosis in Cattle, Sheep and Goats in Maigana And Birnin Gwari Agro-ecological Zones of Kaduna State Based on Breed Using RBPT

In cattle, the two breed identified during the study were White Fulani (Bunaji) and Sokoto Gudali (Bokoloji). White Fulani (Bunaji) recorded the highest seroprevalence, with 60 (19.2%) out of 312 samples tested, while the least seroprevalencewas recorded in Sokoto Gudali (Bokoloji) with 14 (15.9%) out of the 88 samples tested. In sheep, the breeds identified were Balami, Yankasa and Ouda.The highest seroprevalence was recorded in Balami with 27 (18.2%) out of 146 samples tested followed by the seroprevalence of 34 (17.2%) out of 198 Yankasa samples tested. The least seroprevalence wasobtained among Ouda with the seroprevalence of 6 (10.7%) out of 56 samples tested. In goats, the breeds studied were Sahelian, Sokoto Red and West African Dwarf, with the highest seroprevalence observed among Sokoto Red, with 25 (13.4%) out of 186 samples tested, followed by Sahelian goats with a seroprevalence of 17 (11.4%) out of 149 samples tested, and least seroprevalence was obtainedinthe West African Dwarf goats, with 6 (9.2%) out of 65 samples tested(Table 4.5).

4.6 Seroprevalence of Brucellosis in Cattle, Sheep and Goats in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State Based on Breed Using cELISA

Table 4.6 shows the breed distribution of brucellosis in cattle, sheep and goats using competitive enzyme linked immunosorbent assay (cELISA). In cattle, the highest seroprevalence was observed among White Fulani (Bunaji), with 22 (7.1%) out of 312 samples tested and the least was observed among Sokoto Gudali (Bokoloji) with seroprevalence of 5 (5.7%) out of 88 samples tested. There was no statistically significant difference in the seroprevalence rates between the different breeds studied

(P>0.05), (OR= 1.259, 95% CI= 0.463–3.427). In sheep, the highest seroprevalence was recorded in among Yankasa, with 23 (11.6 %) out of 198 samples tested, followed by Balami with the seroprevalence of 15 (10.3%) out of 146 samples tested. The least seroprevalence was observed among Ouda, with 5 (8.9%) out of 56 samples tested by cELISA. There was no statistically significant difference in the seroprevalence rates between the different breed of sheep studied (P>0.05), (OR= 0.856, 95% CI= 0.296–2.478). In goats, the highest seroprevalence was recorded among Sokoto Red, with 15 (8.1%) out of 186 samples tested, this was followed by Sahelian goats with seroprevalence of 9 (6.0%) out of 149 samples tested, while 00(0.0%) was recorded for West African Dwarf goats out of 65 samples tested. There was no statistically significant difference in the seroprevalence rates between the different breedof goats studied (P>0.05), (OR= 0.000, 95% CI= 0.00).

Table 4.5 *Brucella* infection seroprevalence rates in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on breed using RBPT

Species	Breeds	Number Examined	RBPT +ve No. (%)	RBPT –ve No. (%)
Cattle	WF (Bunaji)	312	60 (19.2)	252 (80.8)
	SG (Bokoloji)	88	14 (15.9)	74 (84.1)
	Total	**400**	**74 (18.5)**	**326 (81.5)**
Sheep	Balami	146	27 (18.5)	119 (81.5)
	Yankasa	198	34 (17.2)	164 (82.8)
	Ouda	56	6 (10.7)	50 (89.3)
	Total	**400**	**67 (16.8)**	**333 (83.2)**
Goats	Sahel	149	17 (11.4)	132 (88.6)
	Sokoto Red	186	25 (13.4)	161 (86.6)
	WAD	65	6 (9.2)	59 (90.8)
	Total	**400**	**48 (12.0)**	**352 (88.0)**

Key: WF: White Fulani, SG: Sokoto Gudali, WAD: West African Dwarf.

Table 4.6 Seroprevalence of brucellosis in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on breeds using cELISA

Species	Breed	Number Examined	Number Positive (%)	Number Negative (%)	Chi-square	OR	95% CI lower	upper	P value
Cattle	Bunaji (WF)	312	22 (7.1)	290 (92.9)	0.205	1.259	0.463	3.427	0.651
	Bokoloji (SG)	88	5 (5.7)	83 (94.3)		1*			
	Sub total	**400**	**27 (6.8)**	**373 (93.2)**					
Sheep	Balami	146	15 (10.3)	131 (89.7)	0.383	0.856	0.296	2.478	0.826
	Yankasa	198	23 (11.6)	175 (88.4)		0.746	0.270	2.061	
	Ouda	56	5 (8.9)	51 (91.1)		1*			
	Sub total	**400**	**43 (10.8)**	**357 (89.2)**					
Goats	Sahel	149	2 (3.9)	49 (96.1)	5.555	0.000	0.000	0.000	0.799
	Sokoto Red	186	16 (6.3)	238 (97.3)		0.000	0.000	0.000	
	WAD	65	0 (00)	65 (100)		1*			
	Total	**400**	**24 (6.0)**	**376 (94.0)**					

Key: WF = White Fulani, SG = Sokoto Gudali, WAD = West African Dwarf, 1* = Reference

4.7 Seroprevalence of Q Fever in Cattle, Sheep and Goats in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State Based on Sex UsingiELISA

Of the 1200 sera samples from cattle, sheep and goats tested for the presence of *Coxiella burnetii* antibodies by indirect enzyme linked immunosorbent assays (iELISA), the overall seroprevalence was 7.7%. The highest seroprevalence was recorded in goats with 35 (8.8%) out of the 400 samples tested, followed by sheep with 32 (8.0%) out of 400 samples tested and the least seroprevalence was obtained in cattle, with 25 (6.2%) out of the 400 samples tested. There was no statistically significant difference in the seroprevalence rates between the three species tested (P>0.05), (OR= 1.438, 95% CI= 0.844–2.451). In relation to sex distribution, of the 139 male cattle sampled, a seroprevalence of 7 (5.0%) was observed, while the seroprevalence of 18 (6.9%) was observed out of 261 females sampled. There was no statistically significant difference in the seroprevalence rates between male and female cattle tested (P>0.05), (OR= 0.716, 95% CI= 0.292–1.758). In sheep, the highest seroprevalence rate was observed in females, with 23 (8.9%) out of 259 samples tested, while the seroprevalence of 9 (6.4%) was recorded out of 141 males sampled. There was no statistically significant difference in the seroprevalence rates between the female and male sheep screened (P>0.05), (OR= 0.700, 95% CI= 0.315–1.556). In goats tested for coxiellosis, the highest seroprevalencewas observed in females, with 24 (9.5%) out of 253 samples tested, while the seroprevalence of 11 (7.5%) was observed out of the 147 males sampled. There was no statistically significant difference in the seroprevalence rates between the females and the males goats screened (P>0.05), (OR= 0.772,95% CI= 0.367–1.625) (Table 4.7).

4.8 Seroprevalence of Q Fever in Cattle, Sheep and Goats in Maigana And Birnin Gwari Agro-ecological Zones of Kaduna State Based on Age Using iELISA

Table 4.8 shows the age distribution of *Coxiella burnetii* antibodies in cattle, sheep and goats samples using indirect enzyme linked immunosorbent assay (iELISA). In cattle, the highest seroprevalence was obtained in the age group older 4 years with 15 (6.6%) out of the 226 samples tested, while the least was obtained in age group less than 4 years with the seroprevalence of 10 (5.7%) out of 174 samples tested. There was no statistically significant difference in the seroprevalence rates between the age of cattle screened (P>0.05), (OR = 0.858, 95% CI= 0.376–1.959). In sheep, a seroprevalence of 8.2% was obtained from sheep older than 4 years and a seroprevalence of 7.8%in sheep of the age bracket of 2 to 4 years old, while a seroprevalence of 10.0% was recorded in the age group less than 2 years old. There was no statistically significant difference in the seroprevalence rates between different age group of sheep screened (P>0.05), (OR= 1.050, 95% CI= 0.479–2.303). In goats, a seroprevalence of 13.7% was recorded in the age group older than 4 years, and a seroprevalence of 8.7% was in the age bracket of 2 to 4 years. There was no positive sample, recorded in the age group less than 2 years old. There was statistically significant difference in the seroprevalence rates between the different age groups of goats tested (P<0.05), (OR = 1.672, 95% CI= 0.805–3.471).

Table 4.7 Seroprevalence of Q fever in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on sex using iELISA

Species	Sex	Number Examined	Number Positive (%)	Number Negative (%)	Chi-square	OR	95% CI lower	upper	P value
Cattle	Male	139	7 (5.0)	132 (95.0)	0.536	0.716	0.292	1.758	0.464
	Female	261	18 (6.9)	243 (93.1)		1*			
	Sub total	**400**	**25 (6.2)**	**375 (93.8)**					
Sheep	Male	141	9 (6.4)	132 (93.6)	0.774	0.700	0.315	1.556	0.379
	Female	259	23 (8.9)	236 (91.1)		1*			
	Sub total	**400**	**32 (8.0)**	**368 (92.0)**					
Goats	Male	147	11 (7.5)	136 (92.5)	0.467	0.772	0.367	1.625	0.468
	Female	253	24 (9.5)	229 (90.5)		1*			
	Sub total	**400**	**35 (8.8)**	**365 (91.2)**					
	Grand Total	**1200**	**92 (7.7)**	**1108 (92.3)**	**1.860**	**1.438 1***	**0.844**	**2.451**	**0.395**

1* = Reference

Table 4.8 Seroprevalence of Q fever in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on age using iELISA

Species	Age (Years)	Number Examined	Number Positive (%)	Number Negative (%)	Chi-square	OR	95% CI lower	upper	P value
Cattle	≤ 4 Years	174	10 (5.7)	164 (94.3)	0.133	0.858	0.376	1.959	0.715
	≥ 4 Years	226	15 (6.6)	211 (93.4)		1*			
	Sub total	**400**	**25 (6.2)**	**375 (93.8)**					
Sheep	≤ 2 Years	10	1 (10.0)	9 (90.0)	0.071	0.804	0.092	7.002	0.965
	2 – 4 Years	268	21 (7.8)	247 (92.2)		1.050	0.479	2.303	
	≥ 4 Years	122	10 (8.2)	112 (91.8)		1*			
	Sub total	**400**	**32 (8.0)**	**368 (92.0)**					
Goats	≤ 2 Years	51	00 (0.0)	51 (100)	7.790	> 999	0.000	1.616	0.020
	2 – 4 Years	253	22 (8.7)	232 (91.3)		1.672	0.805	3.471	
	≥ 4 Years	95	13 (13.7)	82 (86.3)		1*			
	Total	**400**	**35 (6.0)**	**365 (94.0)**					

1* = Reference

4.9 Seroprevalence of Q Fever in Cattle, Sheep and Goats in Maigana and Birnin Gwari Agro-cological Zones of Kaduna State Based on Breeds Using iELISA

Table 4.9 shows the distribution of coxiellosis (Q fever) in cattle, sheep and goats by breed using indirect enzyme linked immunosorbent assay (iELISA). In cattle, the highest seroprevalence was observed among White Fulani (Bunaji), with 20 (6.4%) out of 312 samples tested and the least was observed among Sokoto Gudali (Bokoloji) with seroprevalence of 5 (5.7%) out of 88 samples tested. There was no statistically significant difference in the seroprevalence rates between the different breeds of cattle studied (P>0.05), (OR=1.137, 95% CI= 0.414–3.121). In sheep, the highest seroprevalence was recorded in Yankasa breed, with 22 (11.1%) out of 198 samples tested followed by seroprevalence of 4.1% recorded from Balamibreed tested. The least was observed in Ouda breed, with seroprevalence of 7.1%. There was no statistically significant difference in the seroprevalence rates between the different breeds of sheep screened (P>0.05), (OR= 0.615, 95% CI= 0.203–1.866). In goats, the highest seroprevalence was recorded among Sokoto Red, with seroprevalence of 10.2%, while Sahel goats recorded a seroprevalence of 6.7%. Of the 65 samples from West African Dwarf (WAD) goats tested, a seroprevalence of 9.2% recorded. There was no statistically significant difference in the seroprevalence rates between the different breeds of goats screened (P>0.05), (OR= 0.894, 95% CI= 0.341–2.345).

