SEROPREVALENCE OF *BRUCELLA*, KNOWLEDGE AND PRACTICES OF SMALL RUMINANT STAKEHOLDERS IN KADUNA NORTH AND SOUTH LOCAL GOVERNMENT AREAS, KADUNA STATE, NIGERIA

BY

YUSUF BARRY YAKUBU

DEPARTMENT OF VETERINARY MEDICINE
FACULTY OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

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BY

Yusuf Barry YAKUBU, DVM, (ABU), 1991
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DECLARATION

I declare that the work in this Dissertation entitled **"Seroprevalence of *Brucella*, Knowledge and Practices of Small Ruminant Stakeholders in Kaduna North and South Local Government Areas, Kaduna State, Nigeria"** has been carried out by me in the Department of Veterinary Medicine. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Yusuf Barry YAKUBU ---------- ----------

Signature Date

CERTIFICATION

This dissertation entitled **"SEROPREVALENCE OF *BRUCELLA*, KNOWLEDGE AND PRACTICES OF SMALL RUMINANT STAKEHOLDERS IN KADUNA NORTH AND SOUTH LOCAL GOVERNMENT AREAS, KADUNA STATE, NIGERIA"** by Yusuf Barry YAKUBU meets the regulations governing the award of the degree of Master of Science (Food Animal Medicine) of the Ahmadu Bello University Zaria, and is approved for its scholarly contribution to knowledge and literary representation.

Dr. S N A Saidu, Chairman, Supervisory Committee	---------- Signature	---------- Date
Prof. J.O O Bale, Member, Supervisory Committee	---------- Signature	---------- Date
Prof. L B. Tekdek, Head of Department Veterinary Medicine, Ahmadu Bello University, Zaria	---------- Signature	---------- Date
Prof. Kabir Bala, Dean, School of Postgraduate Studies Ahmadu Bello University, Zaria	---------- Signature	---------- Date

DEDICATION

This dissertation is dedicated to my dear wife Evelyn and my wonderful children; Gershimmi, Ghenom, Fennom and Joknom. To God almighty be the glory.

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ABSTRACT

Brucellosis is an infectious disease of domestic and wild animals and one of the commonest zoonosis caused by a facultative, Gram-negative, coccobacilli and intracellular bacteria of the genus *Brucella*. A seroprevalence survey of *Brucella,* knowledge and practices of small ruminants stakeholders was carried out in Kaduna North and South LGAs of Kaduna State, Nigeria. A total of 1,256 serum samples from 696 goats and 560 sheep were used to evaluate for *Brucella* antibodies using Modified Rose Bengal Plate Test (M-RBPT) with *B. melitensis* antigen and Serum Agglutination Test with ethylene diaminotetraacetic acid (SAT-EDTA). Also, 66 structured questionnaires were used to determine the knowledge and practices of livestock owners in the study areas. Similarly, 54 structured questionnaires were also administered to human medical professionals to determine their knowledge of brucellosis in humans. The result revealed a *Brucella* seroprevalence of 8.49 % and 14.85% by M–RBPT and SAT–EDTA in respectively goats, and 9.11% and 17.12% by M–RBPT and SAT–EDTA in respectively sheep. The seroprevalence varied between the two LGAs and between goats and sheep in each LGA. In goats a seroprevalence of 15.12% was recorded in Kaduna North LGA while 14.5% seroprevalence of was recorded for Kaduna South LGA using SAT–EDTA. The difference in seroprevalence between goats and sheep was not statistically significant ($P > 0.05$). Of the 66 structured questionnaires for the livestock owners, 49 (74.24 %) of the respondents indicated having knowledge about brucellosis in animals and of this number, 2 (3.03 %) indicated the media as source of their information (knowledge) of the disease, while 27 (40.91 %) and 19 (28.79 %) other

respondents indicated their source of knowledge of brucellosis in animals to be farmers and veterinary professionals respectively.

All the 54 (100%) human medical profession personnel's interviewed indicated having heard of brucellosis in humans but none of them (0%) had ever encountered the disease in the course of their practice. Regarding knowledge of brucellosis as zoonotic, 42 (77.78%) of the human medical professionals reported knowing brucellosis as a zoonotic disease. In addition 40 (74.10%) of the human medical professionals indicated knowing brucellosis to cause abortion in humans. This study revealed that *Brucella* organism is circulating in small ruminants in the study areas, and also, there is awareness of the disease problem in animals and human beings by the livestock owners and human medical professionals in the study areas. Policy makers in both the human and livestock sectors therefore, need to embark on measures to prevent the occurrence as well as the spread of brucellosis among animals and subsequent transmission to the human populace.

TABLE OF CONTENTS

LIST OF TABLES

Table **Title** **Page**

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Appendix **Title** **Page**

LIST OF ABBREVIATIONS

CDC	Centre for Disease Control
CFT	Complement Fixation Test
CSF	Cerebrospinal Fluid
FAO	Food and Agriculture Organization
FPA	Fluorescent Polarization Assay
FPSR	False Positive Serological Reaction
HIT	Heat Inactivation Test
IFAT	Indirect Immunofluorescent Test
KAP	Knowledge, Attitude and Practices
KDSG	Kaduna State Government
LFA	Lateral Flow Assay
LGA	Local Government Area
LPS	Lipopolysaccharides
MRT	Milk Ring Test
NAD	Nicotinamide Adenine Dinucleotide
OPS	O – Polysaccharides
PCR	Polymerase Chain Reaction
M – RBPT	Modified Rose Bengal Plate Test
RBPT	Rose Bengal Plate Test
RLPS	Rough Lipopolysaccharides
SAT	Serum Agglutination Test
SAT- EDTA	Serum Agglutination Test Ethylene Diamminotetraacetic acid
SLPS	Smooth Lipopolysaccharides
SPAT	Standard Plate Agglutination Test

USA	United States of America
USDA	United States Department of Agriculture
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The socio-economic importance of sheep and goats in Nigeria needs no emphasis. There are about 51 million small ruminants, comprising of 28 million goats and 23 million sheep in Nigeria (FAO, 2006). Goats are very prolific, and are valuable in arid areas (Adamu *et al.*, 2012). This may be due to their ability to subsist on land unsuitable for farming and also to survive drought (Adamu *et al*, 2012). Socially, small ruminants play an important role in the life of the community, being either loaned or exchanged among relatives and friends or slaughtered for social, religious and festive occasions (Bale, 1980). Economically, small ruminants are readily available for sale by farmers to boost family income or meet financial obligations (Bale, 1980).

In Kaduna State, the small ruminant population is estimated at about 1,688, 136 goats and 1,306, 110 sheep (KDSG, 2005). Many families in the State keep goats and sheep as sources of income and manure for farming activities. Their meat is widely accepted by all, thus free from religious or tribal taboos and serve as a major source of meat consumed in Nigeria (Saidu *et al.,* 1991). A major obstacle to improving the production of these highly prolific species of livestock in Nigeria is disease (Saidu *et al*, 1991). Goats and sheep suffer from helminthic, bacterial, viral and fungal diseases which limit their production/productivity and of great importance both from economic and human points of view is brucellosis which is common to these species worldwide (Bale, 1980).

Caprine and ovine brucellosis is endemic in Nigeria and is important not only as a hindrance to increased production but also as a zoonosis (Bale, 1980; Bertu, 2009; Adamu *et al.,* 2012; Kaltungo, 2013).

Brucellosis is an infectious disease of domestic and wild animals with serious implications in humans (Akbarmehr and Ghiyamirad, 2011). The disease is caused by members of the genus *Brucella*, which is a facultative, intracellular, non–capsulated Gram-negative bacterium (Ocholi *et al*., 2005; Junaidu, *et al*., 2010). It affects primarily the genital organs causing inflammation, abortion, stillbirth and infertility (Cadmus *et al.,* 2006). In the female animals, the bacteria are localized in the uterus and udder, followed by excretion via the milk, while in the male, the bacteria are located in the testicles causing orchitis and epididymitis leading to infertility (Gwida *et al*., 2010).

The mode of transmission of the bacteria varies with epidemiological factors like animal reservoirs and occupationally exposed groups (Anonymous, 2001). The prevalence of infection in the animal reservoirs provides a key to the prevalence in humans (Cadmus *et al*., 2006). It is worthy of note that *B. abortus* which primarily infects cattle and *B. suis* infecting pigs usually affect occupational groups while *B. melitensis* primarily infecting goats and sheep occur more frequently than other *Brucella* spp. in the general population of small ruminants (Ocholi *et al*., 2005; Bale *et al*., 2013). Brucellosis occurs world-wide (Akbarmehr and Ghiyamirad, 2011) and has been reported in virtually all animal species in Nigeria (Osinubi *et al*., 2004; Junaidu *et al*., 2006; Cadmus *et al*., 2009; Adamu *et al.,* 2012; Ehizibolo *et al.,* 2013). Despite the advances made in surveillance and control, the prevalence of brucellosis is increasing in many developing countries (Gwida *et al*., 2010).

The incidence of the disease has decreased markedly in some highly industrialised countries due to the policy of test and slaughter of positive reactors, appropriate biosecurity measures and vaccination of young animals between the ages of three months and eight months (Akbarmehr and Ghiyamirad, 2011). In other countries like the United States of America (USA), France, Belgium and Malaysia brucellosis has been eliminated (Palmer *et al*., 1998). In China, *B. melitensis* is prevalent in the Northern Province (Akbarmehr and Ghiyamrad, 2011). In other countries, especially the Middle East, the prevalence of the disease is either high or unknown (Akbarmehr and Ghiyamirad, 2011).

1.2 Statement of the Problem

Small ruminants make up 51 million of the total food animal population in Nigeria. This consists of 28 million goats and 23 million sheep, in addition to 51 million cattle (FAO, 2006). In Kaduna State, small ruminants serve as a source of employment for rural, urban and sub- urban dwellers who engage in the production and marketing of livestock and their by- products (KDSG, 2005). Brucellosis is endemic in Nigeria and has been reported in cattle, sheep, goats, dogs, pigs, horses and camels (Bale *et al*., 2003; Ocholi *et al*., 2004; Osinubi *et al*., 2004; Ehizibolo *et al*., 2011; Kaltungo, 2013; Bertu, 2014; Buhari, 2014). A report on brucellosis has also been documented in wild animals (Davis, 1990).

The economic losses associated with brucellosis in the livestock industry, as well as the health risk it poses to human beings cannot be over-emphasized. The losses in livestock are in terms of abortion, low milk yield, infertility, low conception rate and low neonates survival rates (Osori, 1976; Oyedipe *et al.*, 1981). The prevalence of brucellosis in small ruminants can constitute a barrier in the development of the livestock industry in Nigeria

(Kaltungo *et al.*, 2013). Although a lot of work on bovine brucellosis has been done in Nigeria, not much study has been done on small ruminant brucellosis in Kaduna State.

Human beings contract the disease from animals ignorantly by either direct or indirect contact through infected animals or their products through consumption of unpasteurized milk or milk products and handling animal deliveries with bare hands (Nuru and Denis, 1975). Majority of small ruminant farmers in Nigeria are peasants and least able to contend the loss due to brucellosis in their flocks.

1.3 Justification for the Study

In Kaduna State, Agriculture involving crop production and animal husbandry remains the primary occupation of its populace (KDSG, 2008). Small ruminants are chief sources of animal protein, financial income and manure for farming activities. There is continuous increase in human population with the attendant need of individuals to consume the minimum per caput animal protein requirement of 35 grams per day (FAO, 2006). However, animal diseases like brucellosis are capable of threatening the realization of this goal.

In Kaduna State, mixed–animal husbandry is very common coupled with uncontrolled movement of small ruminants and pastoral cattle herds. This could result in increased prevalence of brucellosis (Ocholi *et al*., 2004), thus, the occurrence of the disease in household small ruminants could be expected. In addition these animals could congregate to eat and drink water from a common source leading to contamination of pasture and water bodies from infected flocks shedding the *Brucella* organism. Markets and selling spots of small ruminants can also serve as a point of infection and redistribution of *Brucella* to apparently healthy areas. It is common for unsold animals of all species to be

returned to the flocks or herds and for newly bought animals whose status are not known to be added to the flocks or herds (Kaltungo, 2013; Buhari, 2014). There are also many cases of abortion, stillbirth, retained placenta and prolonged kidding and lambing intervals in small ruminants whose etiologies have not been conclusively known (Kaltungo, 2013). This could pose a danger for man and animal health and also serve as a means of spreading the disease to other apparently healthy flocks. There is also poor information regarding the status of *Brucella* infection in small ruminants in Kaduna abattoirs, slaughter slabs, livestock markets and households. Hence, the need for this study.

Therefore, there is the need to determine the status of brucellosis in small ruminants in the study areas, along with understanding the knowledge, attitudes and practices of small ruminants farmers, butchers, livestock traders and abattoir personnel with a view to properly strategize for control, prevention and subsequent eradication of the disease in the study area.

1.4 Aim of the Study

The aim of the study was to determine the seroprevalence of *Brucella* antibodies in small ruminants and assess the knowledge and practices of small ruminant farmers and handlers; and to assess knowledge of human medical personnel with regard to its zoonotic potential in Kaduna North and South Local Government Areas, Kaduna State, Nigeria.

1.5 Objectives of the Study

The objectives of the study were to:

i determine the seropravalence of *Brucella* in small ruminants in Kaduna North and South Local Government Areas, Kaduna State, Nigeria.

ii. assess the knowledge and practices of small ruminant farmers, livestock traders, butchers, abattoir workers with regard to brucellosis in Kaduna North and South Local Government Areas, Kaduna State, Nigeria.

iii. assess the knowledge human medical personnel with regard to brucellosis in Kaduna North and South Local Government Areas of Kaduna State, Nigeria.

1.6 Research Questions

i. What is the prevalence of *Brucella* antibodies in small ruminants in abattoirs, livestock markets and households in Kaduna North and South Local Government Areas of Kaduna State, Nigeria

ii. What is the prevalence of *Brucella* antibodies based on age, sex and species in small ruminants brought to the abattoir, slaughter slabs, livestock markets and households in Kaduna North and South Local Government Areas of Kaduna State, Nigeria

iii. What are the levels of knowledge and practices of people involved in small ruminant keeping and business in Kaduna North and South Local Government Areas of Kaduna State, Nigeria

iv. What is the level of knowledge of human medical personnel with regard to brucellosis in Kaduna North and South Local Government Areas of Kaduna State, Nigeria

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Brief History of Brucellosis

Brucellosis is an infectious disease of all warm-blooded animals and man caused by the members of the genus *Brucella (B.) (*Munoz *et al*., 2005*). Brucella* spp. are facultative intracellular organisms with a wide range of hosts including man (Bale 1980; Abdulkadir, 1989). There are six well-known species of the genus namely *B. abortus, B. melitensis, B. ovis, B suis, B. canis and B. neotomae* (Saleem *et al*., 2010), and is found most commonly in cattle, goats, sheep, pigs and dogs, and occasionally in other domestic animals like horses, camels and chickens; as well as wild animals like bison, elk, reindeer and carabou (Jensel *et al*., 1999) and marine animals like whales, dolphins, seals and porpoises (Maria – Laura *et al*., 2001).

The disease was first described by Hippocrates in 450 AD as a febrile disease as cited by Bertu (2009). Bruce in 1870, however, was the first to isolate the organism from the liver of a British Army personnel who died of undulant fever (Malta fever) on the Mediterranean Island of Malta as cited by Megid *et al*., (2010). Then it was called *Micrococcus melitensis.* The disease was known to be associated with milk and milk products of infected goats (FAO/OIE/WHO, 2006). Ten years later in Denmark, Bang and Stribolt (1880) isolated a similar organism from an aborted cow foetus and foetal membrane and named it *Brucella abortus* after Bruce who originally isolated the organism. The relationship of this disease in animals and man was not well understood until Zammit (1905) demonstrated the organism in the blood of goats and isolation of the organism from apparently healthy goats by Harrecks in the same year.

Subsequently, a similar organism named *B. suis* was isolated from an aborted pig foetus by Traun (1914) in the United States of America (USA) cited by Alton (1981). Evans (1918) showed that these organisms were related and were therefore included in the same genus *Brucella* by Meyer and Shaw (1920) in honour of David Bruce who first isolated the organism in 1870 from the liver of a dead British soldier. Another species, *B. ovis* was later described from cases of epididymitis and abortion in sheep in New Zealand by Buddie and Boyes (1953). Furthermore, *Brucella neotomae* was isolated from a desert wood rat *(Neotoma lepida)* by Stoner and Lackman (1957) in Utah, USA while *B. canis* was isolated from cases of canine epizootic abortion and epididymitis in Beagle dogs by Carmuchace and Kenny in 1968 in the USA.

Corbel and Brinley-Morgan (1984) reported the isolation of a *Brucella* specie from free-living seals and crustaceans around the coast of· Scotland and captured Dolphins in the USA (Ewalt *et al*., 1994), and were later , based on molecular studies named as *B. cetaceae* and *B. pinipediae* for cetaceans and seals respectively (Cloekae't *et al*., 2001).

2.2 Aetiology

Brucellosis in goats and sheep is caused mainly by *B. melitensis* and rarely *B. abortus* (Luchsinger and Andarson, 1979; Garin-Bastuji *et al.*, 1998; Bale *et al.*, 2013) with *Brucella ovis* infecting primarily sheep (Garin–Bastuji *et al*., 1998).

