

**SEROLOGICAL AND PARTICIPATORY STUDIES OF BRUCELLA INFECTIONS IN  
SMALL RUMINANTS IN KATSINA AND SOKOTO STATES, NIGERIA**

**BY**

**Bilkisu Yunusa KALTUNGO DVM (UNIMAID), 1997; M. Sc., (ABU), 2013.**

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**FEBRUARY, 2018**

## **DECLARATION**

I declare that the work in this thesis titled “**SEROLOGICAL AND PARTICIPATORY STUDIES OF BRUCELLA INFECTIONS IN SMALL RUMINANTS IN KATSINA AND SOKOTO STATES, NIGERIA**” has been carried out by me in the Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. 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.

**Bilkisu Yunusa KALTUNGO**

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**Signature**

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**Date**

## CERTIFICATION

This thesis titled “**SEROLOGICAL AND PARTICIPATORY STUDIES OF BRUCELLA INFECTIONS IN SMALL RUMINANTS IN KATSINA AND SOKOTO STATES, NIGERIA**” by Bilkisu Yunusa KALTUNGO meets the regulations governing the award of the degree of Doctor of Philosophy (Ph.D. Food Animal Medicine) of the Ahmadu Bello University, Zaria, Nigeria and is approved for its contribution to scientific knowledge and literary presentation.

Dr. S. N. A. Saidu

Chairman, Supervisory Committee

\_\_\_\_\_

Signature

\_\_\_\_\_

Date

Prof. C. A. Kudi

Member, Supervisory Committee

\_\_\_\_\_

Signature

\_\_\_\_\_

Date

Prof. J. O. O. Bale

Member, Supervisory Committee

\_\_\_\_\_

Signature

\_\_\_\_\_

Date

Prof. P. A. Abdu

Head of Department

Vet. Medicine

A. B. U., Zaria

\_\_\_\_\_

Signature

\_\_\_\_\_

Date

Prof. A. Z. Abubakar

Dean, School of Postgraduate Studies

A. B. U., Zaria

\_\_\_\_\_

Signature

\_\_\_\_\_

Date

## **DEDICATION**

This piece of work is dedicated to the golden memory of my late father, Alhaji Yunusa Kaltungo, my compassionate husband, Dr. I. W. Musa and my lovely children, Halima, Aisha, Zainab, Khalil and Sulaiman.

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## ABSTRACT

This study was set out to determine the sero-prevalence of small ruminants brucellosis and to draw spatial maps to show the distribution of *Brucella melitensis* infection by cELISA in Katsina and Sokoto States, Nigeria. Also, the pastoralists' perception on the economics of animal species they kept, prevalence, signs and local perception of small ruminant diseases, traditional treatment and control methods of their ailments were investigated. Local Government Areas (LGAs) and Wards in the study areas were randomly selected. A total of 3,777 (2,066 and 1,711 from Katsina and Sokoto States respectively) blood samples were collected using convenience sampling technique. The sero-prevalence, status (mild or severe) and forms (acute or chronic) of *Brucella* infection were determined using m-RBPT and cELISA, SAT-EDTA, 2-MET respectively. Chi-square test was used to measure associations among categorical variables. ArcGIS version 10.3 was used to draw spatial maps to show the distribution of *B. melitensis* infection as obtained by cELISA in the LGAs under study. Distance Inverse Weighted (DIW) was used to interpolate for unsampled LGAs based on generated data. Participatory Epidemiological (PE) tools were used to determine the economics of animal species kept by pastoralists, prevalence, signs and local perception and remedies to small ruminant diseases. Participatory epidemiology data were subjected to descriptive statistics, Friedman's test and Kendall's coefficient of concordance to determine the level of agreement among informant groups. Of the 3,777 sera tested using m-RBPT, sero-prevalence of 13.5% was obtained for *B. melitensis* in both Katsina and Sokoto States. For *B. abortus* antibodies, 15.8% and 17.7% were obtained for Katsina and Sokoto States respectively. The sero-prevalence for single infection of either *B. melitensis* or *B. abortus* were 21.1% and 20.6% for Katsina and Sokoto States respectively, while 4.1% and 5.0% were obtained for co-infection (*B. melitensis* and *B. abortus*) for Katsina and Sokoto States respectively. Using SAT-EDTA, 4.11% and 3.3% of the

3,777 sera analysed were positive for mild infection in Katsina and Sokoto States respectively while 11.3% and 6.3% were positive for severe infections from the same states respectively. Four hundred and fifty nine sera from Katsina and Sokoto States that were positive at 1:80 and above using SAT-EDTA were tested for acute and chronic infections using 2-Mecaptoethanol test (2MET) and that out of these, 228 (76.5%) and 124 (77.0%) were positive for acute infection from Katsina and Sokoto States respectively while 70 (23.5%) and 37 (23.5%) were positive for chronic infection from the same States respectively. Four hundred of the 459 positive sera samples were further analysed for *B. melitensis* antibodies using cELISA, with 51 (4.0%) and 17 (13.0%) being positive from Katsina and Sokoto States respectively. The spatial map imageries for Katsina and Sokoto States were produced to show the prevalence of *B. melitensis* using cELISA throughout the LGAs sampled. The PE results revealed that cattle constituted the largest proportion of livestock kept by pastoralists (ranked 1) in Katsina and Sokoto States, followed by goats (ranked 2), sheep (ranked 3), poultry (ranked 4) camels (ranked 5<sup>th</sup>) and Donkeys (ranked 6<sup>th</sup>). Benefits derived from the animals kept by pastoralists in Katsina and Sokoto States were fertilizer (manure), means of livelihood, transport, Farm traction, emergency fund, festivities, meat and Milk/milk products. Similarly, Cattle rustling (Rank = 1; 0), effects of transhumance (Rank = 2; 3), lack of watering points (Rank = 3; 7), lack/inadequate shelter (Rank = 4; 2), diseases (Rank = 5; 1), cost of medication (Rank = 6; 5) and feeding (Rank = 7; 6) were identified as common factors militating against livestock production in Katsina and Sokoto States respectively. The median score for factors militating against livestock production was significantly different ( $p < 0.05$ ) in both states. Important small ruminants diseases identified by pastoralists in the two States Gishu/Gurda (PPR), Balku/Hanta (fasciolosis), Matsattsaku (helminthosis), Bakkale/Bari (brucellosis), Chabo (FMD), and Kumburin Guiwa (arthritis). The clinical signs of identified diseases in the states under study

were consistent with the modern veterinary knowledge of such diseases. For traditional treatment of brucellosis, firing of the knee in case of hygroma was the practice, after which a powder of ‘Rai dore’ (*Senna occidentalis*) was applied on the wound. Others drenched affected animals with a solution of the same powder. From the study, it was concluded that small ruminants in the study areas harboured antibodies to *B. melitensis* and *B. abortus* as well as having evidence of acute and chronic and also mild and severe infections. The spatial map imagery may serve as an excellent tool for active surveillance and control strategies for livestock diseases like brucellosis in the study area. Diseases and several management factors were found to militate against livestock production in the study States. Pastoralists were also found to be repository of Veterinary knowledge in Katsina and Sokoto States. There is the need to enlighten pastoralists in the study area on small ruminant brucellosis and the dangers therein. Sero-spatial maps of *Brucella* in all states in Nigeria should be institutionalized as an element of active surveillance and control strategies. Also, ‘Pastoralists’ should be given a voice to enhance understanding of animal health problems in their localities and the options for their prevention, control and surveillance using PE. The active ingredients in the identified plants from this study should be investigated with a view to develop appropriate drugs to improve animal health care delivery.

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## LIST OF ABBREVIATIONS

BCV	<i>Brucella</i> -containing vacuole
BAT	Buffered Antigen Test
BPAT	Buffered Plate Agglutination Test
CDC	Center for Disease Control
CFT	Complement Fixation Test
cELISA	Competitive Enzyme Linked Immunosorbent Assay
CO <sub>2</sub>	Carbon dioxide
CVL	Central Veterinary Laboratory
CCPP	Contagious Caprine Pleuropneumonia
DVS	Director Veterinary Services
DOD	Department of Defence
EDTA	Ethylenediaminetetraacetic acid
EC	European Commission
EVK	Existing Veterinary Knowledge
FAO	Food and Agricultural Organisation
FET	Fishers Exact Test
FMD	Foot and Mouth Disease
FPA	Fluorescent Polarization Assay
GIS	Geographical Information System
GPS	Global Positioning System
HIT	Heat Inactivation Test
IDW	Inverse Distance Weighted
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IFAT	Immuno-flourescent Antibody Test
KAP	Knowledge, Attitudes and Practices
LFA	Lateral Flow Assay
LGA	Local Government Area

LPS	Lipopolysaccharide
2-MET	2-Mercaptoethanol Test
MFC	Mediterranean Fever Commission
MHC	Major Histocompatibility Complex
MAB	Monoclonal Antibody
m-RBPT	Modified Rose Bengal Plate Test
MRT	Milk Ring Test
NAVSTAR	Navigation System Using Timing and Ranging Satellite
OBF	Officially Brucellosis Free
OIE	Office International Des Epizooties
OD	Optical Density
OPS	O polysaccharide
PE	Participatory Epidemiology
PPR	Peste de Petite Ruminante
PLA	Participatory Learning and Action
PBA	Primary Binding Assay
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
RIM	Resource Inventory and Management Limited
RRA	Rapid Rural Appraisal
RLPS	Rough Lipopolysaccharide
RER	Rough Endoplasmic Reticulum
SAT	Serum Agglutination Test
SSI	Semi-Structured Interviews
SLPS	Smooth Lipopolysaccharide
SPSS	Statistical Package for Social Sciences
SPT	Standard Plate Test
TLR	Toll-like receptors
TMP/SMZ	Trimethoprim/Sulphamethoxazole
USA	United States of America
WHO	World Health Organisation

## **CHAPTER ONE**

### **1.0 INTRODUCTION**

#### **1.1 Scope of the study**

Small ruminant production in Nigeria is only second to Sahel countries in the West African Sub-region (Nuru and Dennis, 1975). Sheep and goats form an integral and important component of the pattern of animal production in most rural communities in Nigeria (Gefu, 2002). These species of animals are widely distributed from rural to urban and peri-urban areas, representing about 63.7% of the total grazing domestic animals in Nigeria (Gefu, 2002). In smallholder production systems, goats commonly referred to as “the cattle of the poor” and sheep are imperative because they are highly prolific and are therefore able to multiply the flock size within a short time (Nwafor, 2004). Moreover, they require low initial capital and maintenance costs and are able to utilize marginal land and crop residues (Winrock International, 1983). Thus, sheep and goat farming forms a secured agricultural investment to the Nigerian rural and urban farmers. It forms a source of income that could be easily mobilized for paying some of the household expenditures, particularly in times of need (Kaltungo, 2012).

In the rural areas of northern Nigeria, the contribution of small ruminant meat to total meat consumed is about three times that of beef. Small ruminants also feature prominently in socio-cultural functions such as ceremonies and religious festivals where they are loaned, exchanged among relatives and friends or slaughtered for such occasions (Bale, 1980).

The high fecundity of sheep and goats and their enormous economic prospects in developing countries have greatly been hindered by a range of factors like drought, pests and other environmental challenges including diseases one of which is brucellosis (Ademosun, 1992).