Table 4.9 Seroprevalence of Q fever in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on breeds using iELISA

Species	Breed	Number Examined	Number Positive (%)	Number Negative (%)	Chi-square	OR	95% CI lower upper	P value
Cattle	Bunaji (WF)	312	20 (6.4)	292 (93.6)	0.062	1.137	0.414 3.121	0.803
	Bokoloji (SG)	88	5 (5.7)	83 (94.3)		1*		
	Sub total	**400**	**25 (6.2)**	**375 (93.8)**				
Sheep	Balami	146	6 (4.1)	140 (95.9)	5.662	1.795	0.487 6.616	0.059
	Yankasa	198	22 (11.1)	176 (88.9)		0.615	0.203 1.866	
	Ouda	56	4 (7.1)	52 (92.9)		1*		
	Sub total	**400**	**32 (8.0)**	**368 (92.0)**				
Goats	Sahel	149	10 (6.7)	139 (93.3)	1.294	1.414	0.491 4.068	0.524
	Sokoto Red	186	19 (10.2)	167 (89.8)		0.894	0.341 2.345	
	WAD	65	6 (9.2)	59 (90.8)		1*		
	Total	**400**	**24 (6.0)**	**376 (94.0)**				

Key: WF = White Fulani, SG = Sokoto Gudali, WAD = West African Dwarf, 1* = Reference

4.10 Seroprevalence of Brucellosis in Cattle, Sheep and Goats on the Basis of Two Agro-ecological Zones in Kaduna State UsingRBPT

Table 4.10 shows the distribution of *Brucella*antibodies in cattle, sheep and goats samples tested using RBPT on the basisof the zone. Theseroprevalence of *Brucella* infection in cattle was higher in Birnin Gwari zone (19.5%) than in Maigana zone (17.5%). In sheep, the seroprevalence of *Brucella* infection was higher in Maigana zone (17.5%), than in Birnin Gwari zone(16.0%). In goats, the seroprevalence was higher in Maigana zone (13.0%) than in Birnin Gwari zone (11.0%).

4.11 Seroprevalence of Brucellosis in Cattle, Sheep and Goats on the Basis of Two Agro-ecological Zones in Kaduna State Using cELISA

The distribution of *Brucella*antibodies in cattle, sheep and goats samples tested using cELISA on the basis of the zone. In cattle, the seroprevalence recorded was higher in Birnin Gwari aone (7.0%) than in Maigana Zone(6.5%). There was no statistically significant difference in the distribution of *Brucella* infection in the two zones tested (P>0.05), (OR= 0.924, 95% CI=0.323–2.019). In sheep, the seroprevalence recorded was higher in Maigana zone (11.5%) than in Birnin Gwari zone (10.0%). There was no statistically significant difference in the distribution of *Brucella* infection in the two zones tested (P>0.05), (OR= 1.169, 95% CI= 0.620–2.205).In goats, the seroprevalence recorded was in Maigana zone (7.0%) than in Birnin Gwari zone (5.0%). There was no statistically significant difference in the distribution of *Brucella* infection in the two zones tested (P>0.05), (OR= 1.430, 95% CI= 0.619–3.301) (Table 4.11).

4.12 Seroprevalence of Brucellosis in Cattle, Sheep and Goats on the Basis of Two Agro-ecological Zones in Kaduna State Using iELISA

Table 4.12 shows the distribution of *Coxiella burnetii* antibodies in cattle, sheep and goats samples tested by iELISAon the basis of the zone. In cattle, theseroprevalence of *Coxiella burnetii* infection recorded was higher in Maigana zone(6.5%) than in Birnin Gwari zone (6.0%). There was no statistically significant difference in the distribution of *Coxiella burnetii* infection in the two zones tested (P>0.05), (OR=1.089, 95% CI= 0.484–2.449). In sheep, the seroprevalence of *Coxiella burnetii* infection recorded was higher in Maigana zone (8.5%) than in Birnin Gwari zone (7.5%). There was no statistically significant difference in the distribution of *Coxiella burnetii* infection in the two zones tested (P>0.05), (OR=1.146, 95% CI= 0.556–2.363). In goats, the seroprevalence of *Coxiella burnetii* infection recorded was higher in Maigana zone (9.5%) than in Birnin Gwari zone (8.0%). There was no statistically significant difference in the distribution of *Coxiella burnetii* infection in the two zones tested (P>0.05), (OR= 1.207, 95% CI= 0.602–2.422).

Table 4.10 *Brucella* infection seroprevalence rates in cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on zone using RBPT

Species	Location (zone)	Number Examined	RBPT +ve No. (%)	RBPT –ve No. (%)
Cattle	Maigana	200	35 (17.5)	165 (82.5)
	Birnin Gwari	200	39 (19.5)	161 (80.5)
	Total	**400**	**74 (18.5)**	**326 (81.5)**
Sheep	Maigana	200	35 (17.5)	165 (82.5)
	Birnin Gwari	200	32 (16.0)	168 (84.0)
	Total	**400**	**67 (16.8)**	**333 (83.3)**
Goats	Maigana	200	26 (13.0)	174 (87.0)
	Birnin Gwari	200	22 (11.0)	178 (89.0)
	Total	**400**	**48 (12.0)**	**352 (88.0)**

Table 4.11 Seroprevalence of brucellosis in cattle, sheep and goats on the basis of two agro-ecological zones in Kaduna State using cELISA

Species	Zone	Number Examined	Number Positive (%)	Number Negative (%)	Chi-square	OR	95% CI lower	upper	P value
Cattle	Maigana	200	13 (6.5)	187 (93.5)	0.039	0.924	0.322	2.019	0.842
	B/ Gwari	200	14 (7.0)	186 (93.0)		1*			
	Total	**400**	**27 (6.8)**	**373 (93.3)**					
Sheep	Maigana	200	23 (11.5)	177 (88.5)	0.104	1.169	0.620	2.205	0.746
	B/ Gwari	200	20 (10.0)	180 (90.0)		1*			
	Total	**400**	**43 (10.8)**	**357 (8.3)**					
Goats	Maigana	200	14 (7.0)	186 (93.0)	0.398	1.430	0.619	3.301	0.528
	B/ Gwari	200	10 (5.0)	190 (95.0)		1*			
	Total	**400**	**24 (6.0)**	**376 (94.0)**	**1.860**				

Key:B/ Gwari = Birnin Gwari, 1* = Reference

Table 4.12Seroprevalence of coxiellosis (Q fever) in cattle, sheep and goats on the basis of two agro-ecological zones in Kaduna State using iELISA

Species	Zone	Number Examined	Number Positive (%)	Number Negative (%)	Chi-square	OR	95% CI lower upper	P value
Cattle	Maigana	200	13 (6.5)	187 (93.5)	0.0426	1.089	0.484 2.449	0.836
	B/ Gwari	200	12 (6.0)	188 (94.0)		1*		
	Total	**400**	**25 (6.2)**	**375 (93.8)**				
Sheep	Maigana	200	17 (8.5)	183 (91.5)	0.0339	1.146	0.556 2.363	0.853
	B/ Gwari	200	15 (7.5)	185 (92.5)		1*		
	Total	**400**	**32 (8.0)**	**368 (92.0)**				
Goats	Maigana	200	19 (9.5)	181 (90.5)	0.1252	1.207	0.602 2.422	0.723
	B/ Gwari	200	16 (8.0)	184 (92.0)		1*		
	Total	**400**	**35 (8.8)**	**365 (91.3)**	**1.860**			

Key:B/ Gwari = Birnin Gwari, 1* = Reference

4.13 Seroprevalence of Brucellosis in Lactating and Non-Lactating Cattle, Sheep and Goats in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State Using RBPT

Ofthe 145 lactating cows, 8 (19.3%) were seropositive, while 46 (18.0%) were seropositive out of the 255 non-lactating. Of the 92 lactating ewes, 8 (8.7%) were seropositive, while 59 (19.2%) were seropositive in non-lactating. Similarly, 14 (10.4%) out of 135 lactating doeswere seropositive, while 34 (12.8%) were seropositive of the non-lactating (Table 4.13).

4.14 Seroprevalence of Brucellosis in Lactating and Non-Lactating Cattle, Sheep and Goats in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State Using cELISA

Table 4.14 shows the distribution of *Brucella*antibodies in cattle, sheep and goats samples tested usingcELISA based on lactating and non-lactating. Of the 255 non-lactating cows, 18 (7.1%) were seropositive, while 9 (6.2%) were seropositive out of 145 lactating. There was no statistically significant difference in the seroprevalence rates between the lactating and non-lactating cowstested (P>0.05), (OR= 0.871, 95% CI= 0.381–1.993). In ewes, of the 92 lactating, 6 (6.5%) were seropositive, while out of 308 non-lactating, 37(12.0%) were seropositive. There was no statistically significant difference in the seroprevalence rates between the lactating and non-lactating ewestested (P>0.05), (OR= 0.511, 95% CI= 0.209–1.252). Of the 135 lactating ewes, 5 (3.7%) were seropositive, while out of the 265 non-lactating, 19 (7.2%) were seropositive. There was no statistically significant difference in the seroprevalence rates between the lactating and non-lactating ewes tested (P>0.05), (OR= 0.498, 95% CI= 0.182–1.364).

4.15 Seroprevalence of Q Fever in Lactating and Non-Lactating Cattle, Sheep and Goats in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State Using iELISA

Of the 145 lactating cows, 6 (4.1%) were seropositive, while out of the 255 non-lactating, 19 (7.5%) were seropositive. There was no statistically significant difference in the seroprevalence rates between the lactating and non-lactating cowstested (P>0.05), (OR= 0.536, 95% CI= 0.209–1.375). Of the 92 lactating ewes, 7 (7.6%) were seropositive, while out of the 308 non-lactating, 25 (8.1%) were seropositive. There was no statistically significant difference in the seroprevalence rates between the lactating and non-lactating ewes tested (P>0.05), (OR= 0.932, 95% CI= 0.390–2.231). Of the 135 lactating does, 11 (8.1%) were seropositive, while 24 (9.1%) were seropositive out of the 265 non-lactating. There was no statistically significant difference in the seroprevalence rates between the lactating and non-lactating does tested (P>0.05), (OR= 0.891, 95% CI= 0.423–1.878) (Table 4.15).