2.2.1 Culture and growth characteristics

Morphologically, *Brucellae* are coccobacilli measuring 0.6 to 11.5 µm long and 0.5 - 0.7 µm wide (Corbel, 2006). They occur singly and less frequently in pairs or small groups. The morphology of *Brucellae* are often constant except in old cultures where

pleomorphism is evident. *Brucellae* are non-motile, non-spore forming, non- flagellate, non- encapsulated and have no pili (Young, 2000). The organisms are facultative, intracellular, gram-negative bacteria (Corbel, 1997; Young, 2000). They are not true acid fast but resist decolourization by weak acids. They, therefore stain red by modified Ziehl-Neelsen test that is sometimes used for microscopic diagnosis of brucellosis from smears of solid or liquid specimens (Alton *et al*., 1988). *Brucella* can be isolated as part of the normal flora of the urinogenital tract of a variety of warm blooded animals including cattle, sheep, goats, pig and dogs (Young, 1995).

Members of the genus are aerobic. However, some strains require an atmosphere containing 5-10% carbon dioxide added for growth, especially on primary isolation (Alton *et al*, 1988). On repeated culture, isolates of *Brucella* can lose the need for added carbon dioxide for growth and may grow in air only (Eze, 1981). The optimum pH for growth is from 6.6 - 7.4 and culture media should be adequately buffered near pH 6.8 for optimum growth. The optimum growth temperature range is 36-380C, but most strains can grow between 200C and 400C (Anon, 2001; EC, 2001) *Brucella* require biotin, thiamine and nicotinamide and the growth is improved by serum or blood but haem (v-factor) and NAD are not needed (Alton *et al*., 1988).

The growth of most *Brucella* strains is inhibited by media containing bile salts, tellurite or selenite (Anon, 2001). On suitable solid media, *Brucella* colonies are seen after three days of incubation, but the plates are examined on the fourth or fifth day because there may be a delay of growth on selective media. On examination, *Brucella* colonies are

round, 1-2 mm in diameter, are smooth and cross-react with 5-LPS of *Brucella* antigens in diagnostic tests (Corbel *et al*, 1984; Anon, 2001). *Brucella* selective media became paramount in an attempt to achieve success in the isolation of *Brucella* organisms in clinical veterinary specimens that have been contaminated with other organisms. Furthermore, modifications have been made to this medium by the addition of antibiotics or dyes to the basal medium with the intention to eliminate other bacteria (Corbel *et al*., 1979). These include Farrell medium for B. *abortus* (Farrell, 1974) and Ryan (1967) and Thaye-Martin medium for *B. ovis* (Thaye and Martin, 1964).

Brucella metabolism is oxidative and cultures show no ability to acidify carbohydrate media in conventional tests (Young, 2000). *Brucella* species are catalase positive, oxidase positive, except B. *ovis and* B. *neotomae* (Bale, 1980) and they reduce nitrate to nitrite except B. *canis.* The production of hydrogen sulphide (H2S) from sulfur containing amino acids varies (Alton *et al*., 1988). For example, *B. melitensis* does not produce H2S, Urease activity varies from fast to slow. Indole and acetyl methyl carbinol are not produced from tryptophane and glucose respectively (Anon, 2001). Methyl red and Voges-Proskaeur tests are negative and *Brucella* neither liquify gelatin nor lyse red blood cells (Alton *et al.,* 1988).

2.2.2 The organism in the environment

Under appropriate conditions, *Brucella* organism can persist in the environment for a very long time (Corbel, 1988), and this survival in the environment plays an important role in the epidemiology of the disease, brucellosis. Temperature,

humidity and pH affect the *Brucella* organism's ability to survive (Bercovich, 1988).

According to Corbel (1988), the organisms resist drying, especially in organic substrate and can be viable in the soil for up to ten weeks. In damp soil, *Brucella* can survive for up to 60 days at 200C and 144 days at 40% relative humidity (Bercovich, 1988). In addition, *Brucella* can survive in tap water for several months at 4 – 80C, 30months at 00C and several years in frozen tissues or media (Seifert, 1996). Also *Brucella* survives for up to 4 months in butter, 6weeks in milt and 2weeks in cooled meat and up to 30 days in ice cream (Seifert, 1996). The organism can also survive 30 days in urine, 75 days in aborted foetuses and more than 60 days in uterine discharges (Bercovich, 1998, Corbel, 1988) as well as in cattle faeces for at least one year and in liquid manure and frozen soil for two years (Seifert, 1996), and in contaminated straws for more than one month (Weidmann, 1991).

Brucella organisms are sensitive to direct sunlight, and under dry condition, they survive only when embedded in proteins (Davis and Casey, 1973). They are sensitive to a wide variety of disinfectants including strong acids and alkalis, hypochlorites, iodophores, 1% lysol and formaldehyde (Corbel, 1988, Seifert, 1996). However, low temperatures reduces the efficacy of these chemical disinfectants (Corbel, 1988). Pasteurization of milk at 680C for 10 – 15 minutes destroys the *Brucella* organism (Merchant and Parker, 1975).

2.2.3 Susceptibility to dyes, antibiotics and phages

*Brucella*s are highly sensitive to a wide range of antibiotics and chemotherapeutic agents including penicillins, aminoglycosides, chloramphenicol, tetracyclines and sulphonamides (Corbel, 1988). They are however, resistant to vancomycin, bacitracin, polymyxins, metronidazole, nalidixic acid and antifungal agents (Corbel, 1988).

There are about 40 phages which are lytic and specific to the genus *Brucella* and they are not known to be active against any other bacteria that have been tested (Anonymous, 2001). So, lysis by *Brucella* phages is a useful test to confirm the identity of *Brucella* species and for specialization within the genus (Anonymous 2001). Phages currently in use for *Brucella* typing are Tbilisi (Tb), Weybridge (Wb), Izatnagar (Iz), and *R/C.* The first three are used for differentiation of smooth *Brucella* while *R/C* is used for rough *Brucella (B. ovis, B. canis)* (Corbel *et al.*, 1987). All smooth *Brucella* cross react with one another in agglutination tests with unabsorbed polyclonal antisera, a cross reaction which does not extend to the rough strains. Lipopolysaccharide (LPS) comprises the major surface antigen of the corresponding colonial phase involved in agglutination. The (S-LPS) molecules carry the A and M antigens, which have different quantitative distribution among the smooth *Brucella* strains. This is of value in differentiating biovars of the major species using absorbed mono specific A and M antisera (Anonymous, 2001). *Brucella ovis* and *B. canis* (rough *Brucella*) lack the A and M antigens, as a result their outer surface contains only rough lipopolysacharide (RLPS) and protein antigens (Blasco, 1990).The effects of the dyes thionin and basic fuchsin on various *Brucella*

species and biovars vary (Anonymous, 2001). Susceptibility to dyes at standard concentration (20µg/ml) is used as routine typing test of *Brucella* species (Alton *et al.*, 1988).

Brucella species are sensitive to a wide range of antibiotics. Penicillin is used for the routine differentiation of the vaccinal strain B. *abortus* species biovar 1 strain 19, while streptomycin is used for *B. melitensis* biovar 1 strain rev. 1. The vaccines, widely used for the immunization of cattle and small ruminants respectively, have been obtained from the virulent strains of their respective biovars by virtue of their different sensitivity to these antibiotics (Alton *et al*., 1988). On primary isolation, *Brucella* spp. are usually susceptible in vitro to gentamycin, tetracycline and rifampicin (Anon, 2001). Most *Brucella* strains are also susceptible to ampictilin. chloramphenicol, cotrimoxazole, erythromycin, streptomycin and spectinomycin. Most strains are resistant to β-lactamins, polymyxin B, nystatin, cephalosporin, bacitracin, clendomycin, vancomycin and cycloheximide at the therapeutic concentrations (Anon, 2001).

2.2.4 Antigenic characteristics

All the smooth *Brucella* organisms (*B.melitensis, B. abortus* and *B. suis*) cross-react with one another in agglutination tests with unabsorbed polyclonal antisera (Anon, 2001). Lipopolysaccharide (LPS) comprises the major surface antigens of the corresponding colonial phase involved in agglutination. The (S-LPS) molecule carries the A and M antigens, which have different quantitative distribution among the smooth *Brucella* strains. This is of value in differentiating biovars of the major species using absorbed

monospecific A and M antisera (Anon, 2001). Serological cross- reaction has been reported between the smooth *Brucella* strain and various other gram negative bacteria like *E. coli* 0:116 and 0:157, *Salmonella* group N (0:39) of Kaufmam-white and *Pseudomonas multophilia, Vibrio cholera* and especially *Yersinia enterocolitica* (0: 9) (Anon, 2001). These organisms can induce significant antibodies, which cross – react with S – LPS of *Brucella* antigen in diagnostic tests (Corbel *et al*., 1984; Anonymous, 2001). *Brucella ovis* and *B. canis* lack the OPS component and as a result, their outer surface contains only rough lipopolysacharide (RLPS) and protein antigen (Blasco, 1990).

2.3 Hosts of *Brucella* Species

There are more than 94% similarities among the members of the genus. However, *Brucella* species have different host preferences (Verger *et al.,* 1987). Similarly, Anon (2008) reported that all members of the genus are to be regarded as a single species of *Brucella melitensis* with multiple biovars. The primary host is an important factor in the maintenance of the disease in nature, as most of them are reservoirs of infection for each particular species (Ko and Splitter, 2003).

2.3.1 Goats and sheep

Brucella melitensis has three biovars that mainly affect goats and sheep (OIE, 2009). Most breeds of goats are readily infected, but sheep breeds vary in susceptibility. *Brucella melitensis* has occasionally been reported in cattle, camels, and dogs, but rarely in pigs and horses. Infections in goats and sheep can spill over into other wild ruminants

(EC, 2001).

2.3.2 Cattle

Cattle are considered to be the preferential hosts of *B. abortus* which is classified into seven biovars, namely biovars 1 – 6 and 9 (Marianna *et al*., 2010). *Brucella abortus* can also infect buffaloes, camels, deers, dogs, horses, pigs, goats, sheep and man (Kudi *et al*., 1997).Other species of animals reported to be susceptible *to B. abortus* are chickens, ducks, turkeys, pigeons, geese, and pheasants (Adesiyun and Abdu, 1984; Junaidu *et al*., 2006).

2.3.3 Horses

Horses can be infected with *B. abortus*, with the bacterium having preference for bursae, tendons, muscles and joints (Megid *et al*., 2010) as well as in of fistulous withers and poll evil as secondary infection (Hall, 1977).

2.3.4 Other animal species

Porcine brucellosis caused by *B. suis* biovars 1, 2, and 3 is considered an important re–emerging disease of domestic and wild pigs (Freitin *et al*., 2008). However, this organism may also affect other animal species like cattle, horses, dogs, rabbits and man (Frye, 1991). In addition, *B. suis* biovars 4 and 5 specifically affect reindeer and rodents respectively (Corbel, 2006). Nonetheless, pig can also be infected by other *Brucella spp.* but the infection is self-limiting (OIE, 2009a).

Canine brucellosis caused by *B. canis*, affects dogs, wild carnivores, rarely other

domestic animals and man (Megid *et al*., 2010). The desert wood rat under natural condition is known to be infected with *B. neotomae* and no other cases in addition to the original isolation have been reported (Stoenner and Leckman, 1957).

Since the 1990's, marine strains of *Brucella* have been isolated from a variety of marine mammals, including the seal (*Phoca vitulina*), dolphins (*Tursiops truncates, Delphinus delphis, Lagenorhynchus acutus, Stenella coeruleoalba*), whale (*Balenoptera acutorostrata*), and other marine species (Ewalt *et al.,* 1994; Ross *et al.,* 1994; Foster *et al*., 1996).

Human brucellosis is primarily caused by *B. melitensis, B. suis* and *B. abortus* which have goats and sheep, pigs and cattle as preferential hosts respectively (Godfroid *et al*., 2005). However, *B. melitensis* is considered the most virulent *Brucella* spp. for man with a few organisms (10 to 100) being sufficient to cause a debilitating chronic disease (Fugier *et al.,* 2007). Similarly, two recently identified *Brucella* spp. *B. ceti* and B. *pinnipedialis,* isolated from marine mammals can cause human brucellosis (Foster *et al*., 2007). In addition *B. canis* a pathogen of dogs has a low zoonotic potential, while *B. neotomae* and *B. ovis* which infect desert rat and sheep respectively are not associated with human diseases (Godfroid *et al*., 2005).

2.3.5 Factors of Resistance to *Brucella* infection

Age, sex, breed and stage of pregnancy of affected animals influence resistance to *Brucella* infection (Corbel, 2006). Young animals tend to be less susceptible to infection than mature animals. Available data indicate that pregnant animals are more susceptible

than non-pregnant ones and males because the gravid uterus sustains the organism, thus influencing the progression of the disease (Crawford *et al*., 1990). It also appears that the younger the fetus is at the period of infection, the longer the incubation period (Crawford *et al*., 1990). Data from naturally occurring and experimentally induced *Brucella* infection suggest that natural resistance to brucellosis also influences the course and severity of the disease (Ho and Cheers 1982; Harmon *et al*, 1989).

2.4 Transmission and Pathogenesis

2.4.1 *Brucella melitensis* infection in goats and sheep

In goats and sheep, *B. melitensis* is usually transmitted by contact with the placenta, foetus, foetal fluids and vaginal discharges from infected animals. Small ruminants are able to shed the organism after either abortion or parturition (OIE, 2009). Also goats can shed *B. melitensis* in vaginal discharges for at least 2 to 3 months, but shedding usually ends within three weeks in sheep. *B. melitensis* can be found in milk and semen; and infected does and ewes may shed the organism in milk and semen for prolonged periods of time leading to kids and lambs to become infected (OIE, 2009). The persistent infection of the mammary glands and supra-mammary lymph nodes leads to intermittent shedding of organism in milk in succeeding lactations.

On entering into the body of the host, the organism encounters the cellular defenses of the host but generally arrives at nearest lymph node via the lymph node vessels after escaping the cellular defenses (Ko and Splinter, 2003). The fate of the invading bacteria is determined by cellular defenses of the host mainly macrophages and T-lymphocytes

although specific antibodies play a part (Radostits *et al*., 1997). The outcome depend on the ruminant species infected, age, immune and pregnancy status of the host, and the virulence and number of invading *Brucella* organism (Seifert, 1996).When the invading bacteria prevail over the host's defenses, a bacteraemia is established. The bacteraemia is detected after 10 to 20 days and may persists for from 30 days to more than two months. If the animal is pregnant, bacteraemia often leads to invasion of the uterus (Oslen, 2010). At the same time infection is established in various lymph nodes and organs, often in the udder and sometimes in the spleen (WHO, 1986).

2.4.2 *Brucella ovis* infection in sheep

Brucella ovis transmission can occur by direct contact between rams kept in the same premises for long period of times or through ewes that have mated with an infected ram prior to a susceptible ewe during the same mating season (Hughes, 1972). In ewes, *B. ovis* can uncommonly cause abortion associated with placentitis beginning at 30 days of gestation. Infected ewes give birth to weak lambs and there may be high neonatal death (Meinershagen *et al*., 1974).

Brucella ovis has greater affinity for the reproductive tract of the male than in the female (Megid *et al*., 2010). Epididymitis and sterility are the most frequent and serious consequences of infection with *B. ovis.* In rams, natural infections occur over a long incubation period (16 - 17 weeks) during which time the animals are asymptomatic (Radostits *et al*., 2003). The organism remains confined to the exposure sites and regional lymph nodes for 10 - 14 days before the infection advances to the bacteraemic phase with

a more generalized infection that involves the spleen, kidneys, and lymph nodes far from the exposure site. During the later part of the bacteraemic stage, the organism begins to localize in the genital organs such as the seminal vesicles, ampullae, testes, tail of epididymis and less frequently head of the epididymis (European Commission, 2001). Some organisms may remain in the kidney and such animals continue to shade *B. ovis* in their urinary tracts. These organisms can cause chronic interstitial nephritis in sheep.

In flocks where abortion has occurred, lamb yields have been known to drop from 100% to 59%. About 20% of the ewes may remain barren and up to 16% of lambs born alive die before six weeks of age (Bale, 1982). There is also a prolonged lambing period due to interference with normal breeding operations. In ewes under conditions simulating natural infection, it was found that the organisms disappear from the local sites shortly after exposure, bacteraemia was prolonged and the organisms reappeared in the genital tract in the third month of gestation (Radostits *et al*., 2003). Abortion occurred when there is sufficient necrosis of the placenta and separation from the caruncles. The slow progress of the infection probably accounts for the frequent finding of positive compliment fixation test (CFT) titres, but infrequent occurrence of abortion either by experimental or natural infection (De Massi *et al*., 2005). Foetal lambs can survive *in utero* in the presence of the infection which does not lead to abortion but placentitis, that interferes with foetal nutrition and results in lambs with low birth weights (Seifert, 1990).

Rams are more susceptible than ewes and are more responsible for the transmission of the organisms as well as serving as reservoir for the maintenance of the disease (Okoh,

1980). Contaminated semen from infected rams is the source of the organism. In breeding flocks, the organisms are spread from infected rams to non-infected rams through passive venereal transmission. This occurs when the infected ram mates with an ewe followed by a non-infected ram with the same ewe. Ram to ram infection can occur by direct contract or housing non-infected ram in a barn or shed previously occupied by infected rams (Bertu, 2009) Sodomy is the major source of spread in young rams. Young rams are susceptible to *B. ovis* and in some flocks up to 80% of unmated young rams are believed to have clinical and serological evidence of the infection.