Brucellosis is a contagious systemic bacterial disease primarily of ruminants, characterized by inflammation of the genital organs and foetal membranes, abortion, sterility, and formation of localized lesions in the lymphatic system and joints (WHO, 1971; CDC, 2005). Clinically, there is neutropenia, lymphocytosis and monocytosis (Hope, 1998). Additionally, it has been reported to be one of the most widespread and important zoonotic diseases worldwide (Pappas *et al.*, 2006). Brucellosis has been virtually eliminated from the majority of the developed countries, but it is still endemic in Africa, the Middle East, Central and Southeast Asia, Central and South America and in most of the Southern European countries owing to lack of awareness and knowledge, poor policies or in-appropriate use of resources (Donev *et al.*, 2010). Furthermore, traditional farming practices, beliefs and food habits can further enhance the spread of the pathogen (OIE, 2009; Kaltungo, 2012; Smits, 2013).

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

Exploring the full advantage of small ruminant production in view of the fact that there is a disturbing and noticeable shortage of animal protein in the diet of the average Nigerian is seriously hindered by several factors, one of which is diseases (Ajala *et al.*, 2008). Diseases are very important to farmers as they affect the production of small ruminants in several ways. They include increase in cost of production, low production level, reduction in the quality and quantity of animal products and generally causing great losses to the farmer (Abdullahi *et al.*, 2013). The low educational level of small ruminant keepers, coupled with the poor animal health delivery services and the system of management being mainly semi-intensive to extensive, favours the spread of several diseases including brucellosis especially in the rural communities (Kaltungo, 2012).

Brucellosis is a major bacterial disease which has often been under-diagnosed and under-reported probably due to its insidious nature and risks associated with its diagnosis. However, the disease

has been reported in different species by various authors as being endemic in Africa in general and Nigeria in particular (Falade, 1974; Bale, 1980; Ogundipe *et al.*, 1994; Kaltungo *et al.*, 2013; Bertu, 2014; Buhari, 2014; Baba, 2016). Unfortunately, most epidemiological reports on brucellosis in Nigeria are in the bovine species with only very few being obtainable for small ruminants even though the causative agent of brucellosis in these species remains the most pathogenic species for humans (OIE, 2000). It is interesting to note that in pastoral herds, small ruminants commonly graze along with cattle and it has been reported that there is an increase in the prevalence of bovine brucellosis (Ocholi *et al.*, 2004). There is therefore the possibility of these cattle passing the infection to small ruminants (Ocholi *et al.*, 2004; Kaltungo *et al.*, 2013). Similarly, small ruminants may be exposed to infection as some states in the North-West Geopolitical Zone of Nigeria have international borders that are porous with trans-human and livestock movements either way during most of the year (Bonnet *et al.*, 2011).

In most cases, humans contract brucellosis unknowingly by direct contact with infected animals or indirectly via their products; such as milk, milk products and meat or by handling such deliveries of infected animals (Saleem *et al.*, 2010). Therefore, controlling brucellosis in animals invariably means controlling it in human population.

Unconfirmed cases of abortion, stillbirth and retained placenta in livestock are being reported in Nigeria yearly and these might have been caused by members of the genus *Brucella* (Falade, 1974; Kaltungo *et al.*, 2013). Healthy animals run the risk of exposure to this infection in many ways, as a large number of bacteria are being shed in uterine/vaginal discharges of infected females and these organisms are able to survive for several months in the environment, especially in cold, wet conditions, where they remain infectious to other animals, mainly through ingestion (Díaz-Aparicio, 2013).

Despite the high zoonotic risk associated with small ruminant brucellosis, most small ruminant keepers are neither aware of the disease nor its method of spread. Nevertheless, a few pastoralists have a sparse knowledge of the disease only in cattle (Kaltungo, 2012). The disease is most common in the sub-Saharan Africa and other regions of the developing world (McDermott and Arimi, 2002). Though many countries have eradicated *B. abortus* from cattle, it has been reported that *B. melitensis* has in the recent past re-emerged as a cause of infection in these species (Saleem *et al.*, 2010). This is particularly problematic because *B. abortus* vaccines do not protect effectively against *B. melitensis* infection and that the *B. melitensis* Rev.1 vaccine has not been fully evaluated for use in cattle (Corbel, 1997). Brucellosis in small and even large ruminants might constitute a significant hurdle for the development of the livestock industry in Nigeria.

### **1.3 Justification for the Study**

Small ruminants play a significant role in the food chain (source of protein) and contribute to the overall economy in terms of cash supply, provision of quality leather as well as for festivities among others and are largely the property of women and their children (Fajemisin, 1991; Lebbie *et al.*, 1994). Providing information on evidence of a zoonotic disease-causing agent is relevant in the “one health” perspective and may also be found useful for a better understanding of disease epidemiology, managing outbreaks and setting up efficient preventive and control measures in livestock populations in the study area and in Nigeria in general.

Though advancement in molecular biology and genomics have offered several sophisticated tools for rapid and confirmatory diagnosis of many diseases, disease surveillance, monitoring and the networking approaches using the GIS are much more important for implementing effective control and prevention (Schmitt and Henderson, 2005; Bollo, 2007; Balamurugan *et al.*, 2010; Deb and

Chakraborty, 2012). One of the critical issues in designing a brucellosis surveillance system is to know how extensive the infection is, and when and where is it occurring. This can conveniently be achieved by creating a database using the Geographical Information System (GIS) to show the spatial distribution and mapping of brucellosis infected areas. Its application has been of significant importance in the health systems of developed countries, but to be very limited in developing countries such as Nigeria (Haghdoost *et al.*, 2007).

Most studies on the knowledge, attitude and practices of herd owners in relation to small ruminant brucellosis in Nigeria have been based mainly on structured questionnaires which can be very limiting in terms of allowing any real exploration or understanding of the responses given. However, participatory disease investigation is an effective tool that explores the farmers' traditional practical agricultural knowledge, considers farmers' opinions in intervention strategies thus enabling the development of effective epidemiological surveillance systems for infectious diseases. There does not seem to be a comprehensive data available on surveillance record of small ruminant brucellosis in Katsina and Sokoto States. Therefore, the actual status of the disease in these states cannot be ascertained. Consequently, this makes control programmes difficult to be developed. There is the need to determine the level of spread of the disease in various communities in Nigeria as it is only through that, an efficient and effective control can be initiated. There is, therefore, the need to know the full details of the epidemiological features of the disease especially in the study areas in order to allow for effective control in farms and consequently to ensure optimum production, economic return to production and protection of public health.

## **1.4 Aim and Objectives of the Study**

### **1.4.1 Aim of the study**

The aim of the study was to carry out a sero-prevalence and participatory epidemiological study on small ruminant brucellosis along with mapping out of *Brucella melitensis* infected areas in selected LGAs of Katsina and Sokoto States, Nigeria.

### **1.4.2 Objectives of the study**

The objectives of the study were:

1. Determine the sero-prevalence of antibodies due to classical *Brucella* (*B. abortus*, *B. melitensis*, *B. suis*) infection in small ruminants in selected LGAs of Katsina and Sokoto States, Nigeria.
2. Ascertain if small ruminants in selected LGAs of Katsina and Sokoto States possess antibodies against *B. melitensis*.
3. Determine the status of *Brucella* infection (mild or severe) in small ruminants in selected LGAs of Katsina and Sokoto States.
4. Determine the forms (acute or chronic) of *Brucella* infection in small ruminants in selected LGAs of Katsina and Sokoto States.
5. Draw spatial maps of *B. melitensis* infected areas in selected Local Government Areas (LGAs) in Katsina and Sokoto States, Nigeria.
6. Determine farmers' knowledge and practices in relation to small ruminant diseases along with their management practices in selected LGAs in Katsina and Sokoto States, Nigeria.

## 1.5 Research Questions

1. What is the prevalence of *Brucella* antibodies in small ruminants in selected LGAs of Katsina and Sokoto States, Nigeria?
2. Are small ruminants infected with *B. melitensis* in selected LGAs of Katsina and Sokoto States, Nigeria?
3. Are there mild and severe infections of *Brucella* in small ruminants in selected LGAs of Katsina and Sokoto States, Nigeria?
4. Are there acute and chronic infections of *Brucella* in small ruminants in selected LGAs of Katsina and Sokoto States, Nigeria?
5. What is the distribution of *Brucella* infection in small ruminants in the LGAs under study in Katsina and Sokoto States, Nigeria?
6. What are the farmers' knowledge and practices on small ruminant diseases in the LGAs under study in Katsina and Sokoto States, Nigeria?

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Historical Perspective of Brucellosis

Brucellosis is an infectious disease caused by members of the genus *Brucella* that affect animals and man (Munoz *et al.*, 2005). *Brucella* organisms have a wide host range among domestic and wild animals (Abdulkadir, 1989). The disease in animals is known as infectious abortion, contagious abortion, “slinking of calf”, swine brucellosis and Bang’s disease (Abdulkadir, 1989). In humans, it is called Rock, Gibraltar, Cyprus or Mediterranean fever, Bang's disease, intermittent typhoid or typho-malarial fever, undulant fever, Constantinople fever as well as fevers of Malta, Naples, Cyprus, Crete, Crimea, Levant, Syria, Mediterranean, Gibraltar, Gastric, Undulant, milk, Remittent, Relapsing and Rock (Al Dahouk *et al.*, 2004; Wyatt, 2013). It was also known to the British Royal Army as the Corps disease (Wyatt, 2013).

The disease is an ancient one that can possibly be traced back to the 5<sup>th</sup> plague of Egypt around 1600 BC (Saleem *et al.*, 2010). Evidence of sacroiliitis and other osteoarticular lesions, which are common complications of brucellosis, were unveiled by examination of fossils of Egyptian bones, dating to around 750 BC (Pappas *et al.*, 2006). Historically, Britain maintained a military base on the island of Malta during the 18th and 19th centuries. As British servicemen began to serve in the Mediterranean region of Minorca in Malta after the Crimean War, many of them became ill with mysterious fevers known locally as ‘Malta’ fever, which was often confused with other fevers (Vassallo, 1992). A British Army doctor, David Bruce in 1903 wrote to the Colonial Office asking that the official name be Mediterranean fever, a name he had previously proposed in 1894. This was not actualized until he became the Chairman of the Mediterranean Fever Commission (MFC) in 1904. During the Crimean War, there was much confusion about the diagnosis and cause of

fevers, but in 1861 a British Army surgeon, Dr. Jeffrey Alan Marston gave a very accurate description of the illness in the troops, calling it "Mediterranean Remittent (or) Gastric Remittent Fever". He was able to distinguish between typhoid and 'Undulant Fever' (Wyatt, 2013).