Table 4.13 Brucella infection seroprevalence rates lactating and non-lactating cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State using RBPT

Species	Physiologic Status	Number Examined	RBPT +ve No. (%)	RBPT –ve No. (%)
Cattle	Lactating	145	28 (19.3)	117 (80.7)
	Not Lactating	255	46 (18.0)	209 (82.0)
	Total	**400**	**74 (18.5)**	**326 (81.5)**
Sheep	Lactating	92	8 (8.7)	84 (91.3)
	Not Lactating	308	59 (19.2)	249 (80.8)
	Total	**400**	**67 (16.8)**	**333 (83.2)**
Goats	Lactating	135	14 (10.4)	121 (89.6)
	Not Lactating	265	34 (12.8)	231 (87.2)
	Total	**400**	**48 (12.0)**	**352 (88.0)**

"1* = Reference

Table 4.14 Seroprevalence of brucellosis in lactating and non-lactating cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on lactation and non-lactation using cELISA

Species	Physiologic Status	Number Examined	Number Positive (%)	Number Negative (%)	Chi-square	OR	95% CI lower upper	P value
Cattle	Lactating	145	9 (6.2)	136 (93.8)	0.107	0.871	0.381 1.993	0.744
	Non Lactating	255	18 (7.1)	237 (92.9)		1*		
	Total	**400**	**27 (6.8)**	**373 (93.2)**				
Sheep	Lactating	92	6 (6.5)	86 (93.5)	2.226	0.511	0.209 1.252	0.136
	Non Lactating	308	37 (12.0)	271 (88.0)		1*		
	Total	**400**	**43 (10.8)**	**357 (89.2)**				
Goats	Lactating	135	5 (3.7)	130 (96.3)	1.905	0.498	0.182 1.364	0.168
	Not Lactating	265	19 (7.2)	246 (92.8)		1*		
	Total	**400**	**24 (6.0)**	**376 (94.0)**	**1.860**			

1* = Reference

Table 4.15 Seroprevalence of Q fever in lactating and non-lactating cattle, sheep and goats in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on lactation and non-lactation using iELISA

Species	Physiologic Status	Number Examined	Number Positive (%)	Number Negative (%)	Chi-square	OR	95% CI lower	upper	P value
Cattle	Lactating	145	6 (4.1)	139 (95.9)	1.732	0.536	0.209	1.375	0.188
	Non Lactating	255	19 (7.5)	236 (92.5)		1*			
	Total	**400**	**25 (6.3)**	**375 (93.8)**					
Sheep	Lactating	92	7 (7.6)	85 (92.4)	0.025	0.932	0.390	2.231	0.875
	Non Lactating	308	25 (8.1)	283 (91.9)		1*			
	Total	**400**	**32 (8.0)**	**368 (92.0)**					
Goats	Lactating	135	11 (8.1)	124 (91.9)	0.092	0.891	0.423	1.878	0.761
	Non Lactating	265	24 (9.1)	241 (90.9)		1*			
	Total	**400**	**35 (8.8)**	**365 (91.2)**	**1.860**				

1* = Reference

4.16 Possible Risk Factors Associated with *Brucella* Infection in Cattle Herds in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State

A total of one hundred and ten (110) structured questionnaires were administered to various head of the householdin Birnin Gwari and Maigana Agro-ecological zones of Kaduna State, one to each herd tested. Of the 110 number, 95 respondents returned the filledquestionnaires. The relationship between the variables analyzed and the presence of brucellosis in herds are presented(Appendix III). Seventy three percent (73.2%)of the infected herd ownersdo not routinely utilize veterinary services,while 53.6% of the herd ownerss had previously vaccinated thiercattle against brucellosis. Seventy five percent (75.0%) of the infected herds had abortion cases only once, while 37.5% herds had abortion cases morethan once. There was statistically significant difference in the rates of abortion in the herds tested (p<0.05; OR = 3.857; 95%CI=1.366 – 10.893). It was observed that, 69.8% of the herds that had more than 20 animals were more likely to be infected with brucellosisthan the herds that had less than 20 animals (p< 0.05; OR=3.857; 95% CI=1.366–10.893). Most of the infected breeds in the herds were White Fulani (Bunaji)breed because they are the predominat breed in the study area.Prevalence of brucellosis was higher in the infected herds (71.4%) where the herdsmen disposed aborted foetusesopenly than those bury the aborted foetus (p< 0.05; OR=0.278; 95% CI=0.124–0.620).It was observed that the herdsmen had little knowledge of brucellosis and hence were not aware of the disease.

4.17 Occurrence of Single and Multiple Infections in Various Species due to Brucellosis and Q Fever in Cattle Herds in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State

Of the 78 (6.5%) total number of animals that were positive for brucellosis, 25 (6.3%) were cattle, 17 (4.3%) were goats and 36 (9.0%) were sheep respectively. Of the 76 (6.3%) animals of all species that were positive forQ fever, 23 (5.8%) were cattle, 28 (7.0%) goats and 25 (6.2%) sheep respectively. Of the 16 (1.3%) animals that were positive for both brucellosis and Q fever, 2 (0.5%) were cattle, 7 (1.8%) were goats and 16 (1.3%) were sheep respectively (Fig. 4.1).

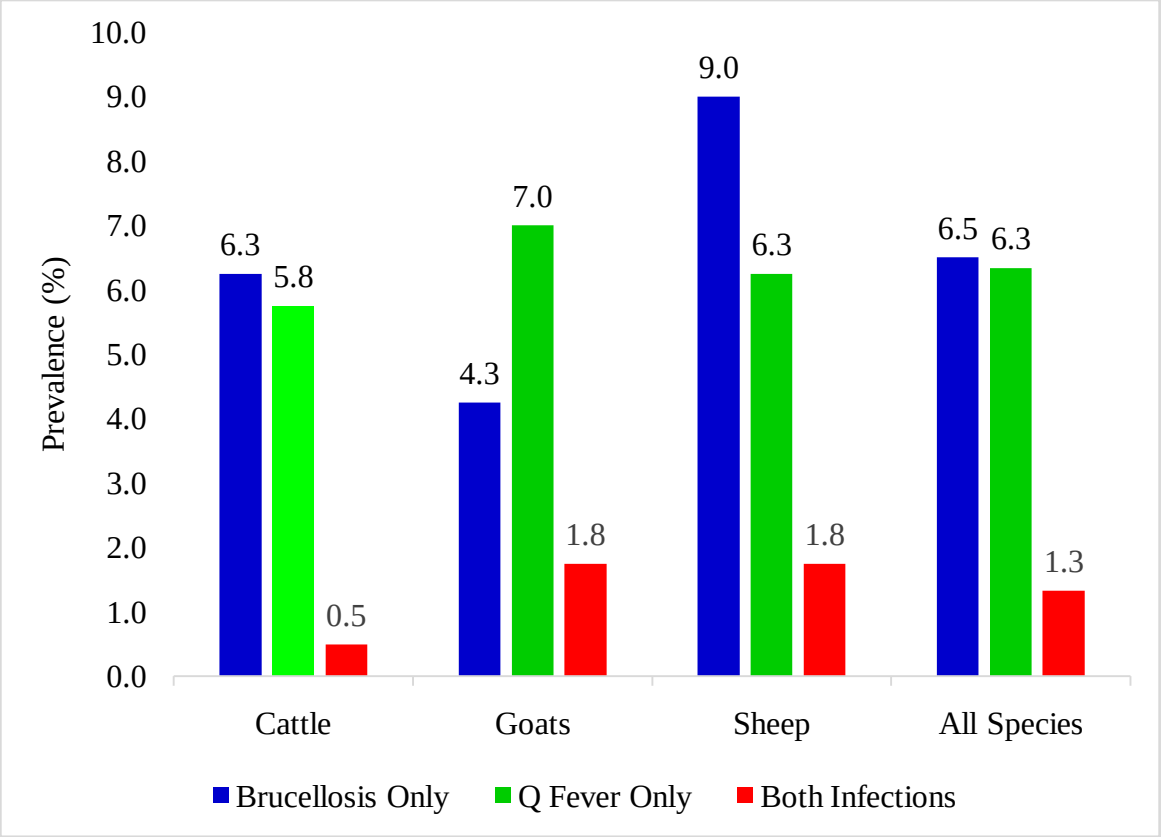


Fig. 4.1 Occurence of single and multiple infections in various species due to brucellosis and Q-Fever in Maigana and Birnin Gwari agro-ecological zones of Kaduna State

4.18 Spatial Distribution of *Brucella*spp. and *Coxiella burnetii* Antibodies in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State

Samples were collected from 13 out of the 23 Local Government Areas (LGAs) of Kaduna State. Animals that were seropositive for brucellosis were found in 11 LGAs out of 13 LGAs sampled with the exception of Kaduna North and Kaduna South LGAs (Fig. 4.2). Similarly, animals that were seropositive for Q feverwere found in 11 LGAs of 13LGAs surveyed with the exception of Chikun and Kaduna North LGAs (Fig. 4.3). Animals with co-infection infections of brucellosis and Q fever were found in 10 LGAs out of 13 LGAs surveyed with the exception of Chikun, Kaduna North and Kaduna South LGAs (Fig. 4.4). Most of the infections were concentrated in the extreme northern part of the State.

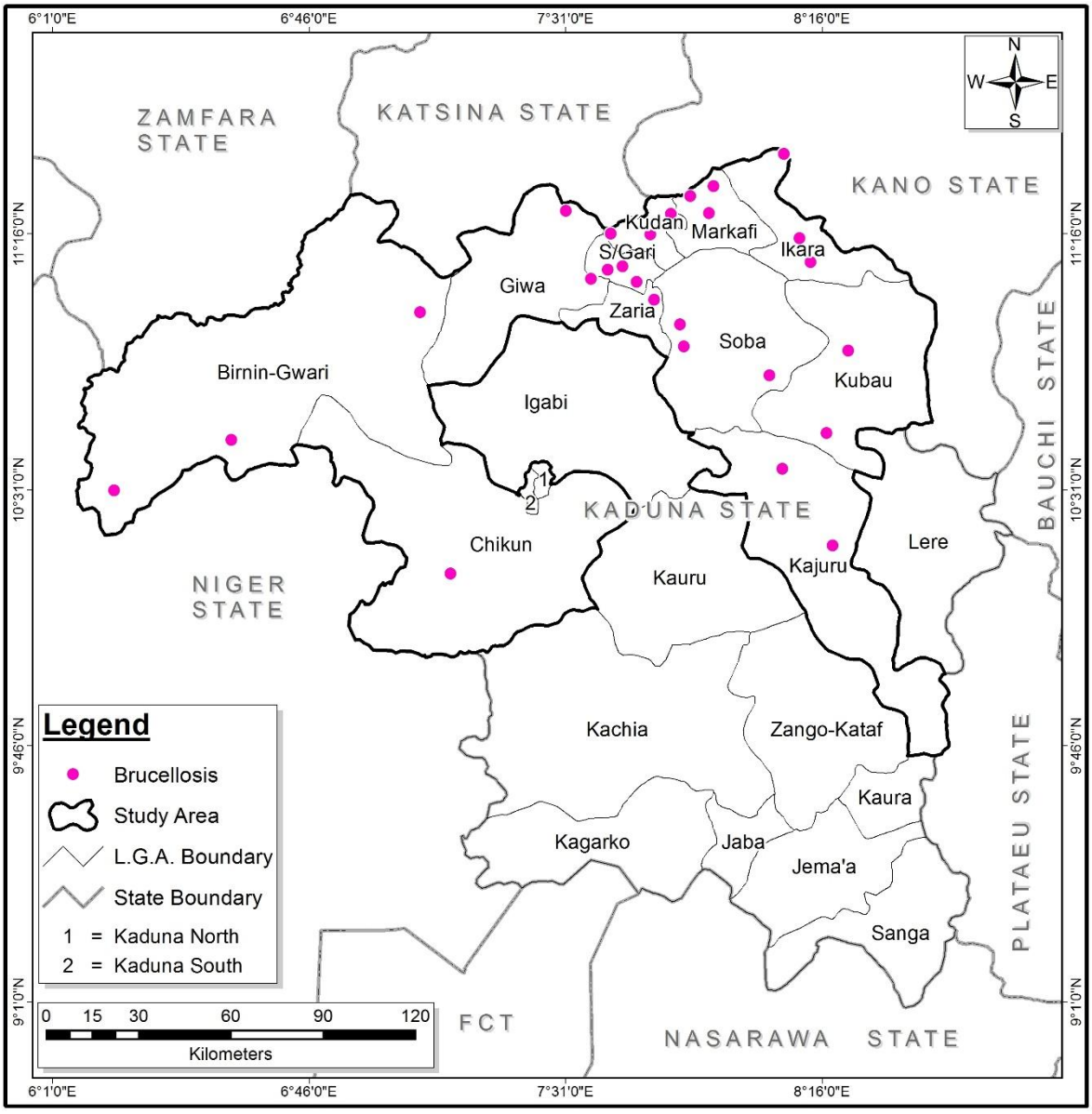


Figure 4.2: Map of study area in Kaduna State showing location of herds that were infected with *Brucella* specie