Experimental transmission has been produced. Another means of infection is through the nasopharynx since rams often nuzzle the genital organs of other rams. Ewes are more resistant compared to rams (Okoh, 1980). In rare cases, direct contact during abortion can lead to infection. Although following abortion or parturition, an infected ewe may excrete *B. ovis* for almost 10 days, it does not maintain the infection from one breeding season to another. The ewe may transmit the infection when it comes on oestrous. *Brucella ovis* infection in rams affects the quality of semen and breeding efficiency (Seifert, 1990).

2.4.3 *Brucella abortus* infection in goats and sheep

Susceptible animals become infected though consumption of contaminated feed or water, or by aerosols as animals tend to leak aborted foetuses or the genital discharges of an aborted dam (Bercovich, 1998). Brucella infection also occurs in-utero or when kids and lambs born to healthy dams are fed with colostrums or milk from infected dams (Bale and Nuru, 2001). Grazing goats and sheep wher infected cattle have grazed or coming in

contact with infected manure or premises may lead to infection (Anonymous, 2001). Infected males shed the organism in the semen especially during the acute phase of the infection which may transmit the infection to the females through coitus (Bercovich, 1998). Dogs have been found to be mechanical and biological vectors of brucellosis (WHO, 1986); as they are involved in transporting infected materials from one place to another thereby introducing the infection to new places and also infecting themselves.

Brucella abortus has predilection for the uterus and placenta (Smith *et al*., 1972; Blood and Radostits, 1989). This predilection has been attributed to the presence of erythritol in the placenta uterus, cotyledons and chorions which have been shown to stimulate the growth of *Brucella* both *in-vivo* and *in-utero* (Smith *et al*., 1972, Corbel, 1988). Erythritol is used by *B. abortus* as an energy source and trace amounts can stimulate its intracellular growth (Corbel, 1988). Erythritol is present in the normal and infected placenta of cattle, sheep, goats and swine as well as the seminal vesicles and testes of the males of these species, but has not been found in the human placenta or in that of rabbit, guinea pig, and rats (Corbel,1988).Erythritol favours the multiplication of *B. abortus* where it causes necrosis and degeneration of the cotyledons leading to abortion (Smith *et al*., 1972; Blood and Radostits, 1989).

During pregnancy, brucellosis is much more acute and a rapid multiplication of the *Brucella* organism occurs in the placenta and lymph nodes leading to bacteraemia and vigorous antibody response (Corbel and Brinely- Morgan 1984). The pathogenesis of the disease in the male is similar to that in the female except that the seminal vesicles are involved. The reason for the predilection is similar to that in the females, the presence of

erythritol (Smith *et al*., 1972).

2.5 Clinical signs of Brucellosis

The incubation period of brucellosis varies from few days to several weeks. According to Seifert (1990), the incubation period varies from 14 days to 120 days. The disease could run an acute or chronic course. When brucellosis is introduced into a clean herd of susceptible animals, it may run an acute course of abortion storm in 50% or more of the pregnant animals. In the male animals, the major signs include; orchitis, and swelling of the scrotum (Seifert, 1990).

2.5.1 Signs in goats and sheep

In sheep and goats, the disease is characterized by high mortality in lambs and kids, mastitis, low milk yield, abortion, epididymitis, orchitis and arthritis, retained placenta, stillbirth birth of weak offspring (European Commission, 2001). Persistent infection of the supramammary lymph nodes is common in goats (Anon, 2001). Alton (1990) observed that in an experiment where goats were infected with high doses of *B. melitensis*, abortion in female goats occurred between 3 and 4 weeks, while in sheep abortion occurred between 4 and 12 weeks after an experimental infection with *B, melitensis* and it seems that ewes were more resistant to infection than the does.

2.5.2 Signs in cattle

The incubation period is often tied up with the stage of pregnancy at the time of exposure (Seifert, 1996). In female animal, the primary clinical signs are abortion at third trimester of pregnancy, stillbirth, birth of weak offspring, dead calf, retained placenta, infertility, sterility and decrease in milk yield (Corbel, 1988; Seifert, 1996; Anon 2001). The aborted foetuses are covered by yellowish, slimy exudates. The placenta is oedematous and the cotyledons are covered with sticky ordourless brownish exudates. Hygroma has been reported as a significant sign of brucellosis in the tropics, especially among transhumant animals (Corbel, 1988; Blood and Radostits, 1989).

2.5.3 Signs in horses

In horses, brucellosis is characterized by arthritis, chronic bursal enlargement of the neck fistulous wither and poll evil, bursitis and lameness (Radostits *et al.*, 1997). In the mare, abortion or infertility may occur and *Brucella* has been isolated from aborted foetuses (Ray *et al*., 1988). Some horses may present general stiffness, fluctuating temperature and lethargy (Macmillan *et al* 1985).

2.5.4 Signs in pigs

The most common signs of brucellosis in sows is abortion, occurring very early or at any time during gestation. Also, vaginal discharges are not very evident, and, in chronic cases, infertility rather than abortion is the most common clinical signs (OIE, 2009a).

In boars, brucellosis is more likely to be persistent with lesions in the genitalia

often interfering with sexual activity which may be temporal or permanent (OIE, 2009a). Also the boar may excrete Brucellae in the semen without any abnormality in the sex organ or interference with sexual activity.

In both sexes there may be swollen joints and tendon shealth wich may develop into lameness and, occasionally posterior paralysis (Holyoake, 2010). According to Acha and Szyfres (2003), abscesses of different sizes often occur in organs and tissues.

2.5.5 Signs in other animals species

Brucellosis in dogs is characterized by abortion and infertility in bitches, epididymitis and scrotal dermatitis in male (EZE, 1978; Okoh, 1978; Corbel, 1988).

2.5.6 Signs in man

In man, brucellosis is characterized by intermittent fever, weakness, depression, night sweating, anorexia, constipation, myalgia and stiffness of the vertebral column (Falade, 1974; Thornes and Morton, 1977; Currier, 1987; Weidmann, 1991).

2.6 Pathology of Brucellosis

In pregnant animals, the disease is characterized principally by necrosis of the placenta and ulcerative endometritis. The placental cotyledons are covered by sticky exudates, swollen and necrotic tissues (Radostits, 1995). The intercotyledonary areas are frequently thickened and opaque with a leathery appearance (Corbel, 1988). Histologically, the

disease is characterized by marked leucocytic infiltration, followed by congestion and cellular proliferation which leads to necrosis (Radostits *et al*., 1995). Infection in foetal lungs produces bronchopneumonia with congestion, fibrinous exudation, and cellular infiltration (Corbel, 1988). In males, the lesion may be confined to the genital organs. The testes, seminal vesicles and epididymis are enlarged and there may be multiple granulomas with infiltration of lymphoid and plasma cells in the epididymis (Smith *et al.*, 1972).

2.7 Diagnosis of Brucellosis

2.7.1 Clinical signs

Brucellosis could be suspected in any herd with history of abortion during the third trimester of pregnancy, infertility, orchitis, epididymitis, stillbirths, neonatal mortality and hygroma but these signs, however, are not pathognomonic (Bercovich, 1998; OIE, 2009a). Different methods have been used in the diagnosis of brucellosis in different animal species in various countries of the world. The methods vary depending on the sample specimen, the urgency of the result, vaccination status of the animal, purpose for screening and the condition of the specimen (fresh or putrefying).

2.7.2 Pathology

Lesions due to brucellosis can be generalized, though they have been mostly reported to be in the reproductive system (Meador *et al.*, 1989). In addition may differ with different Brucella species and the organ or tissue affectd. Some aborted foetuses may look normal while others are autolysed or have variable

quantities of subcutaneous oedema and bloodstained fluid in their cavities (AHA, 2005). In ruminants foetuses, the spleen and/or liver may be enlarged, and the lungs may show signs of pneumonia and fibrous pleuritis (AHA, 2005). Abortion caused by Brucella species are typically accompanied by placentitis. The cotyledons may be red, yellow, normal or necrotic. In cattle or small ruminants, the intercotyledonary region is typically leathery, with a wet appearance focal thickening (OIE, 2009).

2.7.3 Laboratory Diagnosis

2.7.3.1 Samples for laboratory diagnosis

The most useful specimens for the laboratory diagnosis of brucellosis include placenta (most infective) followed by lymph nodes, milk and blood in human (Poiester *et al*., 2010). Other materials include foetal stomach contents, foetal lungs and spleen. From abortive animal (dam) useful materials include uterine discharges, hygroma fluid, serum and vaginal swabs. In male animals, semen, preputial sheath washing and serum are required, while from carcasses specimens used include spleen, testes and seminal vesicle, bone marrow and lesions of bones and joints (Radostits *et al.*, 1995).

2.7.4 Laboratory investigation

2.7.4.1 Direct smear examination

The cultural and microscopic methods described for cattle are applicable to the diagnosis of *B. melitensis* and *B. ovis* in goats and sheep (Poiester *et al*., 2010). The bacteriological diagnosis of *B. melitensis* and *B. ovis* can be made by means of microscopic examination

of smears from vaginal swabs, placenta or aborted foetuses stained with the stamp (1950) modification of Ziehl- Neelsen method (EC, 2001). However, presence of *Chlamydia psittaci* or C*oxiella burnetti* can mislead the diagnosis, they being morphologically related to *Brucella* (Radostis *et al.*, 1997; Poiester *et al*., 2010).

2.7.4.2 Culture, isolation and identification

The three *Brucella* species (*B. abortus, B. melitensis and B. ovis*) can be isolated on a medium containing 50 - 100ml of serum per and an added 5 – 10% carbon – dioxide for growth where the organisms occur only in the rough phase (Corbel, 1988). The specimens to be cultured are semen from rams, vaginal discharges, milk, aborted foetuses and foetal membranes from ewes that have aborted or delivered lambs prematurely. Vaginal swabs and milk from aborted ewes or goats are the best samples/specimens for the isolation of *B. melitensis* while spleen and lymph nodes (iliac, mammary and pre femoral) are the most reliable samples for isolation of the organism in necropsied animals (Marin *et al*., 1996).

Farrell's selective medium developed for isolation of *B. abortus* from milk (Farrell 1974) is also recommended for the isolation of *B. melitensis* (Alton *et al*., 1988). The isolation of *B. melitensis* on appropriate culture media such as Farrell's selective media is recommended for accurate diagnosis (Farrell, 1974). The best samples for the isolation of *B. melitensis* from sheep and goats are vaginal swabs and milk (Marin *et al*., 1996; Bale *et al.,* 2003). However, nalidixic acid and bacitracin concentrations in the medium may inhibit some *B. melitensis* strains (Marin *et al.*, 1996a). Thus, its sensitivity for the

isolation of *B. melitensis* from naturally infected sheep is sometimes lower than that obtained with the less selective Thayer-Martins modified medium (Marin *et al*., 1996a). The sensitivity of bacteriological diagnosis is significantly increased by the simultaneous use of these two media (Marin *et al*., 1966b).

The identification of *Brucella* involves Gram's staining, study of colonial and cellular morphology and routine biochemical tests (Corbel, 1987). Species are distinguished on the basis of lysis by bacteriophage and oxidative reactions on amino acids and carbohydrates substrates. Typing of isolates into biovars is based on carbon-dioxide requirement, sensitivity to thionin and basic fuchsin dyes, H2S and urease production (Alton *et al*., 1988).

2.7.4.3 Laboratory animal inoculation

Laboratory animals can be used for the diagnosis of animals suspected to have brucellosis. Guinea pigs are more susceptible to brucellosis than any other laboratory animal (OIE, 2009). However, mice and rabbits are also susceptible and may be substituted for guinea pigs (Avong, 2000; Ocholi, 2005). A suspension of the suspected specimen (foetal lung, placenta, blood, etc) is injected intraperitonially or intrasmuscularly into guinea pigs or preferably intravenously in mice (Alton *et al*., 1988; OIE, 2009). The spleen of mice is cultured 7 days post inoculation while serum samples of guinea pigs are subjected to a specific test 3 and 6 weeks after inoculation (OIE ,

2009a)

2.7.5 Serology

The current serological tests used for the diagnosis of *B. melitensis* and *B. ovis* in sheep and goats were initially developed for the diagnosis of *B. abortus* in cattle. Though not formally validated for use in sheep and goats, these tests, especially Rose Bengal Plate Test (RBPT), Complement Fixation Fest (CFT) and more recently ELISA, have been used for the serological diagnosis of brucellosis in sheep and goats (Ferina, 1985; Macmillan, 1990). The outer membrane of the bacteria contains the main antigens involved in *Brucella* antibody detection. The resulting reaction is an agglutination where antibodies to *Brucella* are present and this gives a positive result. Where *Brucella* antibodies are absent, there is no agglutination and the test is said to be negative. Body fluids like serum, uterine discharges, vaginal mucus, milk, semen and plasma from suspected animals may contain different quantities of antibodies of the Ig M, Ig G1,IgG2 and Ig A types directed against *Brucella* (Beh, 1974). Because infected animals may or may not produce all antibody types in detectable quantities, several tests are employed to detect brucellosis (FAO, 2005).

Poiester *et al.* (2010) reported that there is no serological test that is 100% accurate and that serological test is presumptive evidence of infection. In addition, there are considerable differences in the accuracy of various serological tests. Furthermore, depending on the sensitivity and specificity, serological tests can be used to screen for or confirm the disease. Screening tests are inexpensive, fast and highly sensitive, but not

necessarily specific. They further reported that confirmatory tests are required to be sensitive and specific; hence eliminate some false positive reactions.

All smooth *Brucella* share common epitopes in the O-polysacharide (OPS) (Poiester *et al*., 2010).Virtually all serological tests for antibodies to those bacteria use *B. abortus* antigen in the form of whole cells smooth lipopolysacharide (SLPS) or OPS (Nielsen and Yu, 2010; Poiester *et al*., 2010) while the rough lipopolysacharide (RLPS) or protein antigens are commonly used as the main antigens for detection of antibodies to *B. ovis.*and *B. canis* (Blasco, 1990; Carmichael, 1990).

2.7.5.1 Rose Bengal plate test

The Rose Bengal Plate Test **(**RBPT**)** is also called card test or buffer *Brucella* antigen test (Bale, 2013). It is a spot agglutination technique which uses a suspension of *B. abortus* smooth cells stained with the Rose Bengal dye, buffered to pH 3.65. A neutral pH RBPT can measure the presence of IgM, IgG1 and IgG2 (OIE, 2009). However, IgM appears to be more active. At a buffered pH of 3.65, RBPT apparently measures only IgG1. It was considered that while the test gave few negative results, it gave many false positives. This may be due to significant reaction with IgM in animals with previous vaccination.

In a situation where there is no routine vaccination, the use of this test can give a good record of exposure of animals to *Brucella* organism. The RBPT is an internationally recognized test for the screening of brucellosis in small ruminants but lacks standardization of the antigen. Low pH of the antigen enhances the specificity of the test

while the temperature of the antigen and the ambient temperature at which reaction takes place may influence the sensitivity and specificity of the test (Alton, 1981; Macmillan, 1990). Corbel (1972) observed that the sensitivity of the test was .associated with fractions containing immunoglobulin IgG, especially the IgG1.

2.7.5.2 Serum agglutination test

Hajdu and Baseda (1974) reported that Serum Agglutination Test **(**SAT**)** measures IgM, IgG1 and IgG2 and IgA. The test is performed at a near neutral pH; hence it detects IgM antibody very well. It is best used to detect acute infections. It is less so for IgGl resulting in low assay specificity (Corbel, 1972; Nielsen *et al*., 1984). As a result, SAT, while very sensitive, is generally not used as a single test but in combination with other tests. The test has some limitations including false positive and false negative (Poiester *et al.,* 2010) hence, SAT is suitable for herd testing, rather than for individual animals. In addition, the presence of post- vaccinal antibodies can complicate the results (Corbel and Brinley-Morgan, 1984). The SAT does not detect antibodies to *B.canis* and *B. ovis* because those rough strains of the organism do not have O Polysacharide on their surfaces (Ndybahenduku and Chu, 1984; Poiester *et al*., 2010). Thus, RLPS is commonly used as the main antigen for detection of antibody to *B. ovis* and *B. canis* (Poiester *et al*., 2010).

2.7.5.3 Complement fixation test

Complement Fixation Test (CFT) is a prescribed test for international-trade (OIE, 2009). It is considered the most specific and valuable serological test for brucellosis (WHO/ MZCP, 1998; Poiester *et al.,* 2010). It detects mainly IgG1 antibodies than it does for the antibody of IgM types, which are partially destroyed during inactivation. Since antibodies of the IgG1 type usually appear after antibodies of IgM type, control and surveillance of this disease is best done with SAT and CFT (WHO/ MZCP, 1998). It shows good correlations with the recovery of *Brucella* organism from artificial infection or naturally infected animals (Madsen, 1994).

Although the test is fast and accurate, it does not allow for discrimination of *B. abortus* S19 derived antibody (Poiester *et al*., 2010). Other problems include the subjectivity of interpretation of results due to difference in technique (Madsen, 1994), occasional direct activation of complement by serum (anti-complementary activity) and the inability of the test to be amenable for use with haemolysed serum samples. Furthermore, it is laborious and requires highly trained personnel with suitable laboratory facilities (FAO, 2005). This makes the CFT less suitable for use in developing countries (FAO, 2005). It is used mainly as a verification tool in the diagnosis of human brucellosis (WHO/ MZCP, 1998). It may also test false negative when the antibodies of the IgG1 types hinder complement fixation (Nielsen *et al*., 1988; Macmillan, 1990).