Surgeon Captain (later Sir) David Bruce, in honour of whom the genus *Brucella* was named, carried out his researches into Malta fever, reporting that it was attacking several hundred soldiers and sailors every year. On July 9, 1887 he isolated the specific organism responsible for the disease from the spleen of a victim, and he went on to prove this fact by isolating the same organism from splenic cultures from seven other fatal cases, and also by animal experiments (Bruce, 1887; 1888; 1892) as cited by Vassallo (1992). In 1893, Bruce named the organism '*Micrococcus melitensis*' from the Roman name for Malta. Adding to the success of Sir David Bruce's work was Dr. Guiseppe Carruana-Secluna the Maltese public health analyst, who played an important technical role in culturing the organism even though Bruce never acknowledged his vital contribution to his research (Rust, 2006; Wyatt, 2013). However, how people became infected remained a mystery, although 'bad air' and drains were suspected. Ten years later, a Professor, (later Sir) Almroth E. Wright and Surgeon Major (later Sir) David Semple successfully applied the method of serum diagnosis, enabling clinicians to differentiate Malta fever from enteric, malarial and other fevers (Wright, 1897a, b). At this stage in time, no one knew the source of infection or the method of spread. The prevailing view was that the disease might be transmitted to man by mosquitoes or other blood-sucking insects (Vassallo, 1992).

Another thrust to the brucellosis history was that in 1897, a Danish veterinarian, Bernhard Bang isolated *Brucella abortus* as the cause of abortion in cattle and named it "Bacillus of abortion". However, the great breakthrough came in June 1905 when Dr (later Sir) Themistocles Zammit, successfully incriminated the Maltese goats as the animal host of *Micrococcus melitensis*, by using

his agglutination test, and by his isolation of the *Micrococcus melitensis* in pure culture from infected blood of goats. Other significant contributors in the research on brucellosis include Dr. M. Louis Hughes, another colleague of Dr. Bruce, who was the first to isolate *B. melitensis* from human brain (Rust, 2006). Horrocks (1905) reported a similar organism from milk and urine of apparently healthy goats. Traum (1914) isolated *Brucella* species from an aborted pig foetus and subsequently named it *Brucella suis*, which later on was recognized by Keefer in 1924 as the cause of undulant fever in man in the USA.

Evans (1918) was the one who showed that *Brucella* organisms were related morphologically, biochemically, and serologically (Vassallo, 1992). Jahans *et al.* (1997) reported other species of *Brucella* of veterinary and public health importance that have since been isolated. For instance, *Brucella ovis*, the cause of epididymitis and abortion in sheep was isolated by Buddles and Boyes (1953) in New Zealand and Australia. Similarly, *Brucella neotomae* was isolated by Stoener and Lackman (1957) from desert wood rat (*Neotoma lepida*) in Arizona, USA. Carmichael in 1967, also in the USA, isolated *B. canis*, which was the cause of canine epizootic abortion and epididymitis from beagle dogs. Much later, another *Brucella* species was isolated from marine mammals and was proposed to be named *Brucella maris* (Anon., 1997).

Snyder (2004) reported that, in 1954, *Brucella suis* became the first biological agent to be weaponized by the United States of America in the days of its offensive biological warfare programme. The infective dose for the organism is very low if acquired via the inhalation route, which makes it potentially an effective bioterrorism agent and a hazard in clinical microbiology laboratories. Ko and Splitter (2003) also reported that lack of human vaccine and its epidemic potential contributes to its efficiency as a bioterrorism agent.

The popular name, undulant fever, originates from the characteristic undulance (or "wave-like" nature) of the fever in brucellosis, which rises and falls over weeks in untreated human patients. In the 20<sup>th</sup> Century, this name, along with brucellosis, gradually replaced the 19<sup>th</sup> Century names of Mediterranean fever and Malta fever. In 1989, neurologists in Saudi Arabia discovered neurobrucellosis, a neurological involvement in human brucellosis (Al-Sous *et al.*, 2004; Malhotra, 2004).

## 2.2 Epidemiology of Brucellosis

There are six well documented species of the genus which include *B. abortus*, *B. melitensis*, *B. ovis*, *B. canis*, *B. neotomae* and *B. suis* which are further divided into biotypes (Alton *et al.*, 1975). Foster *et al.* (2007) reported that a marine species has been identified and was first classified as *B. maris* but was later divided into two species: *B. ceti* and *B. pinnipedialis*, referring to isolates from cetaceans and seals, respectively. A new *Brucella* species was also isolated from systemically infected common voles (*Microtus arvalis*) in South Moravia, Czech Republic which was named *B. microti* (Scholz *et al.*, 2008). They further observed that the organism was similarly isolated from the mandibular lymph nodes of wild red foxes (*Vulpes vulpes*) in Austria. An unnamed strain (*Brucella spp.* NVSL 07-0026) was also isolated from a baboon (Audic *et al.*, 2011). These reports, therefore, indicate that there is still a wide range of information to be explored regarding the genus *Brucella* and its host range.

There is relatively a paucity of science-based evidence on brucellosis in sub-Saharan Africa (Ocholi *et al.*, 1993; McDermott and Arimi, 2002; McDermott *et al.*, 2013) and an appraisal of historical and contemporary epidemiology is fundamental to the implementation of measures for sustainable management of this disease.

### **2.2.1 Distribution of brucellosis**

Brucellosis has a worldwide distribution, though is more common in countries having poorly standardized or ineffective animal and public health programmes (McDermott *et al.*, 2013). Additionally, the geographical distribution of brucellosis is dynamic with new foci emerging or re-emerging (Saleem *et al.*, 2010). The disease occurs worldwide, except in developed countries where bovine brucellosis due to *B. abortus* has been eradicated after a long and expensive eradication campaign. Eradication is usually defined as the absence of any reported cases for at least five years. These countries include Australia, Canada, Cyprus, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden and the United Kingdom (OIE, 2002). Of even greater concern is the fact that brucellosis is considered a re-emerging problem for countries such as Israel, Kuwait, Saudi Arabia, Brazil and Colombia due to a constant change in its geographical distribution, host or vector range (Corbel, 1997a, Saleem *et al.*, 2010).

Brucellosis had not been suspected in Nigeria until contagious abortion was reported in newly established stock farms in 1927 (Earnshaw and O'Brien, 1928). Since then, the existence of the disease as a zoonosis acquired through sheep and goats had been reported by various researchers in Nigeria (Halle and Ajogi, 1997; Ocholi *et al.*, 2005; Ehizibolo *et al.*, 2011; Kaltungo, 2012). The herding of livestock together greatly enhances the possibility of transmission of the disease from one group of animals (species) to another, especially if a member of a herd is currently shedding the organism post-abortion or post-parturition (Ocholi *et al.*, 1993). Esuruoso (1974) reported that generally brucellosis was more prevalent in government-owned farms than in nomadic herds probably due to intensive system of management of the farmer which may favour the survival of *Brucella* organism. Bale (1981) reported that epidemiological investigations revealed that results obtained from different prevalence studies varied which might be attributed

to some factors like sampling technique, diagnostic method used, vaccination status of the animals, tradition or culture of the farmer, history and health status of the animals and other socio-political problems like poverty.

Studies in various parts of Nigeria show that the disease is widespread particularly in ranches, livestock breeding centres, and dairy farms where the prevalence of the infection ranged between 3.7% and 48.8% (Esuruoso and Hill, 1971; Esuruoso and Van Black, 1972; Jajere *et al.*, 2016). Infection rates in nomadic herds have been shown to be between 0.4% and 26.0% (Nuru and Dennis, 1975; Ocholi *et al.*, 1996; Buhari, 2014). There was also a report of prevalence ranging from 0.4% to 8.6% based on abattoir surveys (Chukwu, 1987). In the neighbouring Niger Republic, brucellosis was first reported in humans in 1953, but it was not until 1970 when the first serological study was conducted to report its prevalence in animals (Gidel *et al.*, 1974). Since then there has been reports of an increase in the sero-prevalence ranging between 1.4% and 30.9% in cattle population (Boukary *et al.*, 2013). Though *Brucella melitensis* is the least species-specific of the brucellae, *B. abortus* accounts for the largest number of human and veterinary cases of brucellosis worldwide (Rust, 2006).

### **2.2.2 Aetiology**

*Brucella* is a generic name for a group of organisms which belong to the  $\alpha$ -2 subgroup of the *Proteobacteriaceae*. It also shows close genetic relationship with some plant pathogens and symbionts of the genera *Agrobacterium* and *Rhizobium*, as well as animal pathogens (*Bartonella*) and opportunistic or soil bacteria (*Ochrobactrum*) (OIE, 2009).

*Brucella* organisms are gram-negative, facultative intracellular bacteria (Saleem *et al.*, 2010). They can be isolated as part of the normal flora of the genitourinary tract of a variety of wild and

domestic animals including cattle, goats, sheep, pigs, and dogs (Young, 1995). They do not ferment carbohydrates and have variable urease activity. *Brucella* strains may occur as either smooth or rough, expressing smooth LPS (S-LPS) or rough LPS (RLPS) as major surface antigen (Cardoso *et al.*, 2006). The LPS and LPS-related molecules are extensively used in immunological studies and in the diagnosis of brucellosis (Díaz-Aparicio *et al.*, 1993). They have a lipopolysaccharide coat that is much less pyrogenic than other gram-negative organisms, which accounts for the rare presence of high fever in brucellosis (Gerald and Faaem, 2009).

#### 2.2.2.1 Taxonomic tree

The genus *Brucella* (51BRUC) belongs to the family Brucellaceae (41BRUC), order Rhizobiales (31RHIZ), class Rhodospirilli (21RHOD) in the kingdom Proteobacteria (01PROT) (Kampfer *et al.*, 2014).

#### 2.2.2.2 Morphology

*Brucella* organisms are small (0.5-0.7 by 0.6-1.5  $\mu\text{m}$ ), non-spore forming, non-encapsulated, coccobacilli which occur singly or more rarely in group of short chains or clusters (Ryan and Ray, 2004). Although the brucellae are described as non-motile, they possess all the genes except the chemotactic system, necessary to assemble a functional flagellum. They are not truly acid fast but resist discoloration by weak acids; thus staining red with bluish background when Stamp's modification of Ziehl-Neelsen stain is used (Anon., 2001).

The cellular and colonial morphology of the species of *Brucella* are similar in most respect (Deyoe, 1981). *Brucella* species, except for *B. ovis* and *B. canis*, contain smooth lipopolysaccharide (SLPS) in their outer cell wall. Smooth lipopolysaccharide contains an immunodominant O polysaccharide

(OPS) which has been chemically defined as a homopolymer of 4, 6-dideoxy-4-formamide-alpha-D mannose linked *via* glycosidic linkages (Bundle *et al.*, 1987). *Brucella ovis* and *B. canis* lack the OPS component and as a result, their outer surface contains only rough lipopolysaccharide (RLPS) and protein antigens (Blasco, 1990).

### 2.2.3 Host range

Members of the genus *Brucella* display a marked host range despite the fact that there is more than 94% similarity amongst the members of the genus, and are capable of causing disease in a variety of animal species, including man (Verger *et al.*, 1987, Xavier *et al.*, 2010). It was suggested that all members of the genus should be regarded as a single species *Brucella melitensis* with multiple biovars (Anon., 2008). 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).

Report from the OIE (2009) stated that *Brucella melitensis*, has three biovars that mainly infect sheep and goats. Most breeds of goats are readily infected, but sheep breeds vary greatly in susceptibility. *Brucella melitensis* infections have also been reported occasionally in cattle, camels and dogs, but rarely in horses and pigs (OIE, 2009). Office International Des Epizooties (2009) also reported that, infections in sheep and goats can spill over into wild ruminants. Infections with this organism have also been reported in alpine ibex in Italy and chamois in the French Alps. However, there is no evidence that these animals serve as reservoir hosts for domesticated sheep and goats.