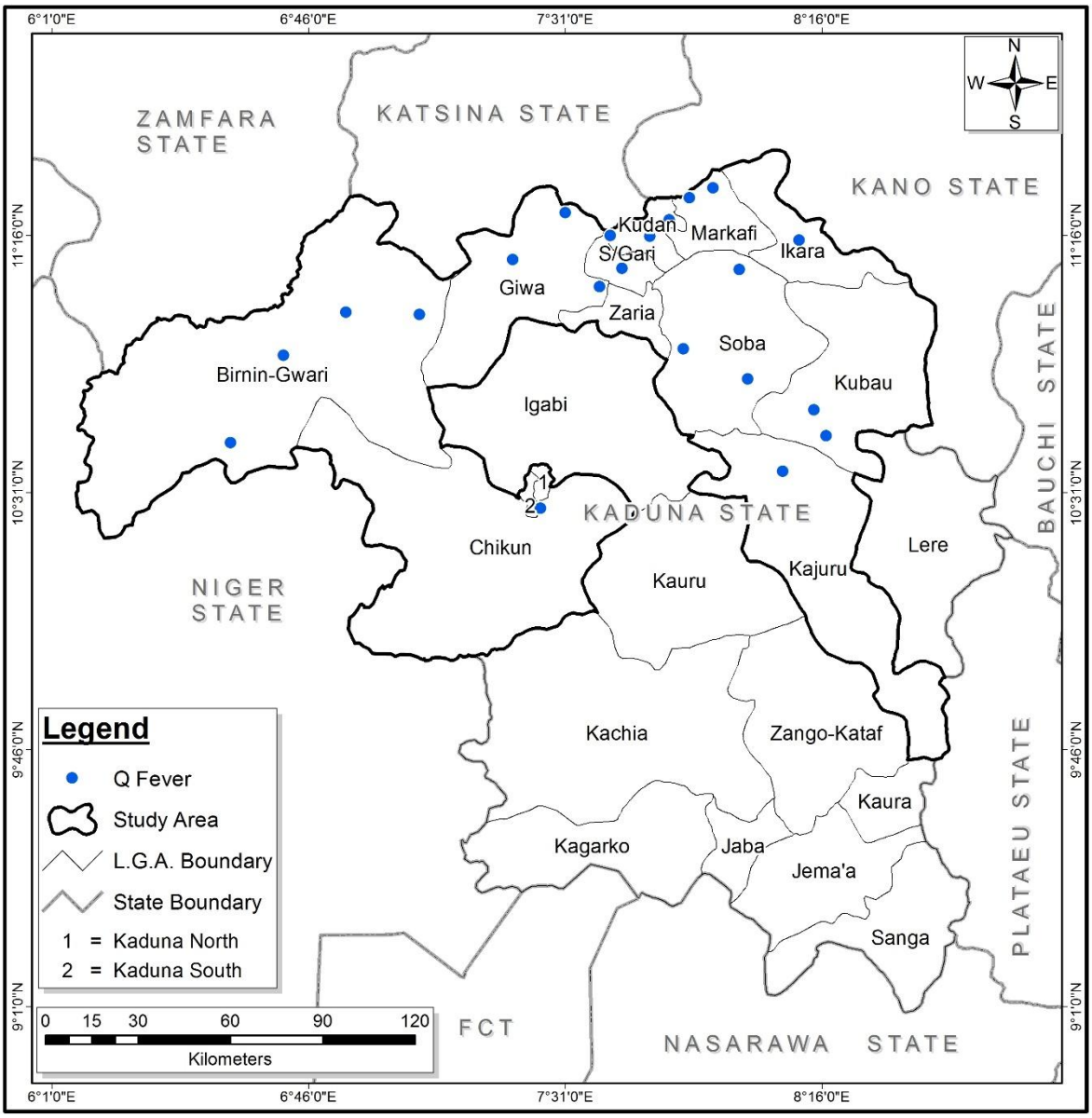


Figure 4.3: Map of the study area in Kaduna State showing location of herds that were infected with *Coxiella burnetii*

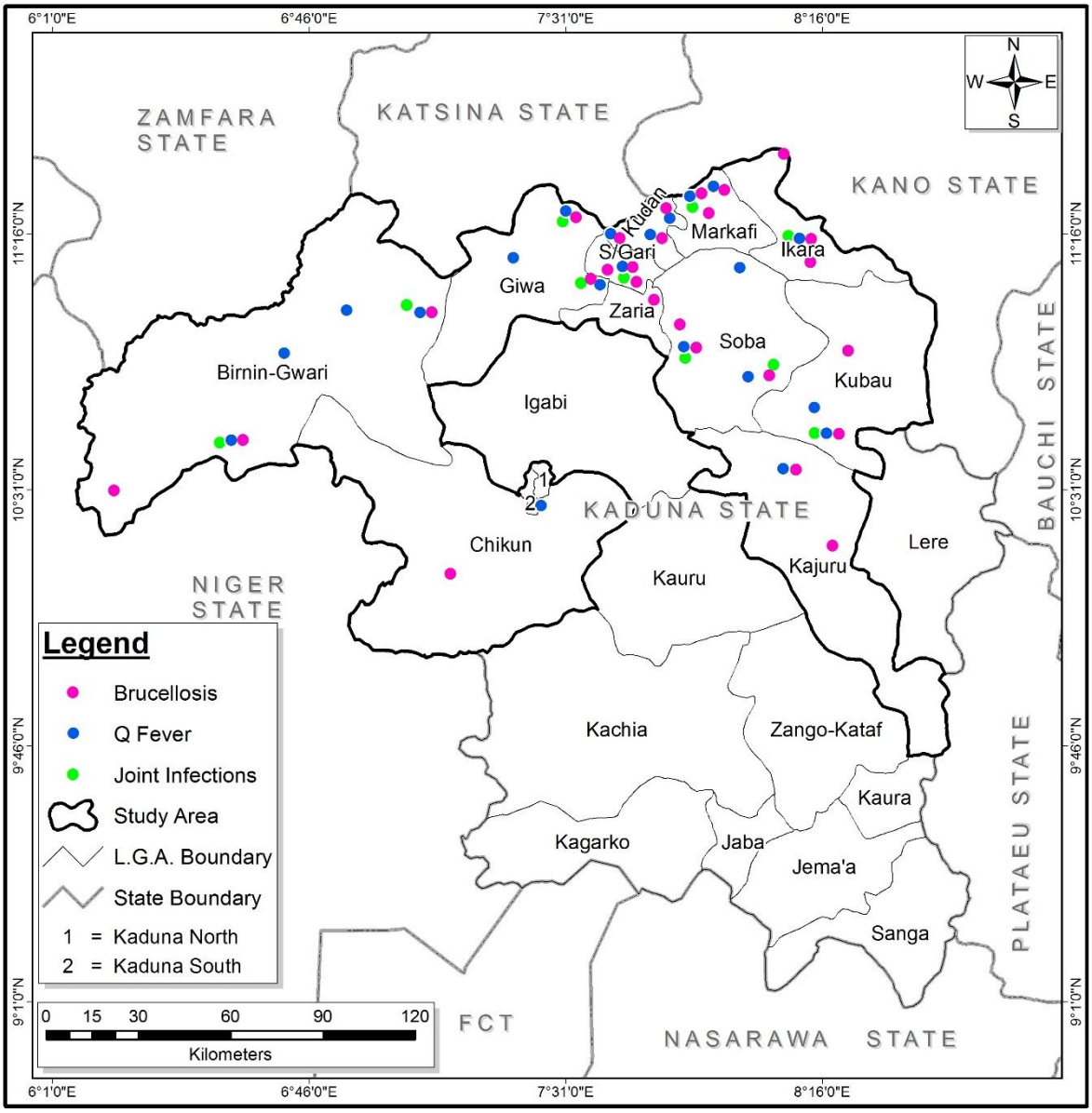


Figure 4.4: Map of the study area in kaduna state showing location of herds that were infected with both *Brucella* specie and *Coxiella burnetii* (co-infections)

4.19 Herds and Flocks that had Previous History of Abortions in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State

Of the 110 herds that were sampled, 36 (32.7%) had previous history of abortions. Of the 67 herds that were sampled from Maiganaagro-ecological zone, 25 (37.3%) had previous history of abortion. Of the 43 herds tha were sampled from Birnin Gwari agro-ecological zone, 11 (25.6) had previous history of abortion. Similarly, of the 112 flocks of sheep and goats sampled, 25 (22.3%) had previous history of abortion, of which 41 flocks were sampled from Birnin Gwari agro-ecological zone. Of the 71 sampled from Maigana agro-ecological zone, 9 (21.9%) had previous history of abortion (Table 4.16).

Table 4.16Herds/flocks that had previous history of abortion in Maigana and Birnin Gwari Agro-ecological zones of Kaduna State

Zone	No. Herd Sampled	Herds with abortion history (%)	No. Flocks Sampled	Flocks with abortion history (%)
Maigana	67	25 (37.3)	71	16 (22.5)
B/Gwari	43	11 (25.6)	41	9 (21.9)
Total	**110**	**36 (32.7)**	**112**	**25 (22.3)**

4.20 Detection of *Brucella* Infection in Blood Samples of Cattle, Sheep and Goats in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State Using PCR

Detection of *Brucella abortus* with JP-F and JP-R primers, was carried out and as expected, the *omp-2* gene amplicon size of 193 bp, was compared with the *Brucella* KGRI reference strain used as control (lane 8 and 19).(Figures 4.5 and 4.6). Of the 40 blood samples confirmed by serology, 12/40 (30.0%) showed positive amplification for *B. abortus* DNA.