2.7.5.4 Enzyme linked immunosorbent assay

The most widely used Enzyme Linked Immunosorbent Assay (ELISA) in detecting brucellosis is indirect ELISA (i ELISA) (OIE, 2009). The method involves binding of the antigen on a solid phase, usually a polystyrene microtitre plate so that antibody, if present in a sample, binds to the immobilized antigen-enzyme conjugate which is a combination with chromogenic substrate that gives coloured reaction indicative of the presence of antibody in the sample (Blasco *et al.,* 1994).

The indirect ELISA, using more or less purified S-LPS of *B. melitensis* as antigen and polyclonal conjugate (anti IgG1 H+L) has been reported to be sensitive for the diagnosis of brucellosis in sheep and goats (Diaz-Aparicio *et al*., 1994; Delgado *et al*., 1995; Jimenezda Bagnes *et al.,* 1995). Recently, a new time resolved fluorescent energy transfer was described and evaluated against sera from infected and un-infected sources. The test matched marched the performance of iELISA which have 100% sensitivity and specificity and surpassed the performance of both cELISA and FPA. It is effective on poor quality sera and may have future applications if validated under field condition, (WHO/OIE, 2009).

2.7.5.5 2–Mercapto ethanol test

The 2–Mercapto Ethanol Test (2-MET) is an adaption of the SAT titre. There are two forms of this test which use either 2-mercaptoethanol (Rose and Roepke, 1964) or dithiothreitol (Klein and Behan, 1981). Dithiothreitol is preferable because of the toxicity of 2- mercaptoethanol (Klein and Behan, 1981). The test measures mainly IgG1 because the disulphide of IgM is being reduced to monometric molecules and therefore not able to

agglutinate. However, IgG1 can also be reduced giving false negative results, though in general, reduction of IgM increases specificity (Poiester *et al*., 2010).

2.7.5.6 Lateral flow assay

The Lateral Flow Assay (LFA) is a simplified form of ELISA for the qualitative detection of antigen specific antibodies in serum or whole blood samples (Christopher *et al*, 2010). The assay is based on the binding of specific antibodies to antigen immobilized on test strip (cellulose membrane matrix). It detects specific IgM and IgG1 antibodies and a high sensitivity is assured for stages of the disease (Nielsen and Yu, 2010). Application of the test does not require expertise, equipment or electricity and the test kits can be kept at room temperature (Christopher *et al*., 2010). However, the interpretation is subjective, depending on the formation of a visible coloured line or reaction and the assay is expensive because of the multiple ingredients/component involved (Nielsen and Yu, 2010).

2.7.5.7 Milk ring test

The Milk Ring Test (MRT) is essentially a rapid agglutination test carried out on whole milk or cream. Haemotoxylin stained *Brucella* cells are added to the whole milk and reactions are allowed to take place (Hubber and Nicoletti, 1986; McCaughey, 1972). Immunoglobulins present in the milk will, in part be attached to fat globules via the Fc portion of the fat molecule (Poiester *et al*., 2010). The immunoglobins detected by MRT are IgM and IgA. This test may be applied to individual animals or to pooled milk samples using larger volume of milk relative to the pooled size (Macmillan, 1990). The

MRT is prone to false reactions caused by abnormal milk derived from mastitis colostrums and late milk from the lactation cycle (Kerr *et al*., 1959;. Cunningham, 1970; McCaughey, 1972). False negatives may also occur in milk with low concentration of lacteal antibodies or lacking fat clustering factors (Bercovich, 1998). In spite of its problems, it is extremely effective and is usually the method of choice on dairy herds and may be used as an inexpensive screening test in conjunction with other tests (Corbel, 2006).

2.7.5.8 Anti–globulin (Coomb's) test

Coomb's test is used to confirm SAT results and in epidemiological survey of brucellosis because of the advantage of detective incomplete and complete antibodies of IgG2 types (WHO/ MZCP, 1998). It also differentiates patients with acute and chronic infections. The limitation of this test is that its results are indicative of infection when its titre is at least two times that of SAT.

2.7.5.9 Skin hypersensitivity test

This test uses brucellin which is injected into the flank or intrapalpebrally and measuring the thickness of the skin (Weidmann, 1991; Cheville *et al*., 1994). However, not all infected animals react. Therefore, this test alone cannot be recommended as the sole diagnostic test for the purpose of international trade (OIE, 2008). The brucellosis test has high specificity such that serologically negative unvaccinated animals that are positive reactors to this test should be regarded as infected animals (Povillot *et al.,* 1997,Seargerman *et al*.,1999). The results of this test may help in the interpretation of

serological reactions thought to be false positive serological reactors due to infection with cross-reacting bacteria, especially in brucellosis free areas (Povillot *et al*., 1997; Seagerman *et al*., 1999; De Massis *et al*., 2005). The test can be relied upon for clinical surveillance and epidemiological survey (FAO/WHO1986). This test has great value in areas with low prevalence and areas known to be free from brucellosis (Bercovich, 1998).

2.7.5.10 other serological tests

Other serological tests for the diagnosis of brucellosis worthy of mention include the Radioimmunoassay, (RIA), Radio- ImmunoDiffusion (RID), Agar Gel Immunodiffusion (AGID), Indirect Haemolgzin Test (IHLT) and Heat Inactivation Test (HIT).

2.7.6 Molecular diagnosis of brucellosis

2.7.6.1 Polymerase chain reaction

Fekete *et al*. (1990) reported that the first Polymerase Chain Reaction (PCR)-based test for brucellosis was introduced in 1990. The first special specific multiplex PCR was called AMOS-PCR assay which was used to identify and differentiate *B. abortus* biovars 1, 2 and 4, *B. melitensis*, *B. ovis* and *B. suis* biovar 1, based on the polymorphism arising from species-specific localization of the insertion sequence IS 711 in the *Brucella* chromosome (Bricker and Halling, 1994). In addition to the commonly used PCR assay, a new multiplex -PCR assay was developed that specifically identified *B. neotomae*, *B.pinnipedialia*, *B. ceti* and *B. macroti* (Huber *et al*., 2009). Furthermore, it differentiated *B. abortus* biovars 1, 2 and 4 from biovars 3, 5, 6 and 9 as well as between

B. suis biovars 3 and 4, biovars 2 and 5. A Bruce-ladder multiplex PCR assay was also developed for identification and differentiation of *Brucella* species and vaccine strains (Lopez-Coni *et al*., 2008).

2.8 Differential diagnosis of Brucellosis

There are many potential causes of abortion in animals which can be confused with brucellosis (AHA, 2005). These include infectious diseases like infectious bovine rhinotracheitis and mucosal disease; and infections with other organisms like *Trichomonas foetus, Neospora caninum, Campylobacter foetus, Listeria monocytogenis* ,*Sacosporidia* spp ,*Leptospira* spp and fungi. Others include viral disease causing abortion in sheep like Rift valley fever and wesselbron disease. There are also potentially non infectious causes of abortion in animals resulting from nutritional and toxic factors.

Brucellosis can be differentiated from these conditions generally by its pathology, presentation of clinical signs/manifestation of the disease, and by laboratory techniques. In humans, brucellosis needs to be differentiated from acute febrile diseases like typhoid fever, Q-fever, enteric fever, rheumatic fever, and cat scratch fever. Others include auto-immune disease, influenza, mononucleosis, cancer, malaria, fungal infections, cholecystitis, HIV, thrombophlebitis, gastroenteritis, meningitis, sacroititis and tuberculosis (Maloney and Fraser, 2006).

2.9 Treatment of Brucellosis

Brucella organisms are facultative intracellular pathogens that infect host macrophages (FAO/ WHO/ OIE, 2001) Therefore, the antibiotic to be used for the treatment must penetrate into the cell (Kaya *et al*., 2012). In addition, a combination of antibiotics must be used to avoid a relapse. Tetracyclines, quinolones, trimethoprim, sulfametha-xazole, rifampicin, and streptomycin are commonly used preparations for the treatment of brucellosis. Unfortunately, as reported by Solera (2010), the relapse rate is 30 %, despite the combination therapy.

2.9.1 Treatment in animals

Presently, there is no practical treatment for brucellosis in infected animals (Anon, 2002). Prolonged antibiotic treatment is sometimes successful in infected dogs (Solera, 2010). However, the disease is difficult to cure (Ettinger and Fieldman, 1995). Also, concurrent treatment with chlortetracycline and streptomycin has affected some level of treatment in sheep, though it is uneconomical except in prized rams (Cynthia, 2005). It has been observed that despite this treatment, fertility remains low even if the organism is eliminated. Radostits *et al*. (2003) reported that treatment of the disease in cattle is generally unsuccessful because of the intracellular sequestration of the organism in lymp h nodes, mammary glands and the reproductive organs. In general, failure in the treatment of brucellosis have been attributed to the use of incorrect doses of antibiotics, inadequate durations of treatment, cost of medication, and failure to cure udder infection which could lead to a relapse (Radwan *et al*., 1992). Subsequently, due to long term treatment regimen, it could lead to antibiotic residues in milk and meat of treated animals

which could be passed onto the food chain of humans.

2.9.2 Treatment in humans

A report by the FAO/WHO (1986) revealed that, in humans despite extensive studies over the past 15 years, the optimum antibiotic treatment for brucellosis is still disputable. The treatment recommended by WHO (1986) for brucellosis in adults is rifampicin at a dose level of 600 to 900 mg and doxycycline at a rate of 200 mg daily for a minimum of 6 weeks. Some practitioners still claim that the long- established combination of intramuscular streptomycin with an oral tetracycline gives fewer relapses (Ariza *et al*., 1985). Alternative treatments were experimented using quinolones, aminoglycosides, streptomycin, gentamycin and rifampicin (Kaya *et al*., 2012).

2.10 Prevention and control of Brucellosis

The prevention of brucellosis can be achieved by using vaccination to increase the flock resistance to the disease. Vaccination of all animals in a flock or region is an important measure for the prevention and control of brucellosis in a livestock (EC, 2001). There are three vaccines strains (*Brucella abortus*, S19, RB51 and *Brucella melitensis* rev.1) which are recommended by the World Organisation for Animal Health (OIE) for used in the prevention of brucellosis in livestock (Garin-Bastuji and Blasco, 2008; Nielson and Ewalt, 2008). In endemic areas, the most important means of keeping a flock free of brucellosis is to prevent its entry into the flock, which could be achieved as follows as reported by several workers:

2.10.1 Livestock movement control

It is possible to establish disease free zones, if the disease is endemic only in a part of a country. Tight control and restriction of movement of livestock between zones will have to be enforced to prevent spread of brucellosis (AHA, 2005).

2.10.2 Avoidance of grazing cattle with goats and sheep

The common practice of rearing cattle along with sheep and goats as practiced by pastoralists should be discouraged. This is because such husbandry practice favours the spread and maintenance of brucellosis in the herd (Kaltungo, 2013; Buhari, 2014). As vaccine is administered to cattle without attending to sheep and goats, they become a source of infection to the cattle when they acquire the infection. Abortion due to *B. abortus,* attributable to this husbandry system has been reported (Ocholi *et al*., 2005).

2.10.3 Sero–surveillance programme against brucellosis

There should be periodic sero-monitoring of the herd/flock to keep tract with the brucellosis status of the animals. Any reactor is a suspect and must be separated and further investigated for elimination. Confirmed positive animals should be culled without delay (AHA, 2005).

2.10.4 Good farm Biosecurity

This is an appropriate measure in a farm that desires to stay free of brucellosis. A high level of biosecurity will prevent the accumulation of the organism in the environment thereby reducing the risk of other animals becoming infected (Ducrotoy *et al*., 2014).

2.10.5 Quarantine of replacement animals

All replacement animals should be quarantined until they are certified brucellosis free before they are introduced into the farm. In any suspicion of brucellosis in a flock or herd, quarantine must be immediately imposed on the suspected herd/flock to contain the spread of infection, after which the flock/herd will require repeated sero surveillance to confirm they are free of brucellosis (AHA, 2005). Movement of latently infected animals present the greater risk and the potential movement of infected materials by dogs or birds should not be ignored (Minas, 2005).

2.10.6 Testing and culling programme

Cattle kept for artificial insemination purposes must be tested often and positive reactors must be culled immediately (FAO, 2005). This removes infected animals from the herd, hence reduces exposure and transmission of the disease within and outside the farm. Animals showing such signs must be isolated and tested for brucellosis. All personnel on the farm must be thoroughly educated on brucellosis, its signs, and symptoms like abortion, retained placenta, stillbirth and infertility. They must report such signs and take appropriate measures when attending to such animals.

2.10.7 Isolation of pregnant animals during parturition

All animals nearing parturition should be considered potential dangers to other animals. They should be isolated and restricted at a maternity pen. After parturition, they are tested and all non-living products of parturition are adequately disposed of and the environment properly cleaned to ensure no traces of contamination are left in the pen.

The animals must be certified free of brucellosis before they leave the pen (AHA, 2005).

2.10.8 Vaccination

The *B. abortus,* S19 and *B. melitensis,* Rev. 1 have been proven to be effective against *B. abortus,* in cattle and *B. melitensis,* and *B. ovis,* in sheep and goats respectively (Elberg, 1996; Nicoletti, 1990). Both vaccines have the disadvantages of causing abortion in a proportion of pregnant animals and of being pathogenic for humans (Nicoletti, 1990a). Similarly, AHA (2005) reported that *B. ovis,* RB51 infection in humans is possible but has not been documented. *Brucella melitensis,* Rev 1 has been evaluated for the vaccination of cattle in countries where *B. melitensis,* infection in goats and sheep is widespread, and experimental studies have shown that *B. melitensis,* Rev. 1 vaccine provided immunity to *B. melitensis,* equal to or superior to the immunity induced by *B. abortus,* with a lower vaccine dose in cattle (Alton, 1990; Blasco, 1997). The optimum age of vaccination for kids and lambs is between 4 – 8 months to avoid stimulating serological reactions that would interfere with the diagnosis (Alton, 1980).

2.10.9 Testing and culling positive reactors

For a successful control of brucellosis, a routine serological testing of livestock should be carried out using approved tests as was practiced in Australia before the disease was eradicated in 1989. All positive reactors to the test are destroyed or confined for slaughter at an approved abattoir and submitted tissues are cultured for *Brucella* (AHA, 2005). However, eradication by test and slaughter is not always successful due to latently infected animals

remaining serologically negative to standard tests until late pregnancy (AH, 2005).

In rams, the prevalence of *B. ovis* can be reduced by examining rams before the breeding season and culling rams with abnormalities (Corbel, 2006). Furthermore, test and removal practices directed at rams can eradicate such organism from the flock (Corbel, 2006).

2.10.10 Public awareness campaign

Stakeholders in the livestock/veterinary business should embark on a media campaign to educate farmers on the importance of inspecting susceptible animals regularly and reporting abortions, the birth of weak or dead animals and infertility cases to the state Veterinary Department (Esuruoso, 1974). An abortion investigation programme that relieves producers on the cost of investigation is a useful strategy. Details of any imposed movement restrictions need to be readily available and clearly explained to the farmers. Given the important zoonotic implications, people at risk must be advised of appropriate occupational health and safety requirements and health authorities alerted to the potential of human infection (Maxwell and Bill, 2008).

2.10.11 Eradication

Usually, the elimination by test and slaughter policy is justified on economic grounds only when the prevalence of infected animals in the area is 2% or less (Nicoletti, 1993). On a national scale, eradication can be done by identifying infected herds and slaughter

all affected animals in the herd. However, this can only be possible when there is adequate compensation arrangement to the affected farmers. This could occur when the animals are under strict surveillance and on restricted movement. On a smaller scale, the animal should be isolated at parturition since all parturitions are considered potential sources of infection (Nicoletti, 1993).

All livestock owners should be properly educated on the aims and objectives of the control programme so that there is total cooperation from them at all levels of the programme. They should be informed of the hazards of the disease to their families and themselves; and the economic benefits of the control programme.

2.11 Public Health Importance of Brucellosis

Brucellosis is a zoonotic disease of public health significance. It is primarily an occupational risk problem in exposed professionals like veterinarians, farmers, abattoir workers, laboratory workers, and others who work with animals and their by-product (Radostits *et al*., 2003). The infection of livestock with *Brucella* organism poses a significant health risk for onward transmission to humans by direct contact or from consumption of unpasteurized milk and other dairy product (Osten and Samartino, 2009). Although there has been great progress in controlling the disease in many countries, there are still some regions where the infection persists in domestic animals; hence subsequent transmission to the human population occurs. Urbanization and expansion of animal industries, coupled with the lack of proper hygiene/biosecurity in animal husbandry and in food handling partly accounts for brucellosis remaining a public health hazard.

Furthermore, the expansion of international travels stimulates the taste for exotic dairy goods such as fresh cheeses that may be contaminated, and the importation of such food into *Brucella* free regions may contribute to the increase in human brucellosis (Corbel, 2006). The economic losses due to human brucellosis, although difficult to estimate, results from physical and psychological suffering due to infection, hospitalization cost of drugs, loss of income, physical incapacity and reduction in productivity (WHO, 1986).

2.12 Economic Importance of Brucellosis

The economic implications associated with brucellosis are extensive and include both public health costs and loss of production in domestic livestock through loss of milk, meat, restriction in international livestock trade and diminished animal working power (Corbel, 2006). Brucellosis affects exports and constrain efforts to import exotic livestock breeds (more susceptible to *Brucella)* to improve the breeds (Saleem *et al*., 2010).