Cattle are considered to be the preferential hosts for *B. abortus* which is classified into seven biovars, namely biovars 1-6 and 9 (Xavier *et al.*, 2010). *Brucella abortus* can also infect buffaloes,

camelids, deer, dogs, horses, goats, sheep, and man. These species play a role in its persistence and transmission (Kudi *et al.*, 1997; Díaz Aparicio, 2013). Species of poultry reported to be susceptible to *B. abortus* are turkeys, pigeons, pheasants, ducks, geese, and chickens (Emmel, 1930; Adesiyun and Abdu, 1984).

Horses can become infected with *B. abortus*, but in this case, the bacterium has a preference for bursae, tendons, muscles, and joints, thus, it is commonly found in cases of fistulous withers and poll evil, probably as a secondary invader (Hall, 1977).

Fretin *et al.* (2008) reported that porcine brucellosis caused by *B. suis* biovars 1, 2 and 3, is considered an important re-emerging disease of domestic and wild pigs and may also affect other animal species such as cattle, horses, rabbits, dogs, and humans. *Brucella suis* biovars 1 and 3, have been shown to have pathogenic potential for humans (Frye, 1991). Furthermore, *Brucella suis* biovars 4 and 5 have been specifically associated with reindeer and rodents, respectively (Corbel, 2006). Nonetheless, swine can also be infected by other *Brucella* species other than *B. suis* but the infection is invariably self-limiting (OIE, 2009a). Canine brucellosis caused by *B. canis*, usually affects domestic dogs, wild carnivores, and rarely other domestic animals and man (Carmichael, 1990).

*Brucella neotomae* is known to infect only the desert wood rat under natural conditions 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 mammal species, including seals (*Phoca vitulina*), dolphins (*Tursiops truncatus*, *Delphinus delphis*, *Lagenorhynchus acutus* and *Stenella coeruleoalba*), whales (*Balaenoptera acutorostrata*), and other species (Ewalt *et al.*, 1994; Ross *et al.*, 1994; Foster *et al.*, 1996).

Human brucellosis is mainly caused by *B. melitensis*, *B. suis* and *B. abortus* which have small ruminants, pigs, and cattle as preferential hosts respectively (Godfroid *et al.*, 2005). However, *B. melitensis* is considered the most virulent *Brucella* spp for humans with a few organisms (10 to 100) being sufficient to cause a debilitating chronic infection (Fugier *et al.*, 2007). In addition, two newly identified *Brucella* species isolated from marine mammals, *B. ceti* and *B. pinnipedialis*, can also cause a mild infection in humans (Foster *et al.*, 2007; Xavier *et al.*, 2010). It has also been reported that, *B. canis*, a pathogen of dogs, has a comparatively low zoonotic potential, while *B. neotomae* and *B. ovis*, which infect desert rats and sheep, respectively, are not associated with human disease (Godfroid *et al.*, 2005; Xavier *et al.*, 2010).

#### 2.2.3.1 Susceptibility to *Brucella* organisms

All domesticated animals have been reported to be susceptible to *Brucella* organisms (FAO/WHO, 1986). Ko and Splitter (2003) reported that the primary host is the important factor in the maintenance of the disease as most of the other hosts serve as reservoirs of the infection for each particular species. In addition, there is the possibility for cross-infection among animal species, especially when they are kept in close contact. It has frequently been reported that adult animals are much more susceptible to infection by *Brucella* spp. than younger ones. However, susceptibility appears to be more strongly linked to sexual maturity rather than age (Corbel, 2006). Therefore, young sexually immature cattle do not generally become infected following exposure or may recover quite rapidly. It should, however, be noted that latent infections can occur and such animals may be hazardous when mature.

Likewise, susceptibility is believed to increase during pregnancy, and animals get more susceptible with the advancement of pregnancy. Bulls are said to be relatively more resistant than sexually

mature heifers and less resistant than sexually immature heifers (Megid *et al.*, 2010). In contrast to bulls, boars are more likely to be sources for introducing *Brucella* organisms into a swine herd (Acha and Szyfres, 2003).

Corbel and Brinley-Morgan (1984) reported that dairy breeds of sheep appear more susceptible than those kept for meat production. Manthei *et al.*, (2010) reported that an important factor other than natural immunity, which also could be responsible for the lower incidence of brucellosis in males than in females, is management. This is because most beef bulls are maintained separately from the herd and their exposure to infection is therefore lessened.

#### **2.2.4 Transmission of brucellosis**

Most infections in animals result from ingestion of the bacteria either from diseased animals or contaminated feedstuffs (Corbel, 2006). Aborted foetuses, placental membranes or fluids, and other vaginal discharges present after an infected animal has aborted or calved are very rich in *Brucella* organisms (Corbel, 2006). Infection may also be acquired by respiratory exposure and by contamination of abraded skin and mucosal surfaces (Noviello *et al.*, 2004; Corbel, 2006). Natural breeding results in infection in swine and dogs and, to a lesser extent in sheep and goats (Corbel, 2006). Dogan *et al.* (2013) also reported that brucellosis can be transmitted transplacentally and that despite occasional exceptions, the general rule is that brucellosis is carried from one herd to another by an infected or exposed animal or purchase of animals from unscreened sources along with sharing of male breeding stock (FAO, 2003).

The use of pooled colostrum for feeding new-born calves may also be responsible for the transmission of the infection (Corbel, 2006). It has been established that brucellosis in bulls does not always result in infertility, although semen quality may be affected (OIE, 2009). Shedding,

however, may cease or become intermittent. In contrast to artificial insemination, bulls used in natural service may fail to spread the infection, as the infected semen is not deposited in the uterus (Ray, 1979).

Animal Health Australia (2005) reported that despite the ability of flies and ticks to experimentally transfer infection, their role in spreading *B. abortus* from infected to uninfected herds has not been established.

In small ruminants, *B. melitensis* is usually transmitted by contact with the placenta, foetus, foetal fluids and vaginal discharges from infected animals. Small ruminants are capable of shedding the organism after either abortion or full-term parturition (OIE, 2009). Goats usually shed *B. melitensis* in vaginal discharges for at least 2 to 3 months, but shedding usually ends within three weeks in sheep. The Office International Des Epizooties (2009) reported that *B. melitensis* can be found in milk and semen of infected animals and that infected ewes and does may shed the organism in milk and semen for prolonged periods leading to lambs and kids becoming infected. Also, infection in sheep may occur mainly through the nasopharyngeal route. Transmission is also possible from dam to kid/lamb *in utero* or via the colostrum or milk (FAO, 2006).

Transmission of brucellosis in pigs occurs by both venereal and oral routes, with *B. suis* being secreted in large numbers for long periods in the semen and urine as well as in uterine discharges and milk (Alton, 1990).

*Brucella ovis* transmission can occur by direct contact between rams kept in the same premises for prolonged periods of time or through ewes that have mated with an infected ram prior to a susceptible one during the same mating season (Hughes, 1972). However, it is unclear whether transmission occurs from direct contact between rams or indirectly by environmental contamination.

In dogs, transmission of brucellosis usually occurs through breeding or by ingestion of contaminated placental tissues. Aborted fetuses or vaginal secretions from infected bitches and urine in males are also sources of infection. Importantly, *B. canis* may be shed for long periods in semen or vaginal secretion after abortion (Carmichael and Kenny, 1970).

Garner *et al.* (1997) reported that the means by which *Brucella* spp are transmitted among marine mammals is unknown. However, the almost exclusive localization of these bacteria within the intestinal lumen and/or uterus of lungworms (*Parafilaroides*, *Phocoena*) in the pulmonary systems of an infected Pacific harbour seal and harbour porpoise suggests the intriguing possibility that these may play a role in transmission of *Brucella* species. Foster *et al.* (2002) were of a different opinion and reported that transmission of brucellosis in marine animals may occur through the mucosa and injured skin, direct contact, or by the oral route due to ingestion of other infected marine mammals. Vertical or horizontal transmission to the foetus also has to be considered as a route of infection, since *Brucella* has been isolated in foetal tissues and in milk from dolphins (Hernandez-Mora *et al.*, 2008). Additionally, it was reported by Rhyan *et al* (2001) that marine *Brucella* species are capable of infecting terrestrial mammal species as demonstrated by experimental infection of cattle.

Brucellosis is primarily a disease of animals in which man is an accidental and final host. Saddler (1960) reported that the occupational source of exposure predisposes farmers, shepherds, hunters, butchers, laboratory workers, veterinarians, and slaughterhouse workers to a greater risk of contracting the disease. The non-occupational sources of exposure include ingestion of contaminated meat, unpasteurized milk and milk products (Williams, 1971). In addition to the foodborne and occupational infection, it is linked to travel and bioterrorism (Young 1995; Greenfield *et al.*, 2002). *Brucella* is considered a biological weapon as a category B pathogen.

Other routes of transmission in humans have been identified to include infection through breast milk (Tikare *et al.*, 2008), sexual intercourse (Ruben and Band, 1991), blood transfusion (Magoffin and Kabler, 1949) and the infection contracted by an obstetrician during the delivery of a transplacentally infected baby (Poulou and Markau, 2006).

### **2.2.5 Effects of management practices on the spread of brucellosis**

Bale (1980) stated that the system of husbandry greatly influences the spread of infection due to *Brucella* spp. Institutional flock animals that abort due to brucellosis have higher chances of infecting other animals within the flock than free range nomadic type of husbandry animals which have lesser chances of remaining or returning to the contaminated environment for some time (Bale, 1980). Corbel (1997b) is of the opinion that, in cold climates, it is the practice to house animals in close space and this also facilitates transmission. However, contrary to this view, and going by the data from a study carried out in South Africa, it was found out that the incidence of brucellosis was higher in pastoral production systems where there was mixing of large numbers of animals, and that it was lowest for the confined farms (Emslie and Nel, 2002). Lambing or kidding in the dark, crowded enclosures favours the spread of the organism, while open air parturition in a dry environment results in decreased transmission (AHA, 2005).

#### *2.2.5.1 Resistance of Brucella organisms to environmental factors and chemicals*

Anon. (2010) reported that the survival of *Brucella* organisms in the environment may play a role in the epidemiology of the disease. The ability of *Brucella* spp. to persist outside mammalian hosts is relatively high compared to most other non-sporing pathogenic bacteria. Temperature, humidity, and pH are factors that can influence the organism's ability to survive in the

environment. *Brucellae* are sensitive to direct sunlight, disinfectant and pasteurization. In dry conditions they survive only if embedded in protein (Davis and Casey, 1973). However, the efficacy of a disinfectant is known to depend on the concentration, time of exposure, temperature, and reaction conditions (Pappas *et al.*, 2005). The organism can survive in tap water for several months at 4 to 8°C, 2.5 years at 0°C, and several years in frozen tissues or media (Bercovich, 1998). *Brucellae* can also survive for up to 60 days in damp soil and for up to 144 days at 20°C, 40% relative humidity and at pH greater than 4. *Brucella* organisms can survive freezing and thawing and also for several weeks in non-fermented milk (OIE, 2009). *Brucellae* can survive for 30 days in urine, 75 days in aborted fetuses and more than 200 days in uterine exudates. In beddings contaminated with infected faecal material, *Brucella* will be destroyed at 56°C to 61°C within 4-5 hours (King, 1957).