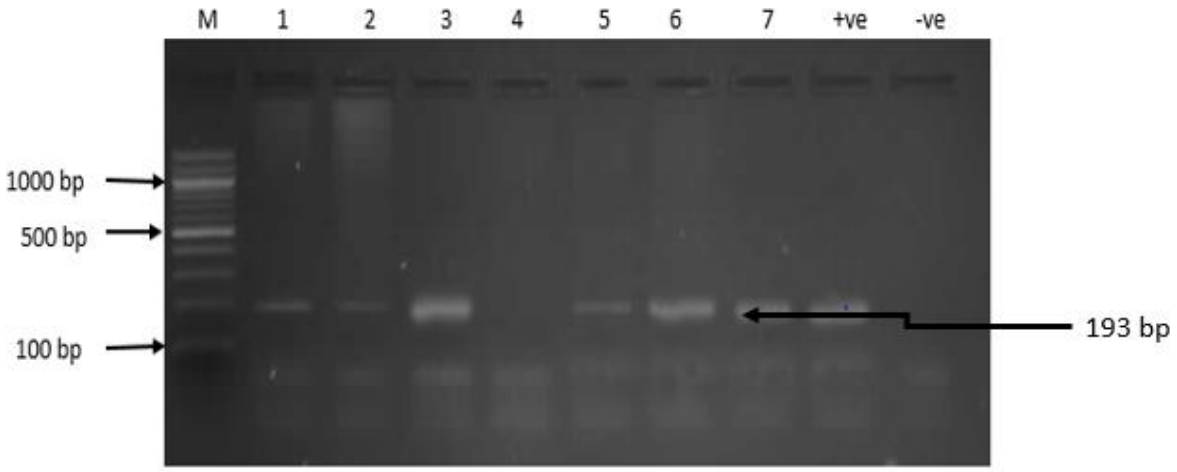


Figure 4.5 Agarose gel showing amplified product of 193 bp for *omp*-2 gene in *B. abortus*. Lane 1–7 aresamples positive for *omp* 2, Lane (+ve): positive control (-ve): negative control, Lane M: 100 bp DNA ladder.

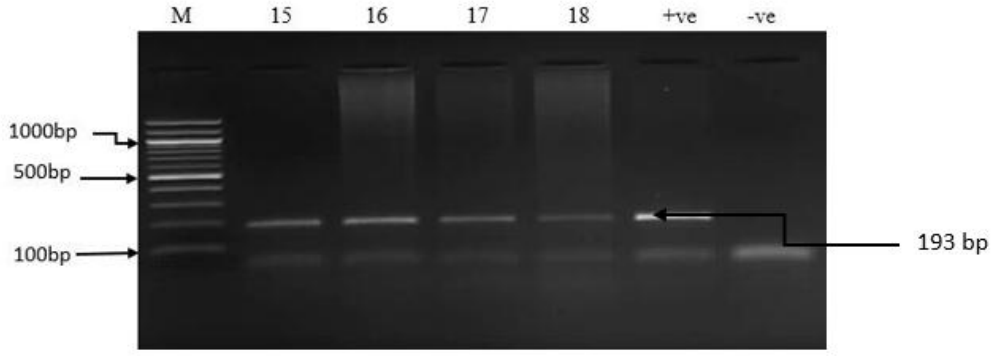


Figure 4.6 Agarose gel showingamplified product of 193 bp for *omp*-2 gene in *B. abortus*. Lane 15–18 are samples positive for *omp* 2, Lane (+ve): positive control, Lane (-ve): negative control, Lane M: 100 bp DNA ladder.

4.21 Detection of *Coxiella burnetii* Infection in Blood Samples of Cattle, Sheep and Goats in Maigana and Birnin GwariAgro-ecological Zones of Kaduna State Using PCR

Detection of *Coxiella burnetii* with CoxP4 and CoxM9 primers, has been carried out using PCR, the *htpAB* gene amplification size of448 bp was compared with the ADN Cb NM reference strain used as control.Positive amplification was obtained, using the primers which amplified the repetitive transposon-like and transposase regions of *C. burnetii*, from blood of cattle, sheep and goats respectively.Of the 48 blood samples confirmed by iELISA, 7/48 (14.6%) showed positive for *C. burnetii* DNA. Of these, (7.1%) were from cattle, (20.0%) from goats, and (30.0%)were from sheep respectively (Figures 4.7 and 4.8).

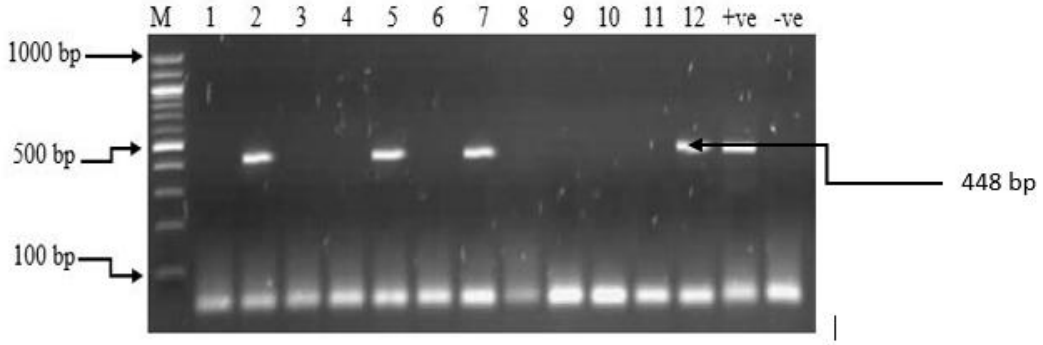


Figure 4.7 Agarose gel showing amplified product of 448 bp for *htpAB* gene in *C. burnetii*. Lane 2, 5, 7 and 12 are samples positive for *htp*AB, Lane (+ve): positive control, Lane (-ve): negative control, Lane M: 100 bp DNA ladder.

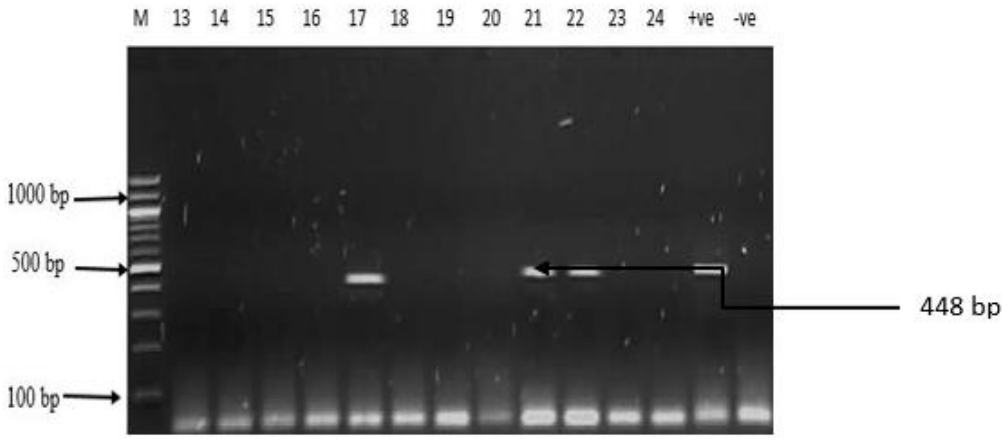


Figure 4.8 Agarose gel showing amplified product of 448 bp for *htpAB* gene in *C. burnetii*. Lane 17, 21–22 are samples positive for *htp*AB, Lane (+ve): positive control, Lane (-ve): negative control, Lane M: 100 bp DNA ladder.

4.22 Persistence of *Brucella* spp. and *Coxiella burnetii* After Six Months in Seropositive Cattle Herds in Maigana and Birnin Gwari Agro-ecological Zones of Kaduna State

Of the 36 herds that had previous history of abortion, 21 (58.3%) was seropositive for *Brucella*by cELISA and 28 (77.8%) was seropositive for *Coxiella burnetii* by iELISA. Of the 21 samples positivefor *Brucella*by cELISA, 15 (60.0%) were from Maigana agro-ecological zone and 6 (54.5%) were from Birnin Gwari agro-ecological zone of Kaduna State. Of the 28 samples positive for *Coxiella burnetii*by iELISA, 8 (72.7%) were from Birnin Gwari agro-ecological zone and 20 (80.0%) were from Maigana agro-ecological zone of Kaduna State (Table 4.17). After six months later, the herds that had previous history of abortion were retested with PCR for the evidence of persistence of infections. Out of the 21*Brucella*positive samplesby cELISA, 12 (57.1%) were positive by PCR. Out of the 6 *Brucella* positive samples from Birnin Gwari agro-ecological zone, 4 (66.7%) were positive by PCR and out of the 15 *Brucella* positive samples from Maigana agro-ecological zone, 8 (53.3%) were positive by PCR. Out of the 28 *Coxiella burnetii*positive samples by iELISA, 2 (7.1%) were positive using PCR. Out of the 20 *Coxiella burnetii* positive samples from Maigana agro-ecological zone, 2 (10.0%) were positive by PCR, and out of 8 *Coxiella burnetii* positive samples from Birnin Gwari agro-ecological zone, none was positive with PCR(Table 4.18).

Table 4.17Distribution of *Brucella*spp. and *Coxiella burnetii*positive animals in cattle herds with history of abortions in Maigana and Birnin Gwari agro-ecological zones of Kaduna State

Location	No. of Herds with Abortion	*Brucella* (cELISA)		*C. burnetii*(iELISA)	
		+ve (%)	-ve (%)	+ve (%)	-ve (%)
B/Gwari	11	6 (54.5)	5 (45.5)	8 (72.7)	3 (27.3)
Maigana	25	15 (60.0)	10 (40.0)	20 (80.0)	5 (20.0)
Total	**36**	**21 (58.3)**	**15 (41.7)**	**28 (77.8)**	**8 (22.2)**

Table 4.18Persistence of *Brucella* and *Coxiella burnetii* Infections in Cattle Herdsin Maigana and Birnin Gwari Agro-ecological zone of Kaduna State After Six Month

Location	*Brucella*			*Coxiella burnetii*		
	cELISA + ve	**PCR +ve (%)**	**PCR -ve (%)**	**iELISA +ve**	**PCR +ve (%)**	**PCR -ve (%)**
B/Gwari	6	4 (66.7)	2 (33.3)	8	0 (00)	0 (00)
Maigana	15	8 (53.3)	7 (46.7)	20	2 (10.0)	18 (90.0)
Total	**21**	**12 (57.1)**	**9 (42.9)**	**28**	**2 (7.1)**	**18 (64.3)**

CHAPTER FIVE

5.0 DISCUSSION

Brucellosis is a zoonotic disease which should be diagnosed at the earliest stage.In order to prevent damages that could arise from infection with organism. Various diagnostic assays are used for the detection of *Brucella* spp. from cattle, sheep and goats which include basic bacteriological, serological and molecular assays (Da Silva Mol *et al*., 2012). Basic bacteriological techniques are laborious and hazardous to the laboratory personnel as the organism is infectious (Sam *et al*., 2012).

The study demonstrated that the overall individual animal level seroprevalence of brucellosis in cattle, sheep and goats were 18.5% and 6.8%, 16.8% and 10.8%, 12.0% and 6.0% by RBPT and cELISA respectively. In cattle, the seroprevalence of 18.5% and 6.8% obtained in this study by RBPT and cELISA respectively, were found to be similar to previous studies in Tanzania (Assenger *et al.*, 2015) which reported7.5% and 6.8% by RBPT and cELISA but lower than the findings of other workers in some parts of Nigeria;37.0% and 26.3% by RBPT and cELISA (Mai *et al*., 2012), 34.0% and 23.0% by RBPT and cELISA(Adamu *et al*., 2014) andin African and othercountries; 24.5% by RBPT and cELISA in Sudan (Angara *et al*., 2010); 20.7% by RBPT and cELISA in Zambia (Muma *et al*., 2013); 21.9% by RBPT and cELISA in Kenya (Nakeel *et al*., 2016); 23.8% by RBPT in Egypt (El-Diasty *et al*. (2016); 21.4% by RBPT and cELISA in Bangladesh (Islam *et al*., 2013).But the seroprevalence were higher than the reports of

some workers in Nigeria; 4.0% and 3.9% by RBPT and cELISA (Farouk*et al*., 2011), 4.9% and 3.8% by RBPT and cELISA(Ogugua *et al*., 2015), in other countries; 5.5% by cELISA in Zimbabwe (Matope *etal*., 2011);3.5% by RBPT and cELISA in Uganda (Nizeyimana *et al*., 2013); 1.2% by RBPT and cELISA in Algeria (Khaldi *et al*., 2016); 7.7% and 6.2% by RBPT and cELISA in India (Raghunatha *etal*., 2014); 11.4% and 2.5% by RBPT and cELISA inBrazil (Borba *et al*., 2013). The high seroprevalence obtained in this study may be attributed to non-vaccination against brucellosis, low patronage of veterinary services and pastoralists low level of western education. Other factors could be the differences in the sample sizes in the various studies, differences in the animal production systems and husbandry and differences in the geographical regions and ecological settings.