2.13 Brucellosis in Nigeria

Brucellosis is endemic in Nigeria having been reported sporadically all over the country since 1927 (Bertu, 2014). Most of the reports were from established farms than from the nomadic cattle herds that constitute over 80% of the livestock in Nigeria. Due to poor disease reporting system, the distribution of the disease is not well documented. Similarly, there is insufficient surveillance of brucellosis leading to poor determination of prevalence and incidence of the disease in the country. Various serological studies over the years revealed that brucellosis is widespread in Nigerian

livestock (MacGregor,1964; Esuruoso, 1965; Banerjee and Bhatty,1970; Nuru,1974; Bale,1980; Ocholi,1990; Bertu *et al*., 2010a; Kaltungo,2013).

2.13.1 Caprine and ovine brucellosis in Nigeria

The first evidence of brucellosis in goats was presented by Adams and Mckay (1966) and later by Krammer *et al*., (1967). Since then little information was available until the first comprehensive reports of brucellosis among goats in Nigeria was presented by Falade, (1974). Other reports on caprine brucellosis presented from various parts of Nigeria were those by Falade (1978; 1980; 1981), Bale (1980) and Bale *et al*. (1982). All these reports revealed a prevalence rate of between 2.1% and 14.5%. A recent study by Cadmus *et al*. (2006) in Ibadan, Oyo State revealed a prevalence of 0.86%. Kaltungo (2013), in a study of brucellosis in small ruminants in the North Senatorial District of Kaduna State, documented a prevalence of 12%. Early efforts at determining causes of abortion in sheep failed until Okoh (1980a) isolated of *B. abortus,* from sheep in an investigation on a farm in Kano State. Subsequent serological and cultural studies have confirmed ovine brucellosis in Nigeria (Bale *et al*., 1982; Ocholi *et al*., 2005a, Okoh 1980a; Falade and Shonekan 1982; Okewole *et al*., 1988)

2.13.2 Knowledge and practices of small ruminants' stakeholders

Brucellosis is an occupational disease of those who come in contact with infected animals and their products such as abattoir workers, veterinarians, slaughterers, laboratory workers, shepherds and farmers (Mukhtar and Kokab, 2008). Humans get infected by direct contact with infected animal products,

ingestion of contaminated food and inhalation of aerosols (Megid et al., 2010). In Nigeria brucellosis is on the increase among nomadic and semi-nomadic individuals who control the highest number of livestock population in the country (Ocholi, 1990). In a report by Adeseji *et al.* (2005), South-western Nigeria, revealed that 13% and 11% of herdsmen and meat vendors were aware of brucellosis, while Adamu *et al*. (2012), revealed a low level of awareness (3.1%) among farmers in the arid zone of Nigeria. Management practices systems for small ruminants vary worldwide and food hygiene practices are also deficient (Grahn, 2013). There are major gaps in knowledge of many livestock producers and their practices are not adequately focused on prevention (Grahn, 2013). Small ruminants are not vaccinated to raise immunity and reduce abortion rate (Bertu, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Kaduna Metropolis which comprises of Kaduna North and South Local Government Areas (LGAs), Kaduna State. The State falls within the southern and northern Guinea Savannah zones of Nigeria (KDSG, 2008). The raining reason is between April and November, with a greater variation in the Northern part. It lies between latitudes 60 and 11' North and longitude 70 and 44' East, and is at 607.076m above sea level (KDSG, 2008). Kaduna State is located within the North–west Geopolitical Zone of Nigeria. The State shares boundaries with Katsina and Zamfara States to the North, Plateau and Bauchi States in the East, Nasarawa and the Federal Capital Territory (FCT), Abuja to the South, Niger State to the West and Kano State to the North–east (KDSG, 2008). Kaduna State occupies about 48, 473 square kilometers land mass, with a human population of 6, 066, 562 (National Population Census Commission, 2006). It is composed of 23 Local Government Areas (LGAs).

The State has rich soil suitable for agriculture with a variety of crops being cultivated. The farm produce and residues serve as a rich source of feed to their livestock, especially sheep and goats. Hence farming and animal husbandry remain the primary occupation of its inhabitants (KDSG, 2008). Kaduna North and Kaduna South Local Government Areas are within the Kaduna Central Senatorial Zone of Kaduna State.

3.2 Study Design

The two LGAs under study, Kaduna North and Kaduna South, were selected based on convenient sampling in view of the high mixing potentials of small ruminants and human habitation. Furthermore, two wards were selected from each of the LGAs based on random sampling without replacement among the wards in each LGA. Similarly, the households used were selected based on random sampling without replacement and acceptance by the household owners. The livestock markets, abattoirs and slaughter houses used in the study were selected based on the fact that these locations routinely received high population of small ruminants for either sale or slaughter than any other location within the LGAs. The individual animals sampled at households, abattoirs, slaughter houses, and livestock markets were sampled based on owner's consent.

A questionnaire was designed to collect information from farmers, abattoir and slaughter slab workers to determine their Knowledge and Practices on brucellosis in ruminants and humans. The questionnaire was comprised of mostly open- ended questions to ensure interaction with the respondents. A similar questionnaire was designed and administered to professional health workers in hospitals and clinics in the study areas, and information on knowledge on brucellosis was collected.

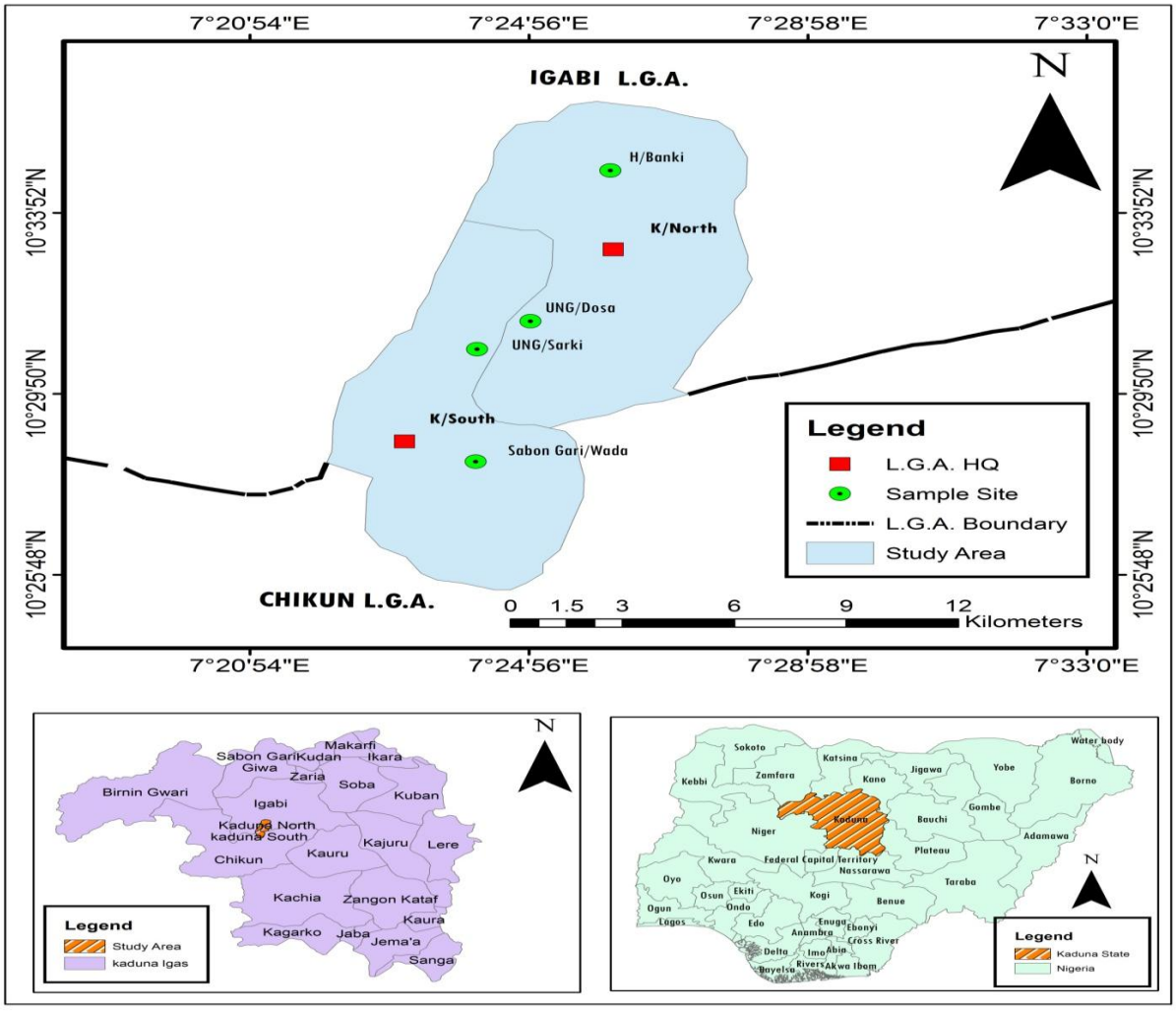

Figure 1: Map of the study area

Source: Modified from the administrative map of Kaduna State, Nigeria

3.3 Sample Size Determination

The sample size was determined by using the formula as described by Michael (2005).

Thus; -

$$n = \frac{Z2pq}{d2}$$

Where: n = minimum sample size

Z = appropriate value for the standard normal deviation set at 95% confidence interval (1.96)

p = prevalence of 14.5% (sheep) and 16.1% (goats) (Bertu *et al*., 2010)

q = complementary probability, 1 – p

d = desired level of significance (0.05)

Thus, sample size for sheep = $\frac{(1.96) \times 0.145 \times (1 - 0.145)}{(0.05)^2}$

$$= \frac{3.8416 \times 0.145 \times 0.885}{0.0025}$$

$$= 190.5 \approx 191$$

Sample size for goats = $\frac{(1.96) \times 0.161 \times 1 - 0.161)}{(0.05)2}$

$$= \frac{3.8416 \times 0.161 \times 0.839}{0.0025}$$

$$= 207.6 \approx 208$$

Thus, the calculated sample size for sheep was ≈ 191 and for goat ≈ 208. But to increase precision the sample sizes were increased to a minimum of 500 for each species.

3.4 Methodology

3.4.1 Blood sample collection

Five millilitres (5ml) of blood sample was collected aseptically through the jugular vein from each sheep and goat using 10ml syringe and 21G needle after proper restraint by an assistant. The blood was transferred into a 10ml EDTA free sampling bottle. The sample was labelled appropriately based on abattoir/slaughter slab and animal species. For example, sample 1 collected from a female goat in Kawo abattoir was labeled KW/AB/G/F/1 and that from a female sheep was labelled KW/AB/S/F/1. Similarly sample one collected from a male goat in Tudunwada abattoir was labelled TW/AB/G/M/1 and that from a male sheep was TW/AB/S/M/1. The labelled bottles containing the blood samples were kept in a slanting position in an ice-box to allow the blood to clot and allow for separation of serum. The samples were transported to the Bacterial Zoonosis Laboratory in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University (ABU) Zaria. They were centrifuged at 3000g for 15 minutes immediately on arrival to allow for proper separation of the serum from the clotted blood, then was siphoned using a sterile pasture pipette into a 5 ml plastic serum bottle and was labelled appropriately. All the serum samples were stored in the freezer at -20°C until used.

3.5 Laboratory Analyses of Samples

3.5.1 Modified Rose Bengal plate test (M-RBPT) using *Brucella melitensis* antigen

The antigen was obtained from the Veterinary Laboratories Agency, New Haw, Weybridge, England (Plate I). The M-RPT was performed based on procedure used by Bertu (2014). Briefly, 25µl of RBPT *Brucella melitensis* antigen were placed on each square of a white tile and 75µl of serum sample was placed alongside (but not onto) the antigen. The antigen and serum were mixed thoroughly using a sterile applicator and the mixture rocked for 4 minutes. The test was examined for agglutination. A sterile applicator stick was used for each serum sample. The result was interpreted as positive (+ve) for any degree of agglutination (pink granules) or negative (-ve) for non-agglutination (no pink granules).

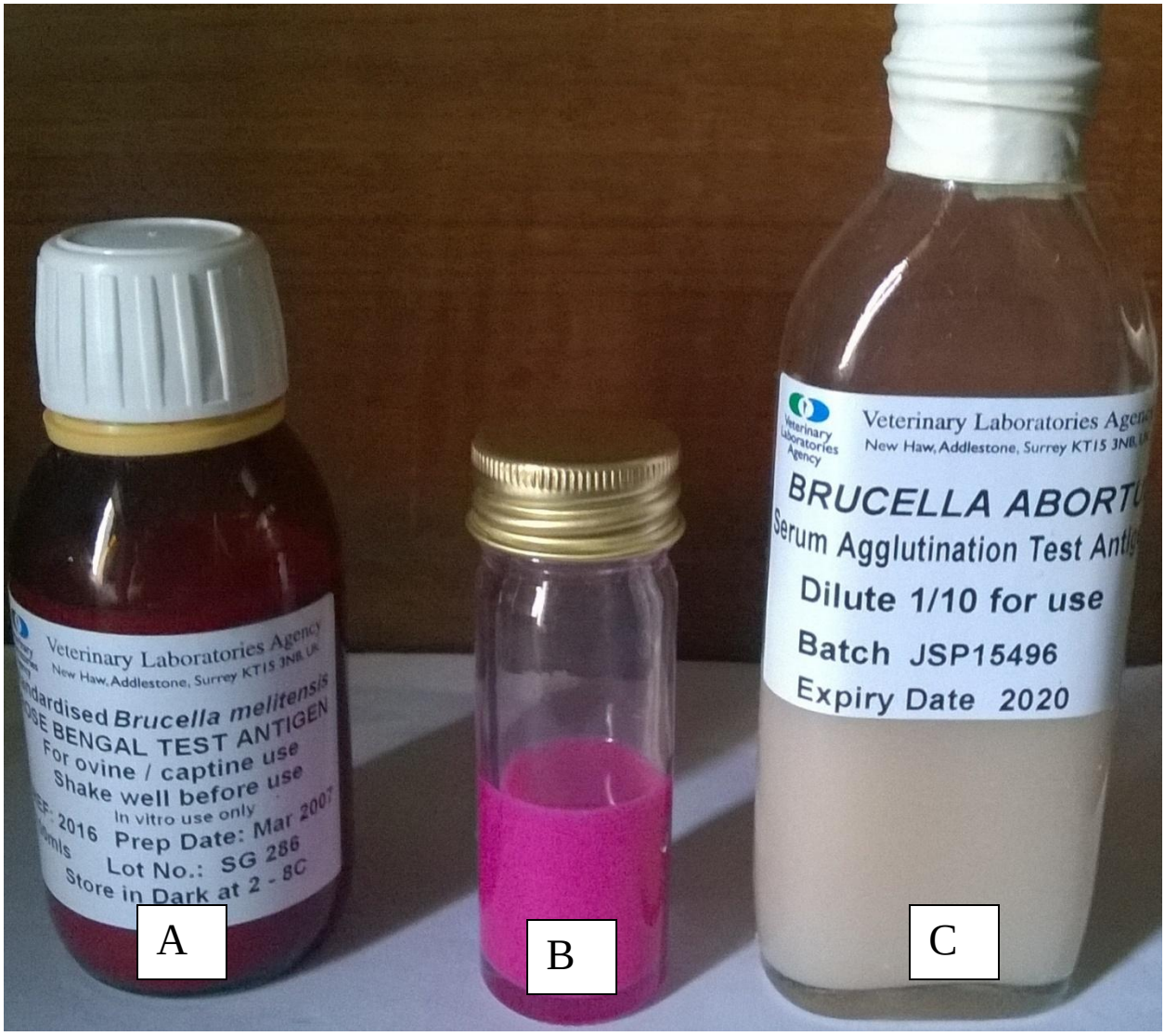

Plate 1: Photograph of the Rose Bengal Plate Test and Serum Agglutination Test antigens used for this study of *Brucella. Where A and B are Brucella melitensis* Antigen for M-RBPT; and C are *Brucella abortus* Antigen for SAT- EDTA

3.5.2 Serum agglutination test-ethylene Diamminotetraacetic acid using *Brucella abortus* antigen

This test was performed using the microtitre technique as described by Brown *et al*., (1981). The antigen was obtained from Veterinary Laboratories Agency, New Haw, Weighbridge, England. Phenol saline with EDTA buffer solution containing 5 g phenol crystals, 8.5 g sodium chloride and 1.86g disodium EDTA were dissolved in a 100ml warm distilled water to prepare the working solution. A 1:10 of the concentrated SAT antigen with the prepared buffer with 0.1µg of 0.02% Safranin O (to provide a contrast to the agglutination reaction) was made for each day's work. A 96–well U–bottom microtitre plate (Cooke Microtitre System) was set up on the work bench. Labelled serum vials were then placed on the work bench close to the microtitre plate according to the positions of the wells already labelled A–H and corresponding 5 vertical numbering of the wells were made. A representative entry of the sample details was made in the laboratory log book.