Susceptibility of *Brucella* species to dyes such as thionin, basic fuchsin, methyl violet, pyronin, and safranin O at standard concentration of 20g/ml has been reported (Anon., 2001). It also grows in a minimal medium containing sodium chloride, sodium thiosulphate, ammonium sulphate, glucose, nicotinic acid, thiamine, panthotenic acid and biotin (Plommet, 1991). Its growth can be improved by the addition of serum or blood while susceptibility to dyes varies among species and biovars, a fact that has been used for routine biotyping tests for members of the genus (Anon, 2001).

Members of the genus *Brucella* are readily killed by most of the commonly available disinfectants including hypochlorite solutions, 70% ethanol, isopropanol, iodophores, phenolic disinfectants, formaldehyde, glutaraldehyde and xylene (Pappas *et al.*, 2005). However, sodium hypochlorite and sodium hydroxide are preferred under dirty conditions and low temperatures (Wang *et al.*, 2015).

### 2.2.5.2 Phenomenon of antigenic interference between *Brucella* species

The phenomenon that there were no detectable infections in animals by *B. canis* occurring in a region epidemic with *B. melitensis* or *B. abortus* infections was first observed in China (Dequi *et al.*, 2002). Experimental studies in mice on the antigenic interference of *Brucella* spp. revealed that only smooth (S) type of *Brucella* could be isolated after the mixture strains of S type *B. abortus* 104M, *B. suis* S2, Rev-1 and rough (R) type of *B. canis* were injected. It was clear that the S type of *Brucella* spp. interfered with the ability of *B. canis* to infect mice. The interference ability of S versus R strains seems to be about 5000-folds, meaning that 1 S type *Brucella* equal to 5000 bacteria of *B. canis*. It was also observed that *B. abortus* 104M infections interfered with those infections by *B. canis* RM6/66. Strain Rev-1 and RM6/66 interfered with each other and no interference was found between S2 and RM6/66 using equal amounts of bacteria (Wang *et al.*, 1999). This suggests that R type of *Brucella* were difficult to isolate from the regions endemic with S type of *Brucella*.

## 2.3 Pathophysiology of *Brucella* infection

The ability of *Brucella* spp. to cause disease requires a few critical steps during infection. *Brucella* spp. can invade epithelial cells of the host, allowing infection through mucosal surfaces (Poester *et al.*, 2013). After invasion, an important aspect of it being a successful infectious agent lies in its ability to persist and replicate within phagocytic cells of the reticulo-endothelial system as well as in non-phagocytic cells such as the trophoblasts (Olsen *et al.*, 2010). *Brucella* has the ability to interfere with intracellular trafficking, thus, preventing fusion of the *Brucella*-containing vacuole (BCV) with lysosome markers, and directing the vacuole towards a compartment that has rough endoplasmic reticulum (RER), which is highly permissive to intracellular replication of *Brucella*

(Poester *et al.*, 2013). These endoplasmic reticulum-associated compartments are the niche for intracellular replication of *Brucella* in macrophages, epithelial cell lines and placental trophoblasts (Celli *et al.*, 2003). The mechanisms that allow host cell invasion by *Brucella* spp. are not fully understood. However, internalisation of *Brucella* into host cells requires cytoskeletal changes (Guzmán-Verri *et al.*, 2001). Interestingly, invasion of the digestive tract by *Brucella* organism does not elicit any inflammatory response from the host (Paixao *et al.*, 2009). Therefore, *Brucella* spp. invade silently or unnoticed by the innate immune system of the host, as it is reported to have mechanisms that prevent activation of the host innate immune system (Barquero-Calvo *et al.*, 2007).

Several studies have shown that *Brucella* virulence factors are directed at the main components of the outer membrane which contains lipopolysaccharide (LPS) (Lapaque *et al.*, 2005). It possesses a peculiar non-classical LPS as compared to the classical LPS from enterobacteria like *Escherichia coli* which exhibits high toxicity and pyrogenicity and induces interferon and tissue necrosis factor (Lapaque *et al.*, 2005). Smooth LPS has a role in cell entry and immune evasion of the infected cell (Christopher *et al.*, 2010). It also alters the capacity of the infected cell to present foreign antigens to the major histocompatibility complex (MHC) class II antigen presentation system, hence, preventing the attack and killing of the infected cell with the help of the immune system (Araya *et al.*, 1989). Wafa (2011) also reported that survival of *Brucella* organism in the host could perhaps be due to adenine and guanine monophosphate production, which inhibits phagosomal fusion and oxidative burst activity. Thus, *Brucella* species replicate without affecting cellular viability. In fact, the ability of the pathogen to switch off cellular apoptosis, practically renders the cell immortal, thus allowing for its own further survival (Gross *et al.*, 2000).

The bacteria can also spread via the blood stream to the foetus and the placenta (Ko and Splitter, 2003). The multiplication of the bacteria is favoured in the pregnant uterus by the presence of the sugar alcohol, erythritol, which is a foetal product concentrated in the chorion and cotyledons in the pregnant uterus of ruminants (Jain *et al.*, 2012). Rapid multiplication of *Brucella* in the placental trophoblasts leads to lesions in the placenta thus interrupting supply of nutrients to the foetus which eventually leads to foetal loss or birth of weak and infected offspring (Cutler *et al.*, 2005). *In vitro* data suggests that *Brucella* metabolizing erythritol have a heightened requirement for iron as well as scavenge through siderophores such as 2, 3-dihydroxybenzoic acid or brucebactin. This may be linked with the requirement for effective iron acquisition for virulence in ruminant hosts. However, it is believed that brucellosis causes fewer spontaneous abortions in humans than it does in animals because of the absence of erythritol in the human placenta and foetus (Gonzalez Carrero *et al.*, 2002; Parent *et al.*, 2002).

#### **2.4 Pathology of brucellosis in small ruminants**

Infection due to *B. melitensis* in small ruminants generally results in granulomatous inflammatory lesions which frequently are found in lymphoid tissues and organs such as the reproductive organs, udder and supramammary lymph nodes and sometimes joints and synovial membranes. However, the lesions, when present, are not pathognomonic. Also necrotising placentitis and palpable testicular alterations could be observed. Acute mastitis with palpable nodules and the production of clotted and necrotising orchitis and epididymitis with subsequent granuloma, necrotising seminal vesiculitis and prostatitis may occur (EC, 2001).

Cameron and Leuerman (1976) reported that rams infected with *B. ovis*, have lesions which are usually limited to the epididymis and testicles. Also, epididymal enlargement can be unilateral or bilateral, and the tail of the epididymis is affected more often than the head or body. Fibrous

atrophy can occur in the testis. The tunica vaginalis is often thickened and fibrous, and can have extensive adhesions. Furthermore, there could be poor semen quality characterized by decreased sperm concentration and sperm abnormalities that are often associated with early infections. Although placentitis is uncommon, it is occasionally seen in infected ewes (OIE, 2009).

## 2.5 Immune response to *Brucella* infection

Antibody response to *B. abortus* will be used as an example because it has been most studied in detail (Nielsen and Yu, 2010). *Brucella* infection in cattle consists of early IgM isotype production, appearing usually 5-15 days after exposure but may be delayed with the timing depending on the route of exposure, the dose of the bacteria and the health status of the animal (Beh, 1973). The IgM antibody response is followed very shortly by the production of IgG1 isotype of antibody and subsequently by IgG2 and IgA in small quantities, thus, theoretically, measuring the IgM isotype production would be best as an indicator of exposure (Corbel, 1972; Beh, 1974).

However, a number of other microorganisms contain antigens with epitopes similar to those of OPS and the main antibody response to these cross reacting antigens is IgM (Corbel and Brinley-Morgan, 1984). Nielsen and Yu (2010) reported that measurement of IgM antibody sometimes gives false positive reactions in serological tests leading to low assay specificity. Further production of IgG2 and IgA isotypes occurs later in infection and so measurement of these antibodies would generally lower assay sensitivity. Therefore, the most useful antibody measurement for serological tests for brucellosis is IgG1 (Nielsen *et al.*, 1984). In addition to cross-reactions, vaccinal antibodies sometimes cause diagnostic problems (Poester *et al.*, 2010).

Nielsen and Yu (2010) reported that *Brucella abortus* S19 is a widely used vaccine. Also, the strain used in vaccine production is antigenically indistinguishable from pathogenic strains of *B. abortus*. Administration of the vaccine to young animals, usually between 3 and 8 months of age,

generally allows the antibody response to wane sufficiently to eliminate some diagnostic problems by the time animals reach sexual maturity and are tested for brucellosis (Nicoletti, 1990). However, some animals were found to have residual antibody leading to higher antibody levels in vaccinated animals. There is a reduction in the host's ability to detect molecular signatures of *Brucella* spp. via Toll-like receptors 2 (TLR2), TLR4 and TLR5 and this impairs the capacity of the body to identify this pathogen as a Gram negative bacterium. As a result, by displaying an incorrect 'barcode', *Brucella* prevents the generation of a typical antibacterial host response (neutrophil recruitment to the site of infection) (De Jong *et al.*, 2013).

## **2.6 Clinical Signs**

Clinical manifestations of brucellosis vary according to the animal species involved and also the incriminating *Brucella* species (Megid *et al.*, 2010). Generally, the disease is characterized by inflammation of the genital organs and foetal membranes, abortion, sterility and formation of localized lesions in the lymphatic system and joints (hygroma) (WHO, 1971, CDC, 2005).

### **2.6.1 Clinical signs in sheep and goats**

There is no evidence that the clinical features of *Brucella melitensis* infection in sheep and goats vary according to the biovar involved (Fensterbank *et al.*, 1987). The main clinical signs of *B. melitensis* infection in ewes and does are abortion in the last 2 months of gestation, placental retention, birth of weak offsprings who usually die during the peripartum period (Aldomy *et al.*, 1992). Approximately two thirds of acute natural infections with *B. melitensis* in goats during pregnancy lead to infection of the udder and excretion of the bacteria in milk during the subsequent lactations (Xavier *et al.*, 2009). Also, intermittent shedding of the agent in milk occurs in animals

with persistent infection of the udder which may result in inflammation of the udder leading to reduced milk production in infected animals (EC, 2001).

In rams and bucks, the infection can produce inflammation of the genital organs (EC, 2001). In the acute phase, it causes orchitis with inflammation of the tunica vaginalis, and the scrotal sac can be distended by an either hemorrhagic or fibrino-purulent exudate (Enright, 1990).

Radostits *et al.* (2003) reported that the initial clinical sign in rams is bacteraemia which is accompanied by a mild systemic response. However, this soon resolves as the organism localises in the epididymis. The epididymitis can be profound, with sperm stasis and secondary spermatocele formation resulting in infertility. This acute phase is characterised by poor semen quality in the presence of scrotal oedema and inflammation. Palpable lesions in the epididymis and tunicae are often the primary clinical findings. However, lesions may not develop until after the acute syndrome has resolved and the latent period has elapsed (Radostits *et al.*, 2003).

According to Jones and Marly (1975), some infected rams showing palpable lesions at one examination may be clinically normal a few weeks later, and that not all *B. ovis* infected rams develop lesions in their external genital organs.