In this study, the seroprevalencesof 16.8% and 10.8% obtained in sheep by RBPTand cELISA respectively, which were lower than the 26.5% by RBPT from Kaduna (Kaltungo *et al*., 2015), 35.2% and 23.8% by RBPT and cELISA from Bauchi(Ya'u *et al*., 2017), 24.0% by RBPT in Libya(Ahmed *et al*., 2010).However, the seroprevalenceswas higher than 11.2% by RBPT in Yobe State, Nigeria (Tijjani *et al*., 2010),11.1% by RBPT in TarabaState, Nigeria (Zubairu *et al*., 2014), 2.5% and 1.5% by RBPT and cELISAin Sudan (Abdallah *et al*., 2015), 8.6% by RBPT and cELISAin Kenya (Nakeel *et al*., 2016) and 2.3% by cELISA in Bangladesh (Rahman *et al*., 2011). In goats, theseroprevalence of 12.0%and 6.0% obtained in this study by RBPT and cELISArespectively, werehigher than 8.2% and 2.5% by RBPT and cELISA in Nigeria (Dogo and Maikai, 2015), 2.0% and 1.6% by RBPT and cELISA in Tanzania (Assenger *et al*., 2015),5.1% by RBPT and cELISA in Egypt (Al-Habaty *et al*., 2015) in Egypt, 1.6% by RBPT in Ethiopia

(Mohammed *et al*., 2015), 7.3% by RBPT and cELISA in Kenya(Nakeel *et al*.,2016) and 3.2% by cELISA in Bangladesh (Rahman *et al*., 2011). But the seroprevalence rateswere lower than 22.9% by RBPT and cELISAin Sokoto State (Junaidu *et al*.,2010), 25.8% by RBPT in Kaduna State (Kaltungo *et al*., 2013), 20.0 % by RBPT in Taraba State (Zubairu *et al*., 2014),23.6% and 13.5% by RBPT and cELISA)in Bauchi State (Ya'u *et al*., 2017), 31.0% by RBPT in Libya (Ahmed *et al*.,2010), 15.0% by RBPT in Ethiopia (Adugna *et al*., 2013),16.3% by cELISA in Sudan (Zein and Adris, 2015). The high rates of brucellosis in sheep and goats may be dueto free grazing and movement of these flocks which contribute to the wide distribution of brucellosis in these animals and to other animal species and due to non-vaccination against brucellosis (Mantur and Amarnath, 2008; Al-Habaty *et al*.,2015).

From the study, the seropositivity were higher in females than male animals studied. These findings agreed with reports of other workers in Nigeriawhich associated brucellosis with sex. For examples Junaidu *et al*. (2010), Farouk *et al*. (2011), Kaltungo *et al*. (2013), Adamu *et al*. (2014),Akinseye *et al*. (2016) and Hashimu *et al*. (2017). In African countries, Niger; Boukary *et al*. (2013), Egypt; Al-Habaty *et al*. (2015),Sudan; Abdallah *et al*. (2015) and Ebrahim *et al*. (2016) and in Bangladesh; Belal and Ansari (2013).These could be due to the fact that female animals are kept for a comparatively longer period within the breeding herds /flocks compared to male animals and so increases the risk of exposure to infections (Dinka and Chala, 2009) but it could also be due to high concentration of erythritol in the placenta and foetal fluids of female which stimulates the growth of the organisms. It could also be due to the fact that the organisms stay long in the

female genitalia and could be as a result of more samples were taken from female.In contrast to this finding, Mai *et al*. (2012) in Nigeria and Shafee *et al*. (2012) in Pakistan reported higher seroprevalence in male than female cattle. Adamu *et al*. (2007) in Nigeria reported that in ruminants, brucellosis infection rates were uniform for both males and females and that there was no significant association between infection rates and sex.

The results of this study revealed the seroprevalencesof 19.0% and 7.1% by RBPT and cELISA respectively, in cattle greater than 4 years of age which were higher than 17.8% and 6.3% obtainedby RBPT and cELISA in the cattle less than 4 years old.This agreed with some reports from Nigeria; Farouk *et al*. (2011) and Ogugua *et al.* (2015), and from Ethiopia; Bashitu *et al.* (2015).*Brucella abortus* infection may occur in cattle of all ages but persists commonly in sexually mature and pregnant cattle (Radostits*et al*., 2000),in sheep and goats, the age category prevalence of brucellosis has shown that it was higher in age bracket of 2 to 4 years old compared to the rest of the age groups.This observed difference agreed with the earlier reports in Nigeria; Junaidu *et al*. (2010) and Dogo and Maikai (2015), Senein and Abdelgadir (2012) in Sudan, Nigatu *et al.* (2014) in Ethiopia, but in contrast with Bashitu *et al.* (2015) in Ethiopia. It has been reported that brucellosis is essentially a disease of sexually mature animals (Quinn *et al*., 1999). Sexually mature and pregnant animals are more prone to *Brucella*infection than sexually immature animals of either sex (Radostits *et al*., 2000). On the other hand, it is also true that younger animals tend to be more resistant to infection and frequently clear an established infection, although latent infections can occur (Walker, 1999). This may result from the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella*organisms, tend to

increase in concentration with age and sexual maturity (Radostits*et al*., 2000). Age is one of the intrinsic factors which can influence susceptibility of *Brucella*infection.

Although not statistically significant, the seroprevalence of brucellosis was lower in Sokoto Gudali (Bokoloji) breeds than amongst White Fulani (Bunaji) breeds.This is in agreementwith the findings of other reports in Nigeria (Mai *et al*., 2012; Olabode *et al*., 2012; Cadmus *et al*. 2013), but in contrast to the report of Junaidu *et al.* (2008), who reported higher seroprevalence of brucellosis in Sokoto Gudali (Bokoloji) breed,Esuruoso (1974) reported that the Ndama breed was the most affected breed in western Nigeria, while Cadmus *et al*. (2008) found the Red Bororo breed to have the most positive reactors followed by Bunaji. Junaidu *et al*. (2011) reported the Sokoto Gudali(Bokoloji) breed to have the highest prevalence, followed by Azuwarq, with Bunaji having the least prevalence. In this study, White Fulani (Bunaji) breed had highest seroprevalence however, they are the predominant breed in the study area. Genetic variation is an important factor in conferring resistance or tolerance of cattle breeds to diseases, whilst the antibody response of animals resistant to infection by *B. abortus* differed significantly from those of susceptible ones (Martínez *et al*. 2010).The seroprevalence was higher in Yankasa and Balami breeds than in Ouda breed. This is in contrast to the finding of Junaidu *et al.* (2008) who reported higher seroprevalence in Ouda breed in Nigeria, but agreed with the findings of Tsegay *et al.* (2015) in Ethiopia.

From this study, a higher seroprevalence of *Brucella* antibodies in Sokoto Red and Sahelian goat breedswere recorded than in the West African Dwarf breed using both RBPT

and cELISA. This agreed with the findings of other researchers in Nigeria (Tijjani *et al*., 2009; Junaidu *et al.*,2010; Ogugua *et al.,* 2014).However, it is in contrast with the findings of Junaidu *et al.* (2008), Dogo *et al.* (2016) in Nigeriaalso reported a higher seroprevalence in Sahelian breed. However, previous reports have shown that *Brucella* infection is not breed specific (Ajogi *et al.*, 2002; Bala, 2013). This might be due to the large number of different breeds of small ruminants owned by these communities, which graze together in communal grazing lands or use the same watering points. Though there was no statistically significant difference in the seroprevalence rates between the animals from the two zones two zones tested, the seroprevalence was however higher among animals sampled in Maigana agro-ecological zone of Kaduna State. Brucellosis in cattle, sheep and goats is therefore, well distributed across the entire Kaduna State. This might well be attributable to the use of similar animal production and management systems throughout the zones of the State, as well as fairly similar agro-ecological conditions. Moreover, unrestricted animal movement in search of pasture and water, trade within and between zones, the mixing of animals at market places and watering points, especially during the dry season might have enhanced the spread of infection.

The herd level prevalence was higher than individual animal level and this characterizes the nature and importance of the disease in the herds. This signifies that brucellosis has significant economic implication in its ability to cause morbidity at herd level. Although, higher seroprevalence was obtained among non-lactating cows and small ruminants, there was no statistically significant association between lactation status and positive serological reactions. Thiscorroborates with the report of Mekonnen *et al.* (2011) and Mai *et al.* (2012)

but inconsistent with findings by Ibrahim *et al*. (2010). A seroprevalence of 6.2% in this study was much lower than 25% obtained in lactating cows in Sokoto State, Nigeria (Junaidu *et al*., 2011). The more fact that lactating cows were seen to have high seroprevalence, it could mean serious public health risks since Nigerian especially those of Northern origin take high measure in consumption of of soured milk with sorghum paste (Buhari *et al*., 2015)

In this study, indirect enzyme linked immunosorbent assay (iELISA) was used to detect antibodies to *Coxiella burnetii*,which is reported to be suitable for epidemiologic studies. Moreover, it is more sensitive, faster and convenient than both Complement Fixation Test (CFT) and Indirect Immunofluorescent Antibody (IFA) (El-Mahallawy *et al.* 2016).From this study it is indicated that cattle, sheep and goats were exposed to *C. burnetii* in Kaduna State, Nigeria, with an overall seropositivity of 6.2%, 8.0% and 8.8%in cattle, sheep and goats, respectively. This was much lower than the previous studies done in the last 4 decades in Northwestern Nigeria. Addo and Schnurenberger (1977) recorded a seroprevalence of 11.0%, 16.4% and 8.8% in cattle, sheep and goats in Nigeria, respectively using Capillary Agglutination Test (CAT). The difference in the seroprevalence between these former studies and this study might be partially attributed to the different techniques used in determining these seroprevalence.

In cattle, the seroprevalence of 6.2% recorded in this study was lower than the seroprevalence of 14.5% recorded in Kaduna State, Nigeria (Tukur *et al*., 2014),in other African countries, 18.0% in Ghana(Adu-Addai *et al.*, 2012),7.8% in Lake Chad (Abakar *et*

al., 2014), 10.5% by in Kenya (Wardrop*et al.*, 2016) and 11.8% in France (Cardinale *et al.*, 2014), 41.3% in Spain (Astobiza *et al.*, 2013).The seroprevalence was higher than4.0% in Senegal (Kamga-Waladjo *et al.*2010), 0.8% in Iran(Edalati-Shokat *et al.*, 2015), 3.6% in Bangladesh (Rahman *et al.*, 2016) and 3.4% in United State of America (McQuiston and Childs, 2002), but comparable to work reported in Bangladesh (Chakrabartty *et al*,.2016).