An automatic micropipette (Dragonmed®, Huawei) was used to measure out 40 µl of the buffer solution into the first well and 25µl into each of the remaining microtitre wells. This was followed by adding 10µl of the test serum into the first well. A fresh disposable pipette plastic tip was used for each test sample which was discarded later. A twofold serial dilution was done by transferring 25 µl aliquots from the first well up to the fifth well. The aliquot of 25µl was discarded after the last well. The content of the working dilution of the SAT antigen was mixed gently and 25 µl were added to each well. The contents of the microtitre plate were mixed gently by tapping the edges for 20 seconds. The plate was then wrapped with aluminum foil to avoid evaporation of the contents and

was incubated for 20 hours at 37°C in a Gallenkemp® incubator after which the result was read. The results were read as follows:

+ + + + 100% agglutination

+ + + 75% agglutination

+ + 50% agglutination

+ 25% agglutination

- No agglutination

The results were interpreted as

a. Positive for any degree of agglutination in wells for 1:40, 1:80, 1:160 dilutions (+)
b. Negative if no agglutination (-)

3.6. Administration of questionnaire

A structured questionnaire aimed at determining the knowledge and practices of small ruminants' keepers, traders, butchers, abattoir workers concerning brucellosis in animals and humans was prepared and administered to the afore- mentioned groups (Appendix 1). Similarly, another set of questionnaire was prepared and administered to health professionals in hospitals and clinics in the study areas to capture their understanding of the disease brucellosis and if they handled suspected cases of brucellosis in their working places (Appendix 2). Permission was sought from the Ministry of Health, Kaduna State for collection of information on brucellosis from its medical personnel (Appendix 3).

3.7 Data Analyses

Data generated from this study were presented in tables, pie charts and figures. The prevalence of *Brucella* antibodies in sheep and goats was calculated as percentage of animals that were positive to the serological tests in relation to the total number of animals tested. The prevalence in goats and sheep, males and females were calculated.

The results obtained were statistically analyzed using Statistical Package for Social Sciences (SPSS) version 17.0 (2009). Chi square (χ^2) was use to analyze categorical variables (age, sex, and species) and odd ratio was used to test association between variables. P value < 0.05 was considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Seroprevalence of *Brucella* by Species (Goats and Sheep)

Of the 696 goats sampled during the study, 61 (8.81%) were seropositive for *Brucella* using the M-RBPT while 103 (14.74%) were seropositive for *Brucella* using the SAT-EDTA. Similarly, of the 560 sheep sampled, 49 (8.76%) and 105 (19.05%) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively (Table 4.1). Of the 410 goats sampled from Kaduna North LGA, 35 (8.53%) were seropositive for *Brucella* using the M-RBPT while 62 (15.12%) were seropositive for *Brucella* using the SAT-EDTA. Also, of the 300 sheep sampled from Kaduna North LGA 26, (8.67%) were seropositive for *Brucella* using the M-RBPT, while 45 (15.0%) were seropositive using the SAT-EDTA. Of the 286 goats sampled from Kaduna South, 26 (9.10%) were seropositive for *Brucella* using the M-RBPT while 41 (14.33%) were seropositive using the SAT-EDTA. Similarly, of the 260 Sheep sampled from Kaduna South LGA, 23 (8.85%) and 60 (23.10%) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively (Table 4.1). There was no significant ($p > 0.05$) association between the species of animals sampled and the prevalence of *Brucella* antibodies detected using the two serological tests, and in the two sampled LGAs.

Of the 180 goats sampled in the abattoir and slaughter slabs in these two LGAs, 19 (10.56%) and 33 (18.13%) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively (Appendix 5). And of the 181 sheep sampled in the abattoir and slaughter slabs in these two LGAs, 14 (7.73%) and 31 (17.13%) were respectively seropositive for *Brucella* using M-RBPT and SAT-EDTA. Three hundred and twenty five

(325) samples were collected from goats in all the markets used in the study. Out of these 26 (8.0%) and 49 (15.08%) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively. Similarly out of the 194 samples from sheep from the markets 21 (10.83%) and 51 (26.29%) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively (Appendix 6). A total of one hundred and ninety one (191) goats were sampled from households in the two LGAs under the study. Of these 15 (7.85%) and 23 (12.04%) were seropositive for M-RBPT and SAT-EDTA respectively. Also, of the 126 sheep sampled from the households, 13 (10.32%) and 23 (18.12%) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively (Appendix 7).

Table 4. 1: Seroprevalance of *Brucella* in Goats and Sheep in Kaduna North and South Local Governments Areas, Kaduna State using M-RBPT and SAT-EDTA

	Goats			Sheep		
		No. positive using			No. positive using	
LGAs	**No. sampled**	**M-RBPT**	**SAT-EDTA**	**No. sampled**	**M-RBPT**	**SAT-EDTA**
Kaduna North	410	35 (8.53)	62 (15.12)	300	26 (8.67)	45 (15.00)
Kaduna South	286	26 (9.10)	41 (14.33)	260	23 (8.85)	60 (23.10)
Total	696	61 (8.81%)	103(14.72%)	560	49 (8.76%)	105 (18.75%)

Goats: M-RBPT P = 0.7792; OR = 0.9333; CI = 0.5485 to 1.588; $\chi 2 = 0.0647$
SAT-EDTA P = 0.7738; OR = 1.065; CI = 0.6945 to 1.1.632; $\chi 2 = 5.964$

P>0.05 – No significant difference

Sheep: M-RBPT P = 0.9402; OR 0.9778; CI = 0.5434 to 1.760; $\chi 2 = 0.00562$
SAT–EDTA P = 0.146; OR = 0.5882; CI = 0.3832 to 0.9031; $\chi 2 = 5.964$

P>0.05 – No significant difference

4.2 Seroprevalence of *Brucella* by Sex

Thirty three (9.24%) and 54 (15.12) of the 357 female goats sampled from households in Kaduna North and South LGAs were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively (Table 4.2). Similarly, of the 339 male goats sampled from these locations, 28 (7.96%) and 49 (14.45%) were seropositive for *Brucella* using M- RBPT and SAT- EDTA respectively. Furthermore, of the 295 female sheep sampled in Kaduna North and South LGAs 21 (7.12%) and 44 (14.92%) were seropositive for *Brucella* using M-RBPT and SAT- EDTA respectively while of the 265 male sheep sampled from Kaduna North and South LGAs, 28 (13.54%) and 33 (34.38%) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively.

Of the 215 female goats sampled from Kaduna North LGA, nineteen (8.84%) and thirty three (15.35%) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively (Table 4.2). Similarly, of the 195 male goats sampled from Kaduna North LGA, fifteen (7.54 %) and twenty nine (14.52 %) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively. Also, fourteen (9.85 %) and 21 (14.79 %) of the one hundred and forty two female goats from Kaduna South LGA were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively. Similarly, thirteen (9.03 %) and twenty (13.88 %) of the male goats from Kaduna South LGA were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively.

As for the sheep, 10 (7.63%) and 19 (12.97%) of the 131 female sheep from the Kaduna North LGA were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively (Table 4.2). Of the 169 male sheep from Kaduna North LGA, 15 (8.87%) and 28 (16.56%) were respectively seropositive using M-RBPT and SAT-EDTA. Similarly, of

the 96 male sheep sampled from Kaduna South LGA, 13 (13.54%) and 33 (34.38%) were seropositive using M-RBPT and SAT-EDTA respectively (Table 4.2). There were no significant ($p > 0.05$) association between the species of animals sampled and the seroprevalence of *Brucella*.

Also of the 93 male goats sampled at the abattoir and slaughter slab, 11 (11.65%) and 20 (21.14%) were seropositive for *Brucella* respectively (Appendix 6). As for the 104 female sheep sampled from abattoirs and slaughter slabs in Kaduna North and South LGAs, 10 (9.79%) and 20 (18.53%) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively, while of the 77 male sheep from the same locations, 4 (5.40%) and 11 (13.26%) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively(Appendix 8).

Of the 152 female goats sampled from the markets in Kaduna North and South LGAs 14 (8.42%) and 26 (16.41%) were respectively seropositive for *Brucella* using M-RBPT and SAT-EDTA. (Appendix 9) while out of the 173 male goats in the same locations 12 (7.31%) and 22 (12.58%) were positive using M-RBPT and SAT-EDTA respectively. Also, of the 81 female sheep sampled in the markets from Kaduna North and South LGAs only 9 (10.21%) and 17 (20.52%) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively, and for the male sheep, 12 (6.83%) and 34 (21.25%) out of the 171sampled in the same locations were respectively seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively (Appendix 9).

Table 4. 2: Seroprevalance of *Brucella* in Goats and Sheep in Kaduna North and South LGAs, Kaduna State by Sex using M-RBPT and SAT-EDTA

	Goats						Sheep					
	Female			Male			Female			Male		
	No. positive			No. positive			No. positive			No. positive		
LGA	**No. sampled**	**M-RBPT**	**SAT-EDTA**	**No. sampled**	**M-RBPT**	**SAT-EDTA**	**No. sampled**	**M-RBPT**	**SAT-EDTA**	**No. sampled**	**M-RBPT**	**SAT-EDTA**
Kaduna North	215	19 (8.84)	33 (15.35)	195	15 (7.69)	29 (14.87)	131	10 (7.63)	19 (12.97)	169	15 (8.87)	28 (16.56)
Kaduna South	142	14 (9.85)	21 (14.79)	144	13(9.03)	20 (13.88)	164	11 (6.71)	27 (16.46)	96	13 (13.54)	33 (34.38)
Total	357	33 (9.24)	54(15.12)	339	28(7.96)	49 (14.45)	295	21 (7.12)	46 (14.92)	265	28 (13.54)	61 (34.38)

Goat: M–RBPT; P = 0.4142; OR = 0.7997; CI = 0.4673 to 1.369; $\chi 2 = 0.6667$
SAT–EDTA; P = 0.7248; OR = 1.078; CI = 0.710 to 1.636; $\chi 2 = 0.1240$

Sheep: M–RBPT; P = 0.2042; OR = 0.6989; CI = 0.4011 to 1.218; $\chi 2 = 1.612$
SAT–EDTA; P = 0.0142; OR = 0.5862; CI = 0.3815 to 0.9008; $\chi 2 = 6.017$

4.3 Seroprevalence of *Brucella* by Age

Of the 696 goats sample in Kaduna North and South LGAs; 31, 352 and 313 were of the age range less than one year (< 1yr), one to three year old (1-3yrs) and above three years old (> 3yrs) (Table 4.3). Similarly, of the 560 sheep sampled, in Kaduna North and South LGAs,36, 254 and 270 were of the age ranges of less than one year (< 1yr), one to three years (1-3yrs) and above three years (> 3yrs) respectively. Sixty one (8.76%) and 103 (14.80%) of the 696 goats from the Kaduna North and South LGAs were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively. Also, 49 (8.75%) and 104 (18.57%) of the 560 sheep from Kaduna North and South LGA were seropositive for *Brucella* using M - RBPT and SAT-EDTA respectively (Table 4.3). Two (6.45%) and 3 (9.68%) out of the 31 of the goats less than one year old from Kaduna North and South LGAs were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively. Similarly, 26 (7.39%) and 53 (16.41%) of the 352 goats within the age of 1 to 3 year old from Kaduna North and South LGAs were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively. Of the 313 goats of over 3years old sampled from Kaduna North and South LGAs, 33 (9.35%) and 47 (15.02%) were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively (Table 4.3).

Two (5.56%) and five (13.89%) of the 36 sheep in the less than one year age bracket sampled from Kaduna North and South were seropositive for *Brucella* using M-RBPT and SAT-EDTA respectively. As for the 254 sheep within the age group 1 to 3 year old sampled in Kaduna North and South LGAs, 24 (9.45%) and 55 (21.65%) were seropositive for *Brucella* using the M-RBPT and SAT-EDTA respectively (Table 4.3). Also of the 270 sheep sampled in Kaduna North and South LGAs which are over 3 years old,,23 (8.52%) and 45 (16.67%) were seropositive for *Brucella* using M-RBPT and

SAT-EDTA respectively. There were no significant association (P>0.05) between the ages of goats and sheep sampled and the prevalence of *Brucella* antibodies detected using the two serological tests

4.4 Stakeholders Knowledge on Brucellosis

In all, 66 respondents were involved in the study. Of these, 15 (22.72%) were livestock owners in the households, 17 (25.76%) were livestock traders and the remaining 34 (51.52%) were abattoir and slaughter slabs workers all from the study areas where blood samples were collected (Table 4.4). Forty-nine (74.24%) of the respondents indicated knowing brucellosis while 17 (25.76%) of them indicated not having any knowledge of the disease (Table 4.5). In addition, 2 (3.03%) of them indicated the media as their source of information on the disease while 27 (40.91%) and 19 (28.79%) indicated their sources for knowing about brucellosis to be farmers and veterinary professionals respectively.

Out of the 15 livestock owners interviewed 11 (73.33%) indicated knowing about the disease, while all the 17 (100.00%) and 34 (100.00%) livestock traders and abattoir workers respectively indicated knowing about the disease (Appendix 11). As for their sources of knowledge, 12 (80.00%), 3 (20.00%) and 0 (0%) of the livestock owners indicated others farmers, professionals and the media respectively to be their sources of knowledge on brucellosis. Similarly, 1 (5.88%), 4 (23.53%) and 12 (70.59%) of the livestock traders mentioned the media, other farmers and professionals respectively to be their sources of knowledge on brucellosis. Furthermore, 34 (100.00%) of the abattoir workers based their sources of knowledge on brucellosis to be veterinarians and other animal health workers (Appendix 11).

Table 4. 3: Seroprevalance of *Brucella* in Goats and Sheep in Kaduna North and South LGAs, Kaduna State by Age using M-RBPT and SAT-EDTA

	Kaduna North and South LGAs Goats			Kaduna North and South LGAs Sheep				
Age	**No. sampled**	**M-RBPT No +ve (%)**	**SAT –EDTA No +ve (%)**	**No. Sampled**	**M-RBPT No. +(%)**	**SAT-EDTA No.+(%)**	**Over all M-RBPT**	**Over all SAT EDTA**
< 1	31	2(6.45)	3(9.68)	36	2(5.56)	5(13.89)	5.97	11.94
1–3	352	26(7.39)	53(16.41)	254	24(9.45)	55(21.65)	8.22	17.43
>3	313	33(10.54)	47(15.02)	270	23(8.52)	45(16.67)	9.64	14.63
Total	**696**	**61(8.76)**	**103(14.80)**	**560**	**49(8.75)**	**104(18.75)**	**8.76**	**15.84**

Goat: M–RBPT: P = 0.2613; $\chi 2 = 2.684$
SAT–EDTA: P = 0.6746; $\chi 2 = 0.7872$

Sheep: M–RBPT: P = 0.6635; $\chi^2 = 0.8205$
SAT–EDTA: P = 0.0624; $\chi 2 = 5.548$

Table 4. 4: Livestock Owners Related Respondents on Their Knowledge on Brucellosis in Kaduna North and South Local Government Areas, Kaduna State

LGAs	Livestock Owners	Livestock Traders	Abattoir Workers	Total No. of Respondents
Kaduna North	9 (60.00%)	8 (47.10%)	21(61.76%)	38
Kaduna South	6 (40.00%)	9 (52.90%)	13 (38.24%)	28
Total	15	17	34	66

KEY

LGAs = Local Government Areas

4.5 Livestock Related Respondents' Views on Transmission of Brucellosis

Enquiries on the means of infection of animals with *Brucella* species indicated that 2 (13.33%) of the 15 livestock owners named ingestion as the source of infection of animals with *Brucella* organisms while 13 (86.67%) of them indicated no knowledge of how the organism is transmitted to animals (Table 4.5). Similarly, 7 (41.18%) and 3 (17.65 %) of the 17 livestock traders indicated ingestion and contact respectively as the sources of infection of animals with the organism while 7 (41.18%) of them did not have any idea on the means of transmission of the organisms to animals. On their part, 8 (23.53%) and 1 (2.94%) of the 34 abattoir workers indicated ingestion and contact respectively as the sources of transmission of the disease to animals (Table 4.5).

Enquiries on the knowledge of signs of brucellosis in animals showed that among livestock owners in Kaduna North LGA, 1 (6.67%) and 2 (13.33%) mentioned still birth, retained placenta and abortion as the main signs of the disease in animal while 4 (26.67%) of them were not aware of the signs of the disease in animals (Table 4.6). On their part none of the 6 (40.00%) livestock owners in Kaduna South LGA knew any sign of brucellosis in animals (Table 4.6).

Similarly, 3 (17.65%), 2 (11.76%) and 1 (5.88%) of the livestock traders in Kaduna North LGA indicated stillbirth, retained placenta and abortion respectively to be the signs of brucellosis in animals while 1 (5.88%), 5 (29.41%), and 2 (11.76%) of those in Kaduna South respectively indicated stillbirth, retained placenta and abortion to be the main signs of brucellosis in animals. With regard to the abattoir workers, 2 (5.88%), 6 (17.65 %), and 4 (11.76 %) of them in Kaduna North LGA revealed that stillbirth, retained placenta and abortion respectively are signs of brucellosis in animals, while 4

(11.76 %), 2 (5.88 %) and 1 (2.94 %) of them in Kaduna South LGA revealed that stillbirth, retained placenta and abortion respectively are signs of brucellosis in animals (Table 4.6).

4.6 Results of the Responses on Management Practices by Small Ruminants Stakeholders from Kaduna North and South LGAs

The management practices employed by the small ruminants' stakeholders from Kaduna North and South LGAs revealed that majority of the stakeholders 16 (24.24 %) out of 66 managed their small ruminants on extensive system, followed by semi-intensive and none by intensive system. Also, most of the respondents 11 (16.67%) indicated buying their animals from their neighbours and 6 (9.10%) from markets while, none of the stakeholders bought their small ruminants from established farms (Appendix 12).