### **2.6.2 Clinical signs in humans**

Human brucellosis is a life-threatening disease that may have variable clinical presentations (Noviello *et al.*, 2004). It is presented as a prolonged febrile illness with flu-like symptoms, such as night sweats, headache, depression, and arthritis. Chronic illness can lead to meningitis and endocarditis. In addition, relapses can occur, even with antibiotic treatment (Megid *et al.*, 2010). Glomerulonephritis which presents as abnormalities in urine sediments, proteinuria and or azotemia have also been reported (Pishva and Salehi, 2008). Notably, humans can remain

asymptomatic for weeks, months, or even years while infected with this pathogen (Yamuk and O'Callaghan, 2012). Neurological complications can occur during the onset of illness, or during the convalescence period or even some months after recovery from an acute infection. In such cases, meningitis, encephalitis, meningoencephalitis, brain abscess, chorea, facial palsy, meningomyeloencephalo-spondylosis, and ischemic attacks have been reported (Tikare *et al.*, 2008).

## 2.7 Diagnosis of brucellosis

Since the original recognition of the causative agent of brucellosis, a large number of diagnostic tests have been developed. It was reported by Gall and Nielsen (2004) and Poiester *et al.* (2010) that the development of the first agglutination test for the detection of antibody to *Brucella* infection was reported by Wright and Smith over 100 years ago. Since then, a great deal of work has been done to improve diagnostic methods and accuracy, culminating in the production of primary binding assays and polymerase chain reaction (PCR) procedures (Poiester *et al.*, 2010). The primary binding assays directly measure the interaction of antibody with antigen while conventional serological tests, such as acidified agglutination tests or the complement fixation test (CFT), measure secondary phenomena such as the agglutination or activation of complement (Nielsen *et al.*, 1996). Smooth lipopolysaccharide (S-LPS) tests are the most sensitive for detecting cattle and small ruminant brucellosis, but they may yield false positive results for these animals if previously vaccinated or exposed to gram-negative bacteria with LPS O-chains similar to those of brucellae (Mittal and Tizard 1979, 1980; Mittal *et al.*, 1985; Perry and Bundle, 1990). These bacteria include *Vibrio cholerae* O1, *Escherichia coli* O157, some strains of *Escherichia hermannii* and *Stenotrophomonas maltophilia*, *Salmonella* group N (O30), and *Yersinia*

*enterocolitica* O9. However, only *Yersinia enterocolitica* O9 is a significant cause of false-positive serological reaction (FPSR) in the diagnosis of bovine brucellosis (Gerbier *et al.*, 1997).

Orally acquired *Y. enterocolitica* O:9 seldom induces high levels of antibodies like *Brucella* spp. S-LPS and the responses are usually transient in cattle (Mittal *et al.*, 1985; Garin-Bastuji *et al.*, 1999), though titres in serum and milk may be high and persistent (Mittal *et al.*, 1985). Currently, there is no diagnostic test adequately sensitive and specific to detect all stages of infection due to *Brucella* species in live animals (McGiven *et al.*, 2003). Hence, the diagnosis of brucellosis is usually performed by a combination of methods with an initial sensitive test used for ‘mass screening’, followed by more specific confirmatory test(s) on animals positive on the screening test. A definitive serological diagnostic technique is not available yet, in spite of being pursued for more than one century (Poester *et al.*, 2010). Direct smear examination, culture and isolation of the organism, animal inoculation and serology are among the diagnostic tests for the detection of brucellosis.

### **2.7.1 Useful specimens for the diagnosis of brucellosis by culture**

For the diagnosis of animal brucellosis by cultural examination, the choice of samples usually depends on the clinical signs observed. The most valuable samples in animals include aborted foetuses (stomach contents, spleen and lung), foetal membranes, vaginal secretions (swabs), milk, semen and arthritis or hygroma fluids. From animal carcasses, the preferred tissues for culture are those of the reticulo-endothelial system (i.e. head, mammary and genital lymph nodes and spleen), the late pregnant or early postparturient uterus, and the udder (OIE, 2009). In humans, blood is the most suitable sample but specimens need to be obtained early in the disease during the bacteraemic phase (Poester *et al.*, 2010; Alikhani *et al.*, 2012). All specimens must be packed separately and

transported immediately to the laboratory under cooled or preferably frozen condition in leak-proof containers. Growth in culture media normally appears after 3–4 days, but cultures should not be discarded as negative until 8–10 days have elapsed as some growth may be seen up to this period (OIE, 2009).

### **2.7.2 Direct smear microscopic examination**

Marin *et al.* (1996) reported that a presumptive bacteriological diagnosis of *Brucella* can be made by means of the microscopic examination of smears from vaginal swabs, placenta or aborted fetuses stained with the Stamp modification of the Ziehl Neelsen staining method. However, morphologically related microorganisms such as; *Chlamydophila abortus*, *Chlamydophila psittaci* and *Coxiella burnetti* can mislead in the diagnosis because they superficially resemble *Brucella* spp. (Marin *et al.*, 1996; Poester *et al.*, 2010). Accordingly, the isolation of *Brucella* organisms on appropriate culture media such as Farrel's selective media is recommended for an accurate diagnosis (Farrel, 1974). Vaginal swabs and milk samples are the best samples to isolate *B. melitensis* from sheep and goats (Marin *et al.*, 1996).

### **2.7.3 Culture and isolation of *Brucella* organism**

This procedure may be performed by culturing body tissues or secretions such as blood, milk and vaginal discharge (Poester *et al.*, 2010). Bone marrow cultures may provide higher sensitivity, yield faster culture times, and may also be superior to blood cultures, when evaluating patients with previous antibiotic use (Mantur *et al.*, 2006). *Brucella* spp. can also be cultured from pus, tissue, cerebrospinal fluid (CSF), and pleural, joint and ascitic fluids. Growth of the bacteria is unequivocal proof of infection (OIE, 2009a; Poester *et al.*, 2010). The culture of blood sample will only work if the animal is bacteraemic which is not always the case. However, milk has often

been found to contain *Brucella* by this test. Post-mortem samples like lymph nodes, liver, spleen, udder and other organs can present positive results associated with negative serological tests. In this respect, the culture test has been widely used in research.

The identification of *Brucella* spp. in culture relies upon a great deal of phenotypic traits such as requirement for CO<sub>2</sub>, phage typing and metabolic tests, which among other problems involve time, biosafety, trained personnel and somewhat ambiguous results (Alikhani *et al.*, 2012). Powder media can be used to prepare either broth or agar medium for culture of *Brucella* organisms while for culturing blood and other body fluids, it is preferred to use broth or a biphasic medium, mainly because *Brucella* is often present in small numbers. However, for other specimens, solid media with 2.5% agar facilitate the recognition of colonies and discourage bacterial dissociation (Poester *et al.*, 2010). Optimum pH for growth of *Brucella* varies from 6.6 to 7.4; culture media should be adequately buffered near pH 6.8 for optimum growth. The optimum growth temperature is 36-38°C. However, most strains grow between 20°C and 40°C (EC, 2001).

Most *Brucella* strains, particularly *B. abortus* biovar 2 and *B. ovis*, grow better in media containing 5-10% of sterile (equine or bovine) serum free from *Brucella* antibodies. Frequently, field samples are contaminated with other bacteria and or fungi (Vicente *et al.*, 2014). Therefore, selective media should be used to avoid overgrowth of fast growing agents. The most widely selective media used are the Kuzdas and Morse medium (Kuzdas and Morse, 1953) and the Farrell's medium (Farrel, 1974). Kuzdas and Morse (1953) used the following antibiotics and quantities per liter of basal medium: 100 mg of cycloheximide (fungistat), 25,000 units of bacitracin (active against gram-positive bacteria) and 6,000 units of polymyxin B (active against gram-negative bacteria). The Farrell's medium is prepared by the addition of the following antibiotics and quantities per liter of basal medium; bacitracin (25 mg), polymyxin B sulphate (5 mg), nalidixic acid (5 mg), nystatin

(100,000 units), vancomycin (20 mg), natamycin (50 mg). As Farrell's medium is rather inhibitory for some strains of *B. abortus*, *B. melitensis*, and *B. ovis*, Modified Thayer-Martin medium shows greater sensitivity than Farrell medium; however, it does not inhibit contaminating microorganisms as well. For this reason, a transparent CITA medium (prepared with GC medium as basal medium supplemented with 1% haemoglobin and the following antibiotics per litre of medium; colistin methanesulphonate (7.5 mg), vancomycin (3mg), nitrofurantoin (10 mg), nystatin (100,000 units) and amphotericin B (2.5 mg) was developed based on Modified Thayer-Martin medium. (Marin *et al.*, 1996; De Miguel *et al.*, 2011). This medium was aimed at inhibiting the growth of contaminants without inhibiting the growth of *Brucella* species.

#### **2.7.4 Laboratory animal inoculation**

Guinea pigs are highly susceptible to *Brucella* spp. (Avong, 2000). Mice are also susceptible and can be substituted for guinea pigs (Avong, 2000; Ocholi, 2005; OIE, 2009a). Animal inoculation may be either subcutaneously or through abraded skin in guinea-pigs or, preferably, intravenously or intraperitoneally in mice (Alton *et al.*, 1988; OIE, 2009). The spleen of mice is cultured 7 days after inoculation while serum samples of guinea pigs are subjected to specific tests 3 and 6 weeks after inoculation (OIE, 2009a).

#### **2.7.5 Serology**

Body fluids such as serum, uterine discharges, vaginal mucus, milk, semen and plasma from a suspected animal may contain different quantities of antibodies of the M, G<sub>1</sub>, G<sub>2</sub>, and A types directed against *Brucella* (Beh, 1974). Because infected animals may or may not produce all

antibody types in detectable quantities, several tests are used to detect brucellosis (FAO/WHO, 1986).

Poiester *et al.* (2010) reported that there is no serological test that is 100% accurate and that serological diagnosis is a presumptive evidence of infection. Furthermore, there are considerable differences in the accuracy of the various serological tests. In addition, depending on the sensitivity and specificity, serological tests can be used to screen for, or confirm the disease. Traditionally, screening tests are inexpensive, fast and highly sensitive, but not necessarily highly specific (Poiester *et al.*, 2010). Confirmatory tests are required to be both sensitive and specific, thereby eliminating some false positive reactions. Most confirmatory tests are more complicated and more expensive to perform than the screening tests (Stemshorn *et al.*, 1985). Generally, diagnosis is made based on the results of two or more tests.

All smooth *Brucella* species share common epitopes in the O-polysaccharride (OPS) (Nielsen and Yu, 2010). Virtually all serological tests for antibody to these bacteria use *B. abortus* antigen in the form of whole cells, smooth lipopolysaccharride (SLPS) or OPS (Nielsen and Yu, 2010; Poiester *et al.*, 2010) while rough lipopolysaccharide (RLPS) or protein antigens are commonly used as the main antigen for detection of antibody to *B. ovis* and *B. canis* (Blasco, 1990; Carmichael, 1990). Other *Brucella* spp. such as *B. neotomae* and *Brucella* of marine mammals can be detected serologically using *B. abortus* antigens (Poiester *et al.*, 2010).