In sheep,theoverall seroprevalence of Q fever obtained in this study was8.0%, which was found to be lower than 16.5% by CAT in Nigeria (Addo and Schnurenberger, 1977), 11.0% by iELISA in Chad (Schelling *et al*., 2003), 18.5% by iELISA in Gambia (Klaasen *et al*., 2014)27.8% by iELISA in Algeria (Abdelkadir *et al*., 2017), 32.7% by iELISA in Egypt (Ghoneim and Abdel-Moein,2012), 57.5% by iELISA in Kenya (Nakeel *et al*., 2016), 62.5% by iELISA in Sudan (Reinthaler *et al*., 1988), 9.5% by iELISA in Bangladesh (Rahman *et al*., 2016), 19.4% by iELISA in Iraq (Kshash, 2012), 24.7% by iELISA in Iran (Mohabbati *et al*., 2017), 30.0% by iELISA in Pakistan (Zahid *et al*., 2016), 12.3% by iELISA in Spain (Ruiz-Fons *et al*., 2010), 15.9% by iELISA in Italy (Rizzo *et al*., 2016),and 28.0% by iELISA in Germany (Hilbert *et al*., 2012). But the seroprevalence was higher than 7.0% by iELISA in Tunisia (Rekiki *et al*., 2005), 5.4% by iELISA in Turkey(Ceylan *et al.*, 2009), and 1.4% by iELISA in France (Cardinale *et al.*, 2014).

In goats, the overall seroprevalence of Q fever obtained in this study was 8.8%, which was lower than 9.5% by CAT in Nigeria (Addo and Schnurenberger, 1977), 13.5% by iELISA in Chad (Schelling *et al*., 2003),14.1% by iELISA in Algeria (Khaled *et al*., 2016), 23.0%

by iELISA in Egypt (Nahed *et al*., 2012), 24.0% by iELISA in Sudan (Hussein *et al*., 2012), 24.2% by iELISA in Gambia (Klaasen *et al*., 2014),19.5% by iELISA in Italy (Rizzo *et al*., 2016), 21.4% by iELISA in the Netherland (Schimmer *et al*., 2011) and 31.9% by iELISA in Iran (Mohabbati *et al*., 2017). The seroprevalence was comparable to 7.6% by iELISA in Bangladesh (Chakrabartty *et al*., 2016), but the seroprevalence was higher than 3.3% by iELISA in Bangladesh (Rahman *et al.*, 2016), and 5.3% by iELISA in Saudi Arabia (Mohammed *et al*., 2014). The differences in seroprevalence rates among different areas could be attributed to climatic variations, frequency of tick population, type of management practices, herd/flock size that might influence the transmission rates and infection with *C. burnetii* and could either be related to the overall cattle, sheep and goats seroprevalence status of the disease or number of cattle, sheep and goats per the studied (herd/flock sizes).

There was no significant association between seroprevalence of infection and sex of cattle, sheep and goats in this study, though the rates of infection were higher among females than males. This may be due to the fact that the organism has a high affinity for foetal membranes, mammary glands and the placenta and the organism is found in large numbers in these tissues. This agreed with the study inNigeria by Tukur *et al*. (2014) in cattle, in Kenya by Wardrop *et al.* (2016) in cattle, in Iran by Edalati-Shokat *et al.* (2015) in sheep and goats, where there was no significant association between seroprevalence of coxiellosis and sex of cattle, sheep and goats in the previous reports.Sakhaee and Khalili (2010) reported seroprevalence of 18.8% in female and 10.6% in male animals from Iran, a difference that was statistically significant ($P<0.05$). But the seroprevalence was in contrast

to the report of Rahman *et al.* (2016) in Bangladesh who reported high seroprevalence of coxiellosis in male cattle than in female cattle, though there was no significant association between the seroprevalence of infection and sex of the animals studied. The hormonal differences between males and females play an important role in determining susceptibility to infection (Cantas *et al*., 2011). Estrogen enhances antibody production and androgen suppresses both T-cell and B-cell immune responses, but immunity in females can be broken down due to various factors such asage, pregnancy and environmental factors (Cantas *et al*., 2011; Porter *et al*., 2011).Cattle greater than 4 years had higher seroprevalence of infection than cattle less than 4 years old. Though there was no statistical significant association between the infection and the age. Cattle greater than 4 years are more likely to be breeding and hence shedding the organism, especially during parturition. Cattle less than 4 years are less likely to shed the organism in high numbers. This agreed with the findings of Tukur *et al.*(2014) in Nigeria andSchimmer *et al.*(2010) in the Netherland.

In sheep and goats, animalsthat were greater than 4 years old exhibited a greater rate of seropositivity for *C. burnetii* than did the two other age groups i.e. less than 2 years old and 2–4 years old. There was a statistically significant difference between the seroprevalence of coxiellosis and age of the goats studied.But there was no statisticallysignificant association in theseroprevalence of coxiellosis and age in sheep.This is in contrast with the reports of Esmaeili *et al.* (2013) in Spain. But the findings were at variance to those of the previous reports from Spain by Ruiz-Fons *et al*. (2010), Gambia (Klaasen *et al*., 2014), and Iran (Ezatkhaha *et al.*,2015)who variously reported high

seroprevalence in the age greater than 4 years old.This findings also suggests the occurrence of horizontal transmission among animals and the maintenance of infection within adult populations (Ruiz-Fons *et al*., 2010, Astobiza *et al*., 2011).Though, there was no statistically significant association between the seroprevalence of coxiellosis and breeds of cattle, sheep and goats studied, but seroprevalence was higher in White Fulani (Bunaji)thanSokoto Gudali (Bokoloji) breeds. In sheep, the seroprevalence was higher in Yanksa followed by in Ouda and the leas was in Balami breed, in goats, the seroprevalence was higher in Sokoto Red followed by West African Dwarf and the least was in Sahel goats.

There was no statistically significant association between the seroprevalence of coxiellosis and the distribution by location in cattle, sheep and goats studied, which suggests that location of the animal is not a risk factor forcoxiellosis. This might be due to the use of similar animal production and management systems throughout the agro-ecological zones of the state, as well as fairly similar agro-ecological conditions. Moreover, unrestricted animal movements may have enhanced the spread of infection, such as movements of animals in search of pasture and water, trade within and between agro-ecological zones, the mixing of animals at market places and watering points, especially during the dry season. The difference in seroprevalence between lactating and non-lactating cows and small ruminants studied was not statistically significant, though it was higher in the non-lactating animals, which is consistent with the report of Schimmer *et al*. (2012).

Despite the fact that all the respondents observed brucellosis related clinical signs in their herds such as abortion which had been seen once and sometimes more than once in some herds. The rates of occurrence of abortionscases only once was 75.0%, and 37.5% abortion cases occurring more than once.It was observed that knowledge about brucellosis was lacking among herdsmen. The level of patronage of veterinary services was very low, while only 21.8% of the herds had previously been vaccinated against brucellosis, the low vaccination rate because the vaccine was expensive and not readily available in Nigeria.The migratory herdsmen were not vaccinating against brucellosis, because the vaccines were not available even in the neighboring countries. These may account for the high prevalence rates of the brucellosis in this study, similar to previousfindings by Negash *et al*. (2011). Up to 71.4% the herdsmen disposed aborted foetuses in the open field using bare hands.

In this study, spatial distribution of brucellosis and Q fever was produced for the study areas,which indicated that the two diseases were widely distributed. The wide distribution could be accounted for by the fact that the livestock owners have very low awareness of the two diseases, especially with regards to their mode of transmission and clinical signs (Adamu *et al*., 2012).The extensive management system for these animals could also account for the spread of the two diseases asKaltungo *et al*. (2013) and Buhari *et al*. (2015)reported livestock owners dumping aborted foetuses which could be contaminated with *Brucella* species.Kaduna State, particularly Northern part share border with Katsina, Kano and Bauchi States, it possible for infection to originate from as far as Niger or Chad Republics into neighbouring states and eventually into Kaduna State. Uncontrolled animal

movements across borders is known to facilitate transmission of infection among animals (Gwida *et al*., 2010; Lounes *et al*., 2014). In our study, the majority of cattle herds, goats and sheep flocks in the semi-arid regions are mobile and represent also a reservoir of the infection to other small ruminants. It is difficult to control the movement of animals kept under nomadic or semi nomadic conditions (Corbel, 2006).Our findings also imply that brucellosis and Q fever were highly associated with population size and distribution of cattle in rural areas of Maigana and Birnin Gwari agro-ecological zones. Most of the affected villages were seen in Maigana agro-ecological zone of Kaduna State which had higher density of cattle.

In the present study, *Brucella abortus*DNA was detected in blood samples of cattle. On the other hand, none of the blood samples from sheep and goats was positive for *Brucellamelitensis*or *Brucella ovis* DNA. Genus specific primers (*omp2*) showed that 12/40 (30.0%) of blood samples were positive. The application of molecular techniques has facilitated the identification of some *Brucella* species. The present study was able to confirm the persistence of brucellosis in the herds of cattle in the study area. This is in line with the report among cattle in Zimbabwe by Gomo *et al*. (2012). The results indicate clearly that cattle, sheep and goats in Nigeria may play an important role as reservoirs of brucellosis and that they could be a significant source for the transmission to human beings. The prevalence of *B. abortus* from whole blood of cattle using PCR was 30.0% showing that *B. abortus* can be detected from whole blood sample. Similar results that DNA can be extracted from whole blood and used for screening animals for brucellosis were reported by Keid *et al*. (2010) in dogs and Khamesipour *et al*. (2013) in cattle and

sheep. Our study revealed that the prevalence rate of brucellosis in Nigeria was in close agreement with a previous report conducted in Jos by Antiabong *et al*. (2009).Various factors like time of sample collection, infection status of the animal, condition of the herds, number of samples collected can all influence the detection rates.

The present study is to our knowledge the first report on the direct detection of *C. burnetii* from the blood of cattle, sheep and goats in Nigeria using PCR. It appears that cattle, sheep and goats are likely to harbor *Coxiella burnetii* organism and that they are capable of shedding it through milk, blood, faeces and urine. In cattle, the prevalence of 14.3% obtained in this study from blood using PCR, is in contrast with the report of Abdel-Rahman(2014), who did not dectect *C. burnetii* DNA in cattle samples tested with PCR in Saudi Arabia. In sheep and goats, the prevalences of 11.1% and 16.7%obtained respectively in this study using PCR was much higher than the work reportedby Edalati-Shokat *et al.* (2015)who detected the prevalence of 2.0% in goats in Iran using PCR. In the present study, it has been shown that the highest concentration of organism was found in the blood of sheep followed, in descending order, by goats and cattle.

The differences between the prevalence of *C. burnetii* DNA in cattle, sheep, and goats in this study may be due to the fact that there are different routes of shedding the organism in different animal species. According to Rodolakis *et al*. (2007), sheep shed the organism mainly in the faeces and vaginal mucus while cattle shed the organism in milk. Goats shed the organism via vaginal discharges, faeces and milk. The few samples that were positivefor*C. burnetii* DNA from the cattle, sheep, and goats obtained in the this study

could be attributed to the fact that in these animal species, the organism is shed primarily via vaginal mucus and faeces.The virginal mucus and faeces were not tested in the present study and this probably confirms that blood is not the preferred routes of discharge for *C. burnetii* in cattle, sheep, and goats. It could be partly attributed to the small number of samples tested. It could also be due to the fact that samples were taken during the bacteremic phase of the infection.Molecular studies in cattle, sheep, goats and other animal species are limited due to lack of simple and sensitive detection tools. The present study has for the first time presented information on the direct detection of *C. burnetii* in cattle, sheep, and goats in Nigeria. The current study again shows clearly that cattle, sheep, and goats may be important reservoirs of *C. burnetii* and the animals are likely to shed *C. burnetii* and pose a significant public health hazard for the transmission of Q fever to humans in areas in which they are reared.

In this study, the occurrence of single and mixed infections due to brucellosis and Q fever has been demonstrated. The occurrence of brucellosis was highest in sheep (9.0%), followed by cattle (6.3%) and the least was observed in goats (4.3%). This could be as result of the fact that sheep are considered to be more prone to brucellosis, however sheep contract *B. abortus* through the grazing together with cattle. The occurrence of Q fever was highest in goats (7.0%) followed by sheep (6.2%) and the least was observed in cattle (5.8%), which could be due to the fact that sheep and goats are the secondary reservoirhost for *Coxiella burnetii*(Webster *et al*., 1995).

The distribution of *Brucella* and *Coxiella burnetii* in cattle herds with history of abortion in the study area was higher in Maigana agro-ecological zone than in Birnin Gwari agro-ecological zone. Out of the 36 herds that had previous history of abortion, and later retested by PCRonly 12 herds showed persistence of *Brucella* infection and 2 herds showed persistence of *Coxiella burnetii* infection.This suggests persistence of infection and that the organisms were present in the animals. It also suggests that infections with the two organisms to be endemic in the study area. This could translate to serious public health risks in the communities around the herds, especially the pastoralists who have the habit of drinking milk directly from the udder of their cattle. The fact that they regard goat milk to have exceptional medicine potentials (Kaltungo *et al*., 2013).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

From this work it was concluded as follows:

1. *Brucella* species and *Coxiella burnetii* are present in cattle, sheep, and goats at seroprevalence rates of 6.8% and 6.2% in cattle, 10.8% and 8.0% in sheep and 6.0% and 6.8% in goats using cELISA and iELISA respectively in Maigana and Birnin Gwari agro-ecological zones of Kaduna State, Nigeria.
2. Presence of *Brucella* species and *Coxiella burnetii* as single infections 6.3% for *Brucella* species and 5.8% for *C. burnetii* in cattle, 4.3% for *Brucella* species and 7.0% for *C. burnetii* in goats and 9.0% for *Brucella* spp and 6.3% for *C. burnetii* in

sheep), and mixed infections (0.5% in cattle, 1.8% in goats and sheep each) in Maigana and Birnin Gwari agro-ecological zones of Kaduna State, Nigeria.

3. *Brucella* species were demonstrated at higher prevalence in older animals (6.9% in cattle, 8.9% in sheep and 9.5% in goats) than in younger ones (5.0% in cattle, 6.4% in sheep and 7.5% in goats).
4. *Coxiella burnetii* antibodies were demonstrated at higher prevalence in older animals (6.6% in cattle, 8.2% in sheep and 13.7% in goats) than in younger ones (5.7% in cattle, 7.8% in sheep and 0.0% in goats).
5. Higher antibodies to *Brucella* species in non-lactating female cattle (7.1%)than lactating ones (6.2%) and non-lactating female sheep (12.0%) than in lactating ones (6.5%) and in non-lactating female goats (7.2%) than in lactating ones (3.7%).
6. Detection of *Brucella* species and *Coxiella burnetii* organisms through the use of PCR in the blood of cattle, sheep and goats were obtained.
7. Production of spatial mapping of the areas with cattle, sheep and goats infected with *Brucella* species and *Coxiella burnetii*.

6.2 RECOMMENDATIONS

Based on the findings, the following recommendations were made:

1. Further serological survey on brucellosis and Q fever in Kaduna State should be carried out in areas that were not included in this study to give a better picture on the occurrence of brucellosis and Q fever in the State.
2. A national study on brucellosis and Q fever should be embarked upon with a view to understanding the epidemiology of these two diseases to enable the

establishment of a national brucellosis and Q fever management programmestowards their eradication.

3. Screening of domestic livestock for brucellosis and Q fever should be performed on a regular basis and the implementation of strict control programmes especially in dairy cattle and livestock farms/ flocks of sheep and goats.
4. Strict quarantine measures should be applied when introducing new animals into herds/flocks.
5. There is the need for educating the livestock owners and the general public on the two diseases (brucellosis and Q fever) with a view to effectively control these diseases not only in animals but also in humans
6. There is need for the government at both National and State levels, to institutionalize effective surveillance for diseases, especially zoonotic ones.

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APPENDIX I

Questionnaire

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Questionnaire on Seroprevalence and Co-occurrence of *Brucella* spp. and *Coxiella burnetii* in cattle in Maigana and Birnin Gwari Agricultural zone of Kaduna State, Nigeria.

This questionnaire is designed to study the epidemiology of brucellosis and Q-fever in cattle in Maigana and Birnin Gwari agricultural zone of Kaduna State. Name or identity of the owner is not required in this questionnaire, all the information gathered will be used for the research purpose only.

Herd Code: _____

A Pastoralist Personal Data

1. Marital status: Married [ ] Single [ ]
2. Age: Adult [ ] Young [ ]
3. Number in the family: _____
4. No. of: Male [ ] Female [ ]
5. Number of children: _____
6. No. of children: Male [ ] Female [ ]

B Educational Background

1. Western nomadic education: [ ]
2. Quranic education (Tsangaya): [ ]
3. None: [ ]

C Type of labour employed

1. Family responsibility: [ ]
2. Head of the family: [ ]
3. Children: [ ]
4. Hired laborers: [ ]

D Location of the herd: _____

E Local Government: _____

F Herd/Flock profile

1. Herd/Flock size: _____

2. Sex Distribution:

Animal species	Number		Total
	Male	Female	
Cattle			
Goats			
Sheep			

3. Age Distribution:

Animal species	< 1 Year	1–2½ Years	>2½ - 5Years	> 6 Years
Cattle				
Goats				
Sheep				

4. Other animal species in herd/Flock:

Camel [ ]

Horses [ ]

Donkeys [ ]

Dogs [ ]

5. Breed of Livestock:

White Fulani (Bunaji) [ ]

Sokoto Gudali(Bokoloji) [ ]

Red Bororo (Rahaji) [ ]

G Herd medical history

1. Do you vaccinate your animals? Yes [ ] No [ ]

If yes: *Brucella*vaccine [ ]

Black quarter vaccine [ ]

Anthrax spore vaccine [ ]

Haemorrhagic septicemia vaccine [ ]

Q fever vaccine [ ]

Others specify_____

None at all [ ]

2. Do you practice tick control? Yes [ ] No [ ]

If yes: Dipping/spray [ ]

Tick bath [ ]

Injectable [ ]

Hand picking [ ]

3. Do you practice routine deworming? Yes [ ] No [ ]

4. Have you had any outbreak of?

Abortion [ ], Hygroma [ ], Still birth [ ], Retained placenta [ ],

Orchitis [ ], Repeat breeder [ ], in the last one year.

5. If yes to the above question, how many

Abortion number _____

Still birth number _____

Retained placenta number_____

Repeat breeders _____

Orchitis _____

Hygroma _____

6. How do you dispose after birth material and aborted fetus?

   Bury [ ], Burn [ ], in refuse bin [ ], others specify _____

7. Do you consume raw milk from your animals? Yes [ ] No [ ]

H Availability of veterinary Services Yes [ ] No [ ]

State veterinary hospital [ ]

Local veterinary clinic [ ]

Private veterinary services [ ]

Local remedies [ ]

None at all [ ]

Thank you for usual cooperation

Dr. Shuaibu Gidado Adamu

APPENDIX II

Individual Animal Record Form

1. Herd/Flock Number: _____
2. Date of Sample Collection: _____
3. Serial Number: _____
4. Age of the Animal: _____

5. Breed of the Animal: _____

6. Sex of the Animal: Male [ ] Female [ ]

7. Physiological Status of the Animal: Milking [ ] not Milking [ ]

8. History of Abortion: Yes [ ] No [ ]

9. Presence of Ticks on the Animal: Yes [ ] No [ ]

APPENDIX III

Possible risk factors associated with *Brucella* infection in cattle herds in Maigana and Birnin Gwari agro-ecological zones of Kaduna State

	***Brucella* Herd Status**					
Factor	**+ve (%)**	**-ve (%)**	**No.**	**OR**	**95% CI**	**P value**
Herd size						
-10–20	23 (48.9)	24 (51.1)	47	0.414	0.189–0.908	0.043
- > 20	44 (69.8)	19 (30.2)	63	1*		

Breeds						
-WF	50 (63.3)	29 (36.7)	79	1.245	0.534–2.907	0.665
-WF+SG	18 (58.1)	13 (41.9)	31	1*		
Abortion						
-No cases	0 (00)	74 (100)	74	3.857	1.366–10.893	0.016
-Once	15 (75.0)	5 (25.0)	20	1*		
-Many	6 (37.5)	10 (62.5)	16			
Vet. Services						
-Yes	15 (53.6)	13 (46.4)	28	0.423	0.174–1.029	0.064
-No	60 (73.2)	22 (26.8)	82	1*		
Vaccination						
-Yes	15 (53.6)	9 (37.5)	24	0.848	0.331–2.170	0.919
-No	57 (66.3)	29 (33.7)	86	1*		
Disposal of Aborted Fetus						
-Bury	25 (40.9)	36 (59.1)	61	0.278	0.124–0.620	0.0027
-Refuse bin	35 (71.4)	14 (28.6)	49	1*		
Awareness of Brucellosis						
-Yes	11 (57.9)	8 (42.1)	19	0.820	0.300–2.241	0.898
-No	57 (62.6)	34 (37.4)	91	1*		

1* = Reference

APPENDIX IV

1. Materials Used in the Research

2. 10 ml hypodermic syringes and needles (21G).
3. Absorbent paper towels.
4. Bottles, tubes and beakers.
5. Chromogen (OPD tablets).

6. Competitive ELISA kits 400 from Veterinary Laboratories Agency, UK.
7. Cool box and ice packs.
8. Disposable hands gloves.
9. Disposable tips.
10. Deep freezer for storage of sera samples / conjugate.
11. DNA Coxiella burnetii control from OIE Reference Laboratory France.
12. DNA extracted kits from Inqaba Biotec, Zymo Research South Africa.
13. EDTA bottle (Bijou).
14. Electric centrifuge machine.
15. Electric incubator.
16. Magnetic stirrer.
17. Masking tape.
18. Methylated spirit.
19. Microtitre plate shaker.
20. Microtitre plate reader with 450nm filter.
21. Oligonucleotides primer sequence for *Brucella* and *Coxiella burnetii.*
22. PCR machine.
23. PCR consumables.
24. Plain sample bottle (Bijou).
25. Plates (Plates pre-coated with *B. melitensis* LPS antigen Lid).
26. Q Fever Indirect ELISA Multi-species kits from Innovative Diagnostics, France.
27. Reagent troughs for multichannel pipetting.
28. Refrigerator for storage of blood samples.

29. Rose Bengal antigen from Animal and Plant Health Agency, United Kingdom.

30. Rotary shaker, capable of 160Rev/Min (or a 37 oC ± 3 oC incubator).

31. Serum sample bottles.

32. Single and multichannel variable volume pipettes.

33. Sterile cotton wools.

34. Sterile distilled water.

35. Test tubes.

36. The reference strains, *B. abortus* KGR1 and JPL1 control from NVRI, Vom.