On the practices employed by the respondents stakeholders (66) on management of aborted foetuses, majority 11(16.67 %) of the respondents from Kaduna North and South LGA throw away the aborted foetuses, whilst 5(7.58 %) of the respondents indicated ignoring the aborted foetus in the environment and only 1(1.58 %) of the respondents burnt the aborted foetuses but none of the respondents ever buried the aborted foetuses of the small ruminants.

Table 4.5: Respondents' views on means of transmission of Brucellosis in animals

		Means of infection								
LGA	**NR**		**LO**			**LT**			**AW**	
		In (%)	**Con (%)**	**NI (%)**	**In (%)**	**Con (%)**	**NI (%)**	**In (%)**	**Con (%)**	**NI (%)**
Kaduna North	38	2 (13.03%)	0	7(46.67)	5(29.41)	1(5.88)	3(17.64)	5(14.71)	0	16(47.70)
Kaduna South	28	0	0	6(40.00)	2(11.76)	2(11.76)	4(23.53)	3(82.2)	1(2.94)	9(26.47)
Total	66	2	0	13	7	3	7	8	1	25

KEY:

LO = Livestock owners

LT = Livestock traders

AW = Abattoir workers

NR = Number of respondents

Con = contact

NI = No idea

In = Ingestion

Table 4.6: Respondents' views on Knowledge of the Signs of Brucellosis in Animals in Kaduna North and South LGAs of Kaduna State

		Signs of brucellosis in animals											
		LO				**LT**				**AW**			
LGA	No	**SB**	**RP**	**AB**	**NI**	**SB**	**RP**	**AB**	**NI**	**SB**	**RP**	**AB**	**NI**
K N	38	1(1.52)	2(3.04)	2(3.04)	4(6.10)	3(4.55)	2(23.04)	1(1.52)	2(3.04)	2(3.04)	6(9.10)	4(6.10)	9(13.64)
K S	28	0	0	0	6(9.10)	1(1.52)	5(7.58)	2(3.04)	1(1.52)	4(6.10)	2(3.04)	1(1.52)	6(9.10)
	66	1(1.52)	2(3.04)	2(3.04)	10(15.15)	4(6.10)	7(10.61)	3(4.60)	3(4.60)	6(9.10)	8(12.12)	5(7.68)	15(22.73)

KEY:

SB = Still birth

RP = Retained placenta

AB = Abortion

NO = No idea

LGAs = Local Government Areas

KN = Kaduna North

KS = Kaduna South

Respondents

LO = Livestock owners

LT = Livestock traders

AW = Abattoir workers

4.7 Medical Personnel's Responses on Brucellosis

In all, 54 human medically related respondents participated in the questionnaire administration (Appendix 13). Thirty one (57.41 %) of them were from the Kaduna North LGA while the remaining 23 (42.59%) were from Kaduna South LGA (Appendix 13). Of the 54 respondents, 43 (79.63%) had tertiary qualifications while 11(20.37%) had postgraduate qualifications. Also, of the 54 respondents 44 (81.48%) were medical doctors while 6 (11.11 %) and 4 (7.41%) were nurses and technologists respectively. Regarding their knowledge on brucellosis as a zoonosis 42 (77.78%) of the respondents reported brucellosis to be zoonotic (Table 4.8). With respect to zoonoses of importance, 19 (35.19%), 25 (46.30%), and 11 (20.37%) of the respondents respectively reported that bird flu, rabies and anthrax were zoonotic. Again a further 9 (6.67%) and 16 (26.62%) said Salmonellosis and tuberculosis respectively were also zoonotic. (Table 4.7; Fig 4.1).

The respondents were also asked if brucellosis causes abortion or not. To this 40 (74.10%) of the 54 respondents indicated knowledge of the disease resulting in abortion and 22 (40%) of the 31 respondents from the Kaduna North LGA while, 18 (33.33%) of the 23 respondents were from Kaduna South LGA (Table 4.8). Regarding their diagnostic ability for the disease as well as knowledge of clinical signs in humans, and the causative agent; none of them showed evidence of diagnosing the disease, 25 (46.30%) mentioned *Brucella abortus* as one of the agents causing brucellosis, 5 (9.26%) of the respondents indicated that *B. melitensis* and *B. suis* do also cause brucellosis; 2 (3.7%) indicated abortion as a clinical sign (Table 4.9).

Regarding knowledge of samples for diagnoses of brucellosis, 2 (3.7%) of the 54 respondents mentioned blood as the sole sample for the diagnosis (Table 4.9). On the

transmission of the disease, 32 (59.26%), 15 (27.78%) and 13 (24.07%) of the 54 respondents mentioned cattle, goats and sheep were respectively involved in the transmission of the disease from animals to human whilst another 5 (9.26%) and 6 (11.11%) of the 54 respondents indicated that the disease can also be transmitted from pigs and dogs respectively to man (Table 4.10).

Table 4. 7: Human Medical personnel respondents' knowledge based on LGA, of some zoonotic diseases

LGA	No. Respondents (% of total)	Brucellosis	Bird flu	Rabies	Anthrax	Salmonellosis	Tuberculosis
Kaduna North	31(57.41%)	23 (74.19%)	8 (25.81%)	13 (24.10)	8 (28.81)	7 (22.58)	8 (14.81)
Kaduna South	23 (42.59%)	19 (82.61%)	11 (47.83%)	12 (22.22)	3 (13.04)	2 (8.70)	8 (14.81)
Total	54 (100.00%)	42 (77.78%)	19 (35.19%)	25 (46.30)	11 (20.37)	9 (6.67)	16 (29.63)

KEY:

LGA = Local Government Area

Table 4.8: Kaduna North and South LGAs Hospital and Clinics Human Medical personnel's response on the ability of brucellosis to cause abortion

LGA	No. of Respondents	Yes to brucellosis as cause of abortion (%) of total respondents
Kaduna North	31	22 (40.00)
Kaduna South	23	18 (33.33)
Total	54	40 (74.10)

KEY

LGA= Local Government Area

Table 4.9: Kaduna North and South LGAs Human Medical personnel's responses based on knowledge of brucellosis, aetiology and clinical samples to be collected in Hospitals and Clinics

LGA	No. of Respondents	Knowledge		Aetiological agents of brucellosis			Clinical samples for diagnosis of brucellosis			
		Yes	No	*B. abortus*	*B. melitensis*	*B. suis*	Blood	Milk	Urine	Aborted materials
Kaduna North	31 (57.41%)	31 (57.41%)	0 (0)	20 (64.52)	2 (6.45)	3 (5.56%)	2 (3.70%)	0 (0.00%)	0 (0.00%)	22 (40.74%)
Kaduna South	23 (42.59%)	23 (42.59%)	0 (0)	5(21.74)	3 (13.04)	2 (3.70%)	0(0.00%)	0 (0.00%)	0 (0.00%)	18 (33.33%)
Total	54 (100.00%)	54 (100%)	0 (0)	25 (46.30)	5 (9.26)	5 (9.26%)	2 (3.70%)	0 (0.00%)	0 (0.00%)	40 (74.07%)

KEY

LGA= Local Government Area

Table 4. 10: Response of Human Medical personnel on Transmission of Brucellosis from Animals to Humans [base in Kaduna North and South LGA in Hospitals/ Clinics]

LGA	No. Respondents	Respondent knowledge on Transmission		Response on Animals involved in transmission				
		Yes	No	Cattle	Goats	Sheep	Pigs	Dogs
Kaduna North	31	31 (57.41%)	0 (0.00)	21(67.74)	11(35.48)	7(22.58)	5(16.13)	3(9.67)
Kaduna South	23	23 (42.59%)	0 (0.00)	11(47.83)	4(17.39)	6(26.09)	0	3(13.04)
Total	54	54(100.00%)	0 (0.00)	32 (59.26%)	15 (27.78%)	13 (24.07%)	5 (9.26%)	6 (11.11%)

KEY

LGA=LocalGovernmentArea

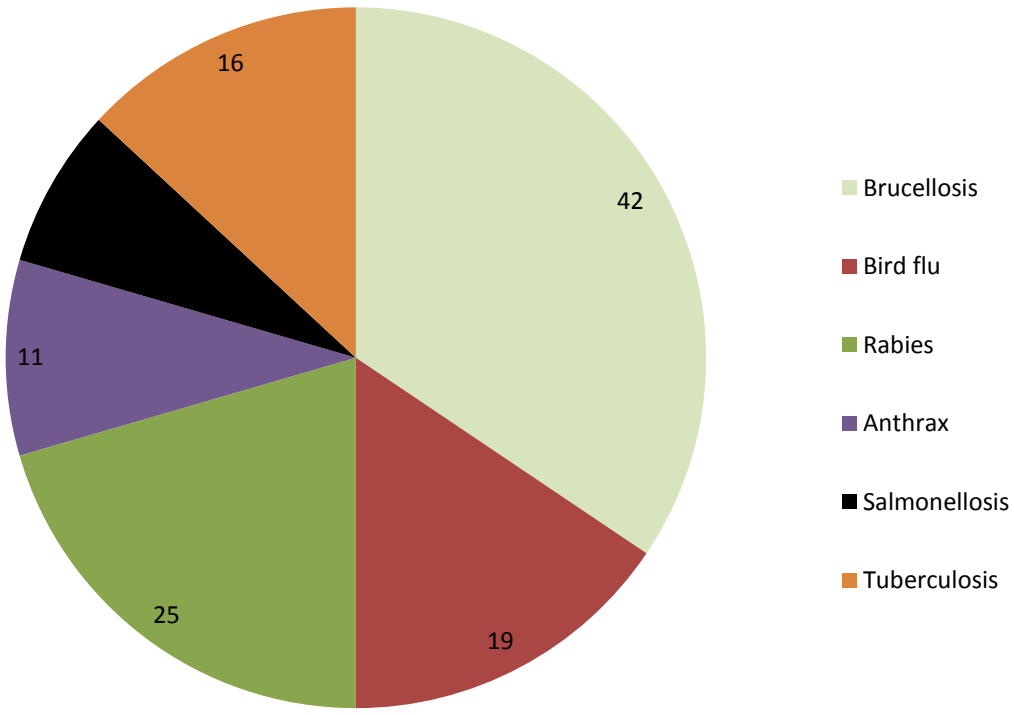

Figure 4.1: Pie chart showing Medical respondents' knowledge on zoonotic diseases in Kaduna North and Kaduna South LGAs

CHAPTER FIVE

5.0 DISCUSSION

The seroprevalence of *Brucella*, knowledge and practices of small ruminants' owners in Kaduna North and South Local Government Areas of Kaduna State, Nigeria were studied. A total of 1,256 serum samples were collected in goats and sheep from abattoirs (199), slaughterhouses (163), livestock markets (577) and households (317). From the samples, seroprevalence of 8.81% and 14.74% using M-RBPT and SAT-EDTA respectively in goats and 8.74 % and 19.05% using M-RBPT and SAT-EDTA respectively in sheep were obtained.

The seroprevalence recorded in this study was higher than those reported by Falade and Shonekan (1982) and Brisbe *et al.* (1993) in Maiduguri, who reported seroprevalence of 2.56% in sheep and 2.05% in goats respectively using Rose Bengal plate test. Similarly, Shehu *et al*. (1999) in Bauchi reported a seroprevalence of 6.6% while Okoh, (1980) recorded a much higher seroprevalence of 14.5% in sheep at a Livestock Investigation and Breeding Centre in Kano using Rose Bengal plate test. Nonetheless, this study has further shown that *Brucella* infection is present in the study areas.

The observed higher seroprevalence of *Brucella* infection among animals sampled in livestock markets and household than the abattoirs could be because those at the household lived routinely freely within village and town streets with no strict

livestock movement control hence easily mixed with possibly infected animals. Furthermore there is no planned breeding as ewes and does breed at the village and town streets indiscriminately and therefore uninfected animals easily get infected from infected ones.

From the present study, female animals were observed to be more seropositive than male animals despite the facts that the abattoirs and livestock markets had more male than female animals sampled. This could be because, traditionally, livestock owners keep more female animals in their flocks than males whilst disposing off most of the males for sale and slaughter but females but female animals are only sent for slaughter if they are old or have reproductive problems (Bertu, 2009; Kaltungo, 2013). Similarly the presence of erythritol in the female reproductive tract increases the chances of infection with *Brucella* organisms since the organisms thrive most in the presence of this chemical (Corbel and Brinley-Morgan, 1984).

The present study also revealed that, seroprevalence increased with age as those older than three years had the highest seroprevalence of 9.64%, followed by those of 1 to 3 years old (8.22%) and least for those under 1 year old (5.97%). This agrees with the fundamental fact that brucellosis is a disease of the older animals. This could be due to the fact that with increase in age there is increased risk of exposure to contaminated feed and water hence increased chances of getting infected with *Brucella* organism.

The high knowledge of the respondents in Kaduna South on brucellosis could be due to the proximity of the livestock market and the abattoir to the Veterinary Health Centre in the study area. Similarly, the use of public enlightenment facilities such as radio and television along with other activities of livestock workshop programmes over the years might have contributed to the increase in knowledge of the livestock owners on many diseases including brucellosis (Saidu *et al*., 1990; Ardo, 1998; El – Nafsaty, 1998).

The understanding of the respondents to the questionnaires that brucellosis is zoonotic may similarly be due to their interaction with more experienced farmers in their communities and with animal health professionals as observed from this study. This finding showed that livestock owners, livestock traders and abattoir workers in the study areas were knowledgeable with regard to small ruminant diseases including brucellosis.

The majority of the small ruminants' farmers were engaged in extensive animal husbandry system while none of the farmers was involved in intensive animal husbandry system. This might be due to their educational level and lack of adequate finances to invest in more efficient intensive system. A similar finding has been reported by Saidu and Isitor (1989).

Also, this study revealed that most of the respondents to the questionnaire threw away the aborted foetuses, followed by those respondents who just ignored the aborted foetuses and placenta in the environment. This practice is risky as

uninfected animals and humans are at risk of contacting *Brucella* infection through such unhealthy practices.

The qualifications of the medically related respondents have shown that the Kaduna State Ministry of Health had adequately qualified personnel to take care of the patients ailments in the study areas. Their knowledge of zoonotic diseases was expected as they are supposed to have learned about them in the medical school.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study it was concluded that:

i. *Brucella spp* antibodies were present in the sera of goats and sheep sampled in Kaduna North and South LGAs of Kaduna State and that there was an overall seroprevalence of 8.81% and 14.72% using M-RBPT in goats and 8.76% and 18.75% seroprevalence in sheep using M-RBPT and SAT-EDTA respectively.

ii. Female goats and sheep were more seroprevalent (9.24% and 7.12%) respectively than their male counterparts (7.96% and 10.57%) respectively.

iii. Older goats and sheep were more susceptible to *Brucella* infection than young ones as animals older than 3 years had the highest seroprevalence (9.64%) followed by those of one to three years old (8.22 %) and least by those less than one year old (5.97%)

iv. Most of the livestock stakeholders 48 (72.72%) from the study areas were aware of brucellosis in small ruminants and its zoonotic implications and their sources of knowledge of the disease were other farmers (40.9 %), animal health professionals (28.79%) and the media (3.03%).

v. The human medical personnel respondents were aware of brucellosis and their zoonotic natures as well as knowing animals were responsible for its transmission but none of the respondents had encountered brucellosis in humans in the study areas.

6.2 Recommendations

From the findings of the study, it is recommended that:

i .Livestock owners, livestock traders, abattoir workers and the general public should be educated on brucellosis, especially its mode of transmission and clinical signs.

ii. There is the need for more research on the epidemiology of the disease in the study areas with a view to advise Government on strategies for the control and prevention of the disease.

iii. There is the need for closer collaboration between veterinarians and those in the human medical field on studies on zoonotic diseases, especially brucellosis in order to better educate the human population on its dangers.

iv. Abattoir workers and other stakeholders of small ruminant production should be educated on the need to use appropriate protective clothing and adoption of good hygienic measures when working with livestock at the abattoirs, farms and livestock markets as *Brucella*, which is pathogenic for humans, has been shown to circulate in animals in the study areas.

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APPENDIX

Appendix 1: Structured Questionnaire for Livestock owners traders and Abattoirs/slaughter slabs Personnel

DEPARTMENT OF VETERINARY MEDICINE
FACULTY OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY, ZARIA

A Structured questionnaire on knowledge of brucellosis in small ruminants in Kaduna North and South Local Government Areas of Kaduna State.

SECTION A: BIODATA OF RESPONDENT

a. Location ……
b. Age (years) : i) <18 ( ), ii) 18 – 27 ( ),iii) 28 – 37 ( ), iv) 38 – 47 ( ), v) 48 – 57 ( ).
c. Sex: i) Male ( ), Female ( ).
d. Literacy level: i) Adult education ( ), ii) Islamiya ( ),iii) Primary ( ),iv) Secondary ( ), iv) Tertiary ( )
e. Occupation (other than livestock farming): i) Livestock vendor ( ) ii), Butcher ( ), iii) Abattoir/slaughter slab worker ( ), iv) Civil servant ( ), v) Others ……
f.Numbers of person in household i) Males …….ii) Females ……...
g. How long have you been in this business …….