The commonly used serological tests include the Milk Ring Test (MRT), Serum Agglutination Test (SAT), Standard Plate Test (SPT), Complement Fixation Test (CFT), 2-Mercaptoethanol Test (2-MET), Buffered Antigen Test (BPAT), and m-RBPT (Bale, 2008). Others include the Card Test (CARD), Rivanol Test, Coombs test, Indirect Immuno-Flourescent Antibody Test (IFAT), Heat

Inactivation Test (HIT), Skin Test, immuneassay and molecular biology techniques. Briefs on each of these tests are presented below.

#### 2.7.5.1 *Milk ring test (MRT)*

The MRT is essentially a rapid agglutination test carried out on whole milk or cream (McCaughey, 1972; Hubber and Nicoletti, 1986). Hematoxylin stained *Brucella* cells are added to whole milk and reaction is allowed to take place (McCaughey, 1972; Hubber and Nicoletti, 1986). 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 immunoglobulins detected by MRT are IgM and IgA. This test may be applied to individual animals or to pooled milk samples using a larger volume of milk relative to the pool size (MacMillan, 1990). Souring of milk in the tropics especially on transit possess a severe limitation to this test (OIE, 2009). However, this can be avoided by the addition of merthiolate (Bandara and Mahipala, 2002). The milk ring test is prone to false positive reactions caused by abnormal milk derived from mastitis, colostrums and late milk from the lactation cycle (Kerr *et al.*, 1959; McCaughey, 1972). False negatives may also occur in milk with a 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 in dairy herds and may be used as an inexpensive screening test in conjunction with other tests (Corbel, 2006).

#### 2.7.5.2 *Serum agglutination test (SAT)*.

Hajdu and Baseda (1974) reported that the SAT measures IgM, IgG<sub>1</sub> and IgG<sub>2</sub> and IgA. This test is performed at a near neutral pH and therefore detects IgM antibody very well. Hence it is best used to detect acute infections. It is less so for IgG resulting in low assay specificity (Corbel, 1972;

Nielsen *et al.*, 1984). As a result, the SAT, while very sensitive, is generally not used as a single test but rather in combination with other tests. The test has a number of defects including false positive and false negative (Poiester *et al.*, 2010). For this reason, the test is only suitable for herd testing, rather than for individual animals. Furthermore, the presence of post-vaccinal antibody can complicate the results (Corbel and Brinley-Morgan, 1984). The SAT does not detect antibodies to *B. canis* and *B. ovis* because these rough strains of the organism do not have O-polysaccharide on their surfaces (Ndyabahinduka 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 Serum agglutination test-ethylenediaminetetraacetic acid (SAT-EDTA)

Because of the lack of specificity of the SAT, an adaptation of the test, which involves the addition of EDTA, has been proven to significantly increase the test specificity (Poiester *et al.*, 2010). This test works on the principle that the pH of the serum is altered to the isoelectric point of IgM to prevent its agglutination. Furthermore, the test has been used widely because it eliminated some non-specific reactions though has the disadvantage that fresh antigen is needed daily (Rose and Roepke, 1964). Nonetheless, the test has been improved by use of a stable buffered antigen (Nielsen and Yu, 2010).

#### 2.7.5.4 Standard plate agglutination test (SPAT)

The SPAT was standardized to give similar results as that of the SAT titre. Later a dye-stained antigen was used for ease of reading and could be used under field conditions. In this test, a series of dilutions like in the SAT is not used but it is standardized to give a result equivalent to a SAT titre of 1:100 (positive). Stemshorn *et al.* (1985) considered that it could give positive results when

SAT was negative by virtue of its use of high saline (8%) and higher serum concentrations. It is also resistant to the prozone effect.

#### 2.7.5.5 *Buffered plate agglutination test (BPAT)*

The main advantage of this test is the reduction of nonspecific test reactions. It is directed at testing for IgG (Angus and Barton, 1984). Antigen for the BPAT is prepared from *B. abortus* strain 1119-3 stained with crystal violet and brilliant green and buffered to pH  $3.65 \pm 0.02$ .

#### 2.7.5.6 *Rose Bengal plate test (RBPT)*

The m-RBPT is a spot agglutination technique. It is also known as card test or buffered *Brucella* antigen test and uses a suspension of *B. abortus* smooth cells stained with the Rose Bengal dye, buffered to pH 3.65-6.7 (OIE, 2009). At neutral pH, this test can measure the presence of IgM, IgG<sub>1</sub> and IgG<sub>2</sub>. However, IgM appears the most active. At the buffered pH of 3.65, RBPT prevents agglutinations by IgM and encourages agglutination by IgG<sub>1</sub> hereby reducing non-specific reactions (Corbel, 1972; Alton, 1981; Macmillan, 1990). It was considered that while the test gave few false negative results, it gave many false positives, possibly due, in significant part, to reaction with IgM in animals with previous vaccination (Alton, 1981). In situations where vaccination is not routinely conducted, the use of this test can give a good record of exposure of animals to *Brucella* organisms. It is an internationally recommended test for the screening of brucellosis in small ruminants but lacks standardization of the antigen.

#### 2.7.5.7 *Brucellosis card test*

It is a version of the m-RBPT test developed for use in Canada and used in the USA. It is a test in which the antigen, usually dyed, is impregnated into absorbent paper. The subject specimen of urine, blood, plasma or serum is placed on the impregnated card and a colour or similarly easily seen change is recorded. (Stemshorn *et al.*, 1985).

#### 2.7.5.8 *The rivanol test*

Some non-specific reactivity may be removed by precipitation of high molecular weight serum glycoprotein which is the basis for this test (Poester *et al.*, 2010). Acridine dye such as rivanol (2-ethoxy-6, 9-diaminoacridine lactate) is used to precipitate glycoproteins from serum solutions which in this case is mainly IgM leaving mostly IgG in the serum (Nicoletti, 1969). The precipitate is then removed by centrifugation. The supernatant is tested using rapid plate agglutination test with undiluted serum or a tube test using serum dilutions starting from 1:25 and because the protocol is fairly labour intensive, precipitation tests are generally used as confirmatory tests (Poester *et al.*, 2010) The test is capable of distinguishing between vaccinated and infected animals and chronic carriers. Interpretation of the test is, however, difficult (Abdulkadir, 1989).

#### 2.7.5.9 *Complement fixation test (CFT)*

The CFT is a prescribed test for international trade (OIE, 2009). It is considered as the most specific and valuable serological test for brucellosis (WHO/MZCP, 1998; Poester *et al.*, 2010). It detects mainly IgG<sub>1</sub> antibodies than it does for antibodies of the IgM type which are partially destroyed during inactivation. Since antibodies of the IgG<sub>1</sub> type usually appear after antibodies of

the 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* organisms from artificial recovery 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 the interpretation of results due to differences in techniques (Madsen, 1994), occasional direct activation of complement by serum (anticomplementary activity) and the inability of the test to be amenable for use with haemolysed serum samples (OIE, 2009). Furthermore, it is laborious and requires highly trained personnel as well as suitable laboratory facilities. This makes the CFT less suitable for use in developing countries (Kiros *et al.*, 2016). It is used mainly as a verification tool in the diagnosis of human brucellosis (WHO/MZCP, 1998). It may also test false negative when antibodies of the IgG<sub>2</sub> type hinder complement fixation (Nielsen *et al.*, 1988; Macmillan, 1990).

#### 2.7.5.10 The 2-mercaptoethanol test (2-MET)

The 2-MET is an adaption of the SAT titre. There are 2 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 and mutagenicity of 2-mercaptoethanol. The test measures mainly IgG because the disulphide bridge of IgM is being reduced to monometric molecules and therefore unable to agglutinate. However, IgG can also be reduced giving false negative results, though in general, reduction of IgM increases specificity (Poiester *et al.*, 2010).

#### 2.7.5.11 Anti-globulin (Coombs) test

The Coombs test, which is used to confirm SAT result, is useful in epidemiological survey of brucellosis because of the advantage of detecting incomplete and complete antibodies of the IgG<sub>2</sub> types (WHO/MZCP, 1998). It can also differentiate patients with acute and chronic infections. However, results of this test are indicative only for infection when its titre is at least two times the titre of the SAT. This is the main limitation of the test as not all infected cattle show this titre.

#### 2.7.5.12 Immunofluorescent antibody test (IFAT)

The IFAT test is specific, rapid and sensitive and is used as confirmatory test. It is used for detecting antibodies in sera of humans (Dhar *et al.*, 1988).

#### 2.7.5.13 Heat inactivation test (HIT)

With experimental cases, the HIT is very sensitive at early stages of infection. The test is based on the observation that two types of *Brucella* agglutinins IgM and IgG are found and can be differentiated on the basis of stability at 65°C for 15 minutes, cooled to 18°C. The test is just like the tube agglutination test, the only difference being the heating (Amerault *et al.*, 1961).

#### 2.7.5.14 Skin test

The brucellin skin test has a very high specificity such that serologically negative unvaccinated animals that are positive reactors to this test should be regarded as infected animals (Pouillot *et al.*, 1997; Saergerman *et al.*, 1999). Results of this test may aid 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 (Pouillot *et al.*, 1997; Saegerman *et al.*, 1999; De Massis *et al.*, 2005). Bercovich (1999) reported that it should be the test of choice in developing countries, as cattle in these countries are usually not tagged so that serological test results could be related to the individual animal. The test can be relied upon for clinical surveillance and epidemiological surveys (FAO/WHO, 1986). It is of great importance in areas with low prevalence and areas known to be free from brucellosis (Bercovich, 1998). The test uses brucellin which involves injecting it into the flank or intrapalpebrally and measuring the thickness of the skin (Weildmann, 1991; Cheville *et al.*, 1994). Not all infected animals react, therefore this test alone cannot be recommended as the sole diagnostic test or for the purposes of international trade (OIE, 2009). Similarly, Cutler *et al.* (2005) reported that the specificity of the test is reduced following vaccination, and the necessity for two farm visits, delay between repeat tests, and subjective nature of the interpretation of the result, make this type of test impractical for high throughput diagnosis.

#### *2.7.5.15 Lateral flow assay (LFA)*

The LFA is a simplified 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 a test strip (cellulose membrane matrix). It allows for the detection of specific IgM as well as specific IgG antibodies and that a high sensitivity is assured for all stages of the disease (Nielsen and Yu, 2010).

Application of the assay does not require specific expertise, equipment or electricity, and test kits may be kept in stock without the need for refrigeration (Kaltungo *et al.*, 2013). However, the interpretation is subjective, depending on the formation of a visible coloured line of reaction and

the assay itself tends to be expensive because of the multiple reagents/components involved (Nielsen and Yu, 2010).

#### *2.7.5.16 Fluorescent polarization assay (FPA)*

The FPA assay was developed by Nielsen *et al.* (1996). It is a simple technique for measuring antigen/antibody interaction and may be performed in a laboratory setting or in the field. It is a homogeneous assay in which analytes are not separated and it is therefore very rapid (OIE, 2009). The principle for the fluorescence polarization assay is that the rate of rotation of a molecule in solution is inversely proportional to its size (Poiester *et al.*, 2010). In brucellosis serology, small molecular weight subunit of OPS is labelled with fluorescein isothiocyanate and used as the antigen. When testing serum, blood or milk, if antibody to the OPS is present, the rate of rotation of the labelled antigen will be reduced at a rate which is proportional to the amount of antibody present. The FPA is very accurate and the sensitivity/specificity can be manipulated by altering the cut-off value between positive and negative reactions to provide a highly sensitive screening test as well as a highly specific confirmatory test (OIE, 2009). The FPA can distinguish vaccinal antibody in most vaccinated animals and it can eliminate reactivity by some cross-reacting antibodies as well (Nielsen and Yu, 2010).

#### *2.7.5.17 Competitive immunoassays*

Poiester *et al.* (2010) reported that competitive enzyme immunoassays were developed in order to overcome some of the problems arising from residual vaccinal antibody and from cross-reacting antibody. By selecting a monoclonal antibody (MAb) with slightly higher affinity for the antigen than most of the vaccinal/cross reacting antibody but with lower affinity than antibody arising from

infection, reactivity by vaccinal antibody could be eliminated in the majority of cases. The specificity of the competitive enzyme immunoassay is very high. However, it is slightly less sensitive than the indirect enzyme immunoassay (Munoz *et al.*, 2005). This assay is an excellent confirmatory assay for the diagnosis of brucellosis in most mammalian species.

### **2.7.6 Molecular Biology Techniques**

Basically with regards to large scale investigations, laboratory diagnosis of brucellosis is mainly based on serological tests (Poester *et al.* 2010). However, the similarity of the O-antigenic side chain of *Brucella* LPS with other microbes, particularly *Yersinia enterocolitica* O: 9, has restricted the specificity of most serological diagnostics (Garin-Bastuji *et al.*, 1999). On the other hand, the culture of *Brucella* is time-consuming, expensive, of low sensitivity, requires highly skilled personnel, sometimes difficult in interpretation of results, and requires biohazard conditions for handling highly contagious materials (Kiros *et al.*, 2016). Due to these disadvantages, the new methods based on molecular biology (PCR) are introduced into routine diagnostics. The first brucellosis polymerase chain reaction-based (PCR-based) test was introduced in 1990 (Fekete *et al.*, 1990). The first species-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 IS711 in the *Brucella* chromosome (Bricker and Halling, 1994). In addition to the commonly used PCR assays, a new Multiplex-PCR assay was developed that specifically identified *B. neotomae*, *B. pinnipedialis*, *B. ceti*, and *B. microti* (Hubber *et al.*, 2009). Furthermore, it differentiated *B. abortus* biovars 1, 2, 4 from biovars 3, 5, 6, 9, as well as between *B. suis* biovar 1, biovars 3 and

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

## **2.8 Treatment of Brucellosis**

*Brucella* strains are intracellular pathogens that infect host macrophages. Hence, the antibiotics to be used for treatment should penetrate adequately into the cell (Kaya *et al.*, 2012; Kiros *et al.*, 2016). A combination of antibiotics should also be used to prevent relapse (Skalsky *et al.*, 2008; Alavi and Alavi, 2013). Thus, tetracyclines, quinolones, trimethoprim/sulfamethoxazole, rifampicin, and streptomycin are commonly used preparations for this treatment. Unfortunately, despite these combinations, the relapse rate is almost 30% (Solera, 2010).

### **2.8.1 Treatment in Animals**

Anon (2002) reported that there is no practical treatment for infected animals. Long-term antibiotic treatment is sometimes successful in infected dogs. However, it is difficult to cure (Ettinger and Feldman, 1995). Also, a concurrent treatment with chlortetracycline and streptomycin has effected some level of cure in sheep, though it is usually not economically feasible except in valuable animals (Khan, 2005). Radostits *et al.* (2003) reported that the treatment of this disease in cattle is generally unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland and the reproductive organs. In general, failure in treatment of brucellosis has been attributed to reasons like the use of incorrect doses of antibiotics, inadequate durations of treatment, high cost of medication, and failure to cure udder infection which could lead to a relapse (Radwan *et al.*, 1992). It is noteworthy that long

term-treatment regimen could lead to antibiotics residues in milk and meat of treated animals which could be passed onto the food chain of humans (Girardi and Odore, 2008; AU-IBAR, 2013).

### **2.8.2 Treatment in humans**

The choice and duration of the antibiotic regimen for treatment of brucellosis depends on the phase and severity of the disease, and the physiologic and medical state of the patient (Solera, 2010). Corbel (1997) reported that, in humans, despite extensive studies over the past 15 years, the optimum antibiotic therapy for brucellosis is still disputed due to several relapse cases. However, the predominant therapeutic regimen is a combination of doxycycline and aminoglycosides (streptomycin or gentamycin) or rifampicin for 6 weeks (Alavi and Alavi, 2013). The duration of therapy in cases with complications, such as endocarditis, bone focusing (spondylitis) and other focal forms, is extended for several months (Alavi and Alavi, 2013). In cases of endocarditis, surgical intervention could be needed to remove the infected valve. The most efficient therapy for infants, small children and pregnant women is yet to be defined. It is, however, advocated that in children under the age of 8, a combination treatment using trimethoprim/sulphamethoxazole (TMP/SMZ) combination with streptomycin or gentamycin presents satisfactory results (Roushan *et al.*, 2006). Alternatively, TMP/SMZ can be administered together with rifampicin or the latter with an aminoglycoside. In cases of pregnancy, positive results have been achieved with the use of TMP/SMZ or rifampicin. Caution is needed with the use of rifampicin as *Brucella* strains seem to acquire resistance to this antibiotic, and this may then offer cross-resistance to TMP/SMZ (Sandalakis *et al.*, 2012). Some researchers claimed that the long-established combination of intramuscular streptomycin with an oral tetracycline gives fewer relapses (Mantur *et al.*, 2006; Al-

Tawfiq, 2014). Alternative treatments were tried using the quinolones, aminoglycosides, streptomycin, gentamycin and rifampicin (Kaya *et al.*, 2012).

## **2.9 Prevention and Control of Brucellosis**

Control of brucellosis in the livestock of pastoralist communities is quite challenging not only because of the number and complexity of risk factors involved, but also because these risk factors are tightly linked and often inherent to their farming practices (Smits, 2013). Migration and contact with other animals on common grazing grounds or at water sources form a major risk for transmission of brucellosis in pastoral livestock, but other factors such as poor farm hygiene, exchange of infected animals and contact with wildlife could also be contributing factors (Munoz *et al.*, 2010). Before a control programme can be developed, a situation analysis and needs assessment need to be performed. A situation analysis is essential, as most endemic countries have little information on the prevalence of brucellosis, its geographical distribution, the major risk factors involved in transmission, and the knowledge, attitudes and practices (KAP) of farmers and livestock owners (Smits, 2013). Strategies for the prevention and control of brucellosis have been severally used and they include surveillance, test and slaughter and vaccination programmes (Smith, 2013). Others include quarantine, livestock movement control, publicity campaigns and back tracing along with eradication programmes.

### **2.9.1 Surveillance**

The surveillance system determines baseline prevalence, monitors the progress and effectiveness of control programmes, and is crucial for disease monitoring after the cessation of vaccination (Corbel, 2006). Mass vaccination should minimize further spread of the disease, but small foci may persist and post- vaccination surveillance is essential for their early detection (Smits, 2013).

A quick resurgence of brucellosis has been seen in countries where lack of resources, or allocation of the limited resources to other livestock problems, leads to the ending of mass-vaccination programmes. Surveillance is carried out to identify any infected herds not already identified by tracing and investigation of neighbouring properties. It provides an assurance that the infection has not spread to other herds in the immediate neighbourhood. Additional surveillance may be needed to assist the design and implementation of the control strategy. Surveillance for evidence of antibodies to *Brucella* spp in livestock by MRT and other serological testing of high-risk herds is the preferred approach (Poester *et al.*, 2010).

Similarly, MacDiarmid and Hellstrom (1988) reported that the brucellin skin test has been used in several countries and has been found to be cheap and reliable for defining the size and distribution of a brucellosis outbreak and for post-eradication surveillance.

On-farm activities also include examination of production records for evidence of abortions and/or infertility. In addition, accurate livestock census information is essential for the interpretation of epidemiological data and planning (Smits, 2013). To make optimal use of the collected information, a properly designed electronic data storage and analysis system is essential. The use of a Geographical Information System (GIS) could be useful for planning control actions, for monitoring progress, and for localization of problematic areas during vaccination and post-control surveillance (Esri, 2011).

### **2.9.2 Test and culling of reactors**

Serological testing of livestock should be routinely carried out using approved serological tests as in a test and slaughter strategy which was practiced in Australia before the total eradication of the disease from the country in 1989 (AHA, 2005). All animals that react positive to such a test are destroyed or consigned for immediate slaughter at an approved abattoir, and tissues were submitted

for *Brucella* culture. Quarantine time should be long enough to ensure that all breeding animals complete gestation without test evidence of infection (Radostits *et al.*, 2003). Eradication by test and slaughter is not always successful because in some cases latently infected young animals remain serologically negative to standard tests until late into their first pregnancy (AHA, 2005). Corbel (2006) reported that the prevalence of *B. ovis* can be decreased by examining rams before the breeding season and culling rams with palpable abnormalities. However, the palpable lesions are not always found in all infected rams and therefore laboratory testing of rams should also be considered. Test and removal methods directed at rams can eradicate this organism from a flock. Also *B. ovis* infections in ewes are generally prevented by controlling infections in rams (Corbel, 2006).

### **2.9.3 Quarantine and movement controls**

The greatest risk to brucellosis control is most likely the introduction of infected animals through migration or trade from neighboring non-vaccinated areas or countries (Smits, 2013). Unless strict measures are taken to control trade and prevent introduction of potentially infected animals, it will be necessary to continue vaccinating susceptible animals (Corbel, 2006). To minimize the risk of reintroduction from neighboring unvaccinated areas, a region-wide approach would be instituted. Quarantine and movement controls contributed immensely to brucellosis eradication in France, Germany, Norway, Sweden, The Netherlands, Japan, Canada, Australia, New Zealand and Australia, as immediate quarantine was placed on all suspicious herds/flocks with the ultimate aim of containment of any possible infection (Perez-Sancho *et al.*, 2015). These countries have thus been declared Officially Brucellosis Free (OBF) (OIE, 2009). This was followed by sero-monitoring of such herds/flocks to confirm their status with regards to *Brucella* infection (AHA, 2005; Perez-

Sancho *et al.*, 2015). Movement of latently infected animals presents the greatest risk, and that the potential for movement of infected material by dogs or birds should not be ignored (Kaltungo *et al.*, 2013).

#### **2.9.4 Vaccination**

The mainstay of control of brucellosis is mass vaccination and it is considered to be cost effective and cost–benefit analysis may help to convince policy-makers of the need to control the disease and provide funds (Smits, 2013). Vaccination is an extremely important and effective facet of most control strategies for brucellosis (Corbel, 2006). It however has the disadvantage of confusing diagnosis by stimulating the production of antibodies notably IgG<sub>1</sub> which is detectable by some serological tests (Poiester *et al.*, 2010). Thus, antibody titres may persist for a prolonged period in a small proportion of vaccinated animals and this proportion increases with age at vaccination (Cheville *et al.*, 1994). However, Nicoletti (1990) and AHA (2005) reported that live attenuated vaccines have up till now proved superior to inactivated products for the prevention of animal brucellosis because of their high immunogenicity.

*Brucella abortus* S19 and *B. melitensis* Rev.1 vaccines have proven to