SECTION B: BIODATA OF SMALL RUMINANTS

a) What type of small ruminants do you keep i) …….. ii) ……
b) Total number kept, including kids/lambs i) ……. ii) …….
c) What are the breeds: Goats i) ……. ii) ……Sheep i) …… ii) ……
d) Sex i) Goats: Males ……., Females ……. ii) Sheep: Males ……. ii) Females ……
e) Age (months): Goats i) < 12 ( ), ii) 12 – 25 ( ), iii) 26 -36 ( ), iv) > 36 ( )
Sheep i) < 12 ( ), ii) 12 – 25 ( ), iii) 26 – 36 ( ), iv) > 36 ( ).
f) Other animals kept ……

SECTION C: KNOWLEDGE OF THE DISEASE

a) List the diseases of small ruminants that you know i) …….. ii) …….. iii) …… iv) ……
b) Have you heard of brucellosis? : Yes ( ), No ( )
c) If Yes from what source did you hear of brucellosis? i) media ( ), ii) Animal health worker ( ) iii) Friend or Neighbour ( ), iv) others ……..
d) What are the manifestation/ clinical signs of the disease i) …….. ii) …….. iii) …….. iv) ……..
e) Have you seen any case before? i) Yes ( ), ii) No ( )

f) If Yes how many animals have you seen with such signs in your flock? i) Goats …….. ii) Sheep ……..

g) Can human beings be infected with brucellosis? i) Yes ( ), ii) No ( )

h) How do you think someone can be infected with brucellosis? i) ……. ii) ……. iii) ……..

SECTION D: INFORMATION ON MANAGEMRNT PRACTICES

a) How do you keep/rear your animals? i) Intensive( ) ii) Semi intensive( ) iii) Extensive ( )

b) Where is the source of your animals? i) Market ( ), ii) established farm ( ), iii) friend/neighbor ( )

c) Have you added more animals recently? i)Yes ( ), ii) No ( )

d) If yes, how many? ……..

e) Where is the source of the new animals? i) ……. ii) ……

f) Do you borrow or give out males animals breeding? i) Yes ( ), ii) No ( )

g) Have you seen any case of abortion, retained placenta, or still birth in your flock? i) Yes( ) ii) No ( )

h) What do you do with aborted fetuses, still birth or retained placenta? i) feed my dogs ( ) ii) bury it ( ), iii) burn it ( ), iv) throw it away ( ), v) ignore it on the farm ( )

i) Where do you keep your animals? ……

j) Do you wear protective clothing when dealing with your animals? i) Yes ( ), ii) No ( )

k) If Yes, list the proactive clothing. i) …….. ii) …… iii) …… iv) ……

l)Do you house your animals separately based on breed, age or sex? i) Yes ( ),ii) No ( )

m)How do you feed/water your animals? i) separately ( ), ii) combined ( ), iii) …….

n)Where is the source of your water? i) public water ( ), ii) river/pond ( ), borehole/well (

o)Where is the source of your feed for your animals? i) Market ( ), ii) farm residues ( ) iii) scavenge ( ) iv) others …….

p)Do you allow your animals to roam about or associate with other animals? Yes/No

q)Do you routinely vaccinate your animals? Yes/No

r)Which disease do you vaccinate against? i) ……. ii) …… iii) ……..

s)What do you do with a sick animal? i) treat by myself ( ), ii) call a vet/animal health worker ( ), iii) sell it ( ), iv) others ……

Appendix 2: Structured Questionnaire for Human Medical Personnel

DEPARTMENT OF VETERINARY MEDICINE
FACULTY OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY, ZARIA

Dear Sir/Madam, I will like to obtain information about your knowledge on brucellosis, please. Every information given will be treated with respect and high confidence.

SECTION A: BIODATA OF RESPONDENT

a) Town ……. Sex ……. Age ……..
b) Education i) Tertiary ( ) , b) Post – tertiary ( )
Designation i) Dr. ( ), ii) Nurse ( ), iii) Technologist ( )
Years in practice, i) < 5 ( ), ii) 5 – 10 ( ), iii) 10 – 15 ( ), iv) 15 – 20 ( ), > 20 ( ).

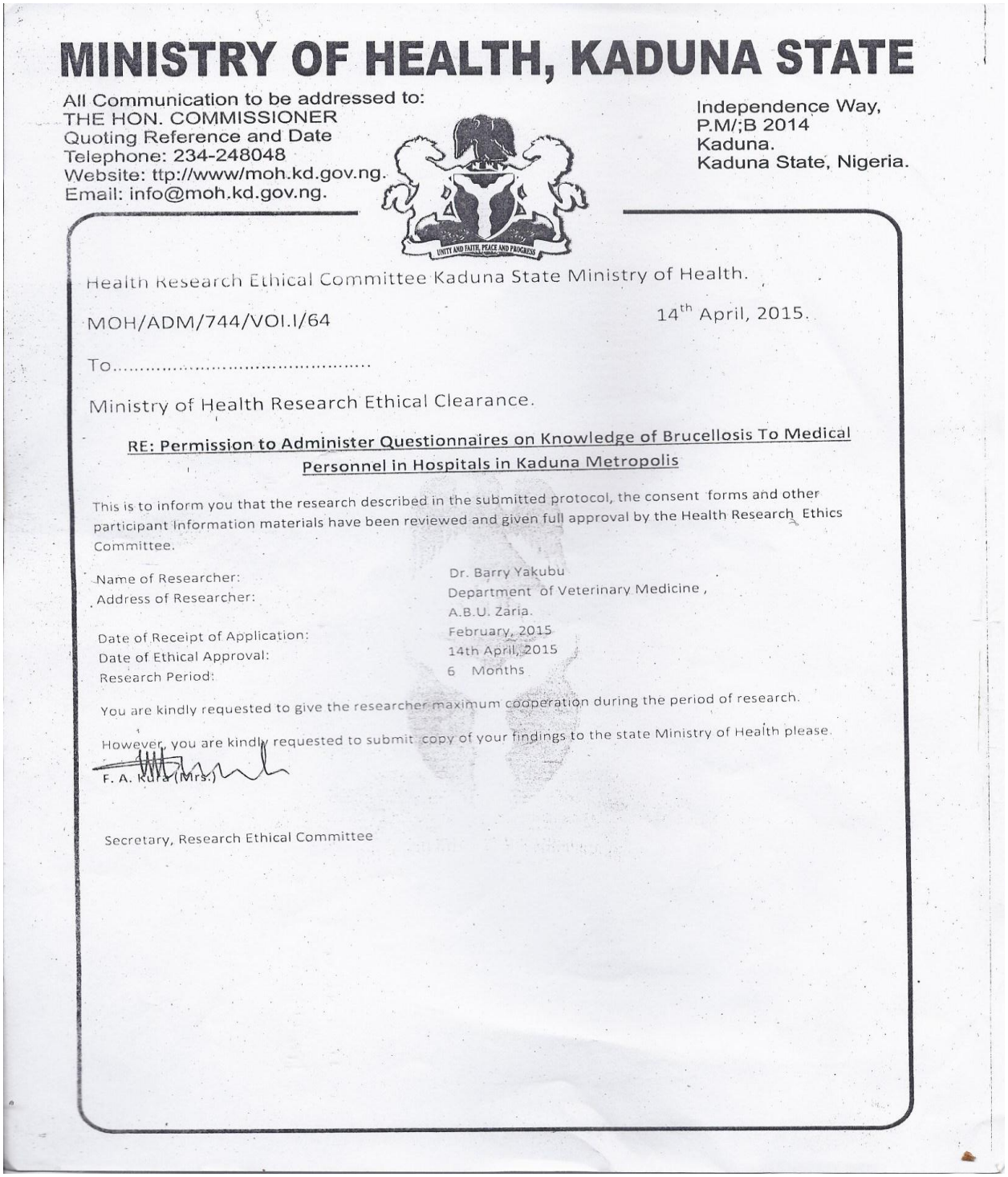
SECTION B: INFORMATION ON THE KNOWLEDGE OF BRUCELLOSIS

a) Kindly name the zoonotic diseases you know: i) …… ii) ……. iii) …….. iv) ……. v) ……..
b) Which of these causes abortion? i) …….. ii) …….. iii) …….
c) Which would say is very common …….
d) Have you ever diagnosed brucellosis in your practice of medicine? Yes ( ), No ( )
e) If yes, do you know the causative agent(s) of brucellosis that infect(s) man? …….
f) Do you think animals can transmit brucellosis? Yes/No
g) If yes which animals are responsible? …….. ……. ……..
h) Do you routinely diagnose brucellosis? Yes/No
i) If yes which specimen do you send to the laboratory for diagnosis? i) ……. ii) …….. iii) …….
j) How do you manage a case of brucellosis in humans? …….

Thank you Sir/Madam for your time and cooperation.

DR. BARRY, Y. Y.

Appendix 3: Ethical clearance letter



MINISTRY OF HEALTH, KADUNA STATE

All Communication to be addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Telephone: 234-248048
Website: ttp://www/moh.kd.gov.ng.
Email: info@moh.kd.gov.ng.

Independence Way,
P.M/;B 2014
Kaduna.
Kaduna State, Nigeria.

UNITY AND FAITH, PEACE AND PROGRESS

Health Research Ethical Committee Kaduna State Ministry of Health.

MOH/ADM/744/VOI.I/64 — 14th April, 2015.

To.....

Ministry of Health Research Ethical Clearance.

RE: Permission to Administer Questionnaires on Knowledge of Brucellosis To Medical Personnel in Hospitals in Kaduna Metropolis

This is to inform you that the research described in the submitted protocol, the consent forms and other participant information materials have been reviewed and given full approval by the Health Research Ethics Committee.

Name of Researcher:	Dr. Barry Yakubu
Address of Researcher:	Department of Veterinary Medicine, A.B.U. Zaria.
Date of Receipt of Application:	February, 2015
Date of Ethical Approval:	14th April, 2015
Research Period:	6 Months

You are kindly requested to give the researcher maximum cooperation during the period of research.

However, you are kindly requested to submit copy of your findings to the state Ministry of Health please.

F. A. Kura (Mrs.)

Secretary, Research Ethical Committee

Appendix 4: Distribution of Goats and Sheep by sampling locations in Kaduna North and South LGAs, Kaduna State

	No. Sampled per location						
	Abattoir		Market		Household		Total
LGA	**Goats**	**Sheep**	**Goats**	**Sheep**	**Goats**	**Sheep**	
Kaduna North	117	106	210	129	83	65	710
Kaduna South	63	76	115	123	108	61	546
Total	180 (14.33)	182 (14.49)	325 (25.88)	252 (20.06)	191 (15.21)	126 (10.03)	1256 (100)

KEY

LGA = Local Government Area

Appendix 5: Seroprevalance of *Brucella* in Goats and Sheep sampled in Abattoir and Slaughter Slab in Kaduna North and South LGAs, Kaduna Sate

	Goats			Sheep		
LGA	**No. sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**	**No. Sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**
Kaduna North	117	12 (10.26)	22 (18.80)	105	8 (7.61)	15(14.29)
Kaduna South	63	7 (11.11)	11 (17.46)	76	6 (7.89)	16(21.10)
Total	180	19 (10.56)	33 (18.13)	181	14 (7.73)	31(17.13)

KEY

LGA = Local Government Area

Appendix 6: Seroprevalance of *Brucella* in Goats and Sheep sampled in Markets in Kaduna North and South LGA Kaduna Sate

	Goats			Sheep		
LGA	**No. Sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**	**No. Sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**
Kaduna North	210	17(8.10)	33(15.71)	71	10(14.10)	20(28.17)
Kaduna South	115	9(7.83)	16(13.91)	123	11(8.94)	31(25.20)
Total	325	26(8.0)	49(15.08)	194	21(10.83)	51(26.29)

KEY

LGA = Local Government Area

Appendix 7: Seroprevalance of *Brucella* in Goats and Sheep sampled in House Holds in Kaduna North and South LGA Kaduna Sate

	Goats			Sheep		
LGA	**No. Sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**	**No. Sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**
Kaduna North	83	5(6.02)	7(8.43)	65	8(12.03)	10(15.38)
Kaduna South	108	10(9.26)	16(14.81)	61	5(8.20)	13(21.31)
Total	191	15(7.64)	23(11.62)	126	13(10.32)	23(18.12)

KEY

LGA = Local Government Area

Appendix 8: Seroprevalance of *Brucella* in Goats and Sheep sampled in Abattoir and Slaughter Slab in Kaduna North and South LGA Kaduna Sate by Sex

	Goats						Sheep					
	Female			**Male**			**Female**			**Male**		
LGA	**No. sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**	**No. sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**	**No. Sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**	**No. sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**
Kaduna North	67	5 (7.46)	9 (13.49)	50	7 (14.00)	13 (26.00)	45	5 (11.11)	6 (13.33)	61	3 (4.92)	9 (14.75)
Kaduna South	20	3 (15.00)	4 (20.00)	43	4 (9.30)	7 (16.28)	59	5 (8.47)	14 (23.73)	17	1 (5.88)	2 (11.76)
Total	87	8 (11.23)	13 (16.7)	93	11 (11.65)	20 (21.41)	104	10 (9.79)	20 (18.53)	78	4 (5.54)	11 (13.26)

KEY

+ = Number positive LGA = Local Government Area

(%) = Percentage positive

Appendix 9: Seroprevalance of *Brucella* in Goats and Sheep sampled in Markets in Kaduna north and South Kaduna Sate

	Goats						Sheep					
	Female			**Male**			**Female**			**Male**		
LGA	**No. sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**	**No. sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**	**No. Sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**	**No. sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**
Kaduna North	104	11(10.58)	19(18.27)	106	6(5.66)	14(13.21)	26	2(7.69)	5(19.23)	103	8(7.77)	15(14.56)
Kaduna South	48	3(6.25)	7(14.58)	67	6(8.96)	8(11.94)	55	7(12.72)	12(21.82)	68	4(5.88)	19(27.94)
Total	152	14(8.42)	26(16.43)	173	12(7.3)	22(12.58)	81	9(10.21)	17(20.52)	171	12(6.82)	34(21.25)

KEY

+ = Number positive , LGA = Local Government Area

(%) = Percentage positive

Appendix 10: Seroprevalance of Brucella antibodies in Goats and Sheep sampled in Households in Kaduna North and South, Local Government Areas Kaduna State

	Goats						Sheep					
	Female			**Male**			**Female**			**Male**		
LGA	**No. sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**	**No. sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**	**No. Sampled**	**M-RBPT +(%)**	**SAT-EDTA +(%)**	**No. sampled**	**M-RBPT +(%)**	**SAT-EDTA**
Kaduna North	61	3(4.92)	5(8.20)	22	2(9.10)	2(9.10)	44	4(9.10)	6(13.64)	21	4(19.04)	4(19.04)
Kaduna South	34	8(23.52)	11(32.35)	72	8(11.11)	11(15.28)	50	3(6.0)	12(24.0)	11	2(18.18)	1(9.10)
Total	95	11(14.22)	16(20.28)	94	10(10.11)	13(12.91)	94	7(7.55)	18(18.82)	32	6(18.75)	5(15.63)

KEY

+ = Number positive

(%) = Percentage positive

LGA = Local Government Area

Appendix 11: Livestock owners Related Respondents knowledge and source of Information on Brucellosis in Kaduna North and South Local Government Areas, Kaduna State

	Knowledge of Brucellosis							Source of information									
LGA		LO		LT		AW			LO			LT			AW		
	NR	Yes	No	Yes	No	Yes	No	NR	M	F	P	M	F	P	M	F	P
Kaduna North	38	9	0	8	0	21	0	38	0	7	2	1	2	5	0	0	21
Kaduna South	28	2	4	9	0	13	0	28	0	5	1	0	2	7	0	0	12
Total	66	11	4	17	0	34	0	66	0	12	3	1	4	12	0	0	33

Key: LO = Livestock owners; LT = Livestock traders; AW = Abattoir workers; NR = Number of respondents

M = Media, F = Farmers, P = Professional LGA = Local Government Area

Appendix 12 : Results of the Questionnaire on some management practices in households in Kaduna North and South Local Government Areas of Kaduna State

		Management practices			Animal sourcing			History of abortion			Management of aborted foetuses			
LGA	Respondents	**Intensive**	**Semi intensive**	**Extensive**	**Market**	**Neighbour**	**Farm**	**Yes**	**No**	**No idea**	**Burry**	**Burn**	**Throw away**	**Ignore**
Kaduna north	**38**	**0**	**1(1.51)**	**9(13.64)**	**4(6.10)**	**6(9.10)**	**0**	**6(9.10)**	**4(6.10)**	**0**	**0**	**1**	**6**	**3**
Kaduna south	**28**	**0**	**0**	**7(10.61)**	**2(2.76)**	**5(7.58)**	**0**	**4(6.10)**	**2(2.76)**	**1(1.51**	**0**	**0**	**5**	**2**
Total	**66**	**0**	**1**	**16**	**6**	**11**	**0**	**10**	**6**	**1**	**0**	**1**	**11**	**5**

Appendix 13: Qualifications and types of Human Medical Personnel as Respondents' to Questionnaire on Brucellosis from Kaduna North and Kaduna South Local Government Areas, Kaduna State using Hospitals and Clinics

LGA	No. of Respondents (%)	Qualification		Designation		
		Tertiary	Post graduate	Medical Doctor	Nurse	Technologist
Kaduna North	31 (57.41%)	24 (44.44%)	7 (12.96%)	26 (48.15%)	2 (23.70%)	3 (5.56%)
Kaduna South	23 (42.59%)	19 (35.19%)	4 (7.41%)	18 (33.33%)	4 (7.41%)	1 (1.85%)
Total	54 (100.00%)	43 (79.63%)	11 (20.37%)	44 (81.48%)	6 (11.11%)	4 (7.41%)

KEY:

LGA = Local Government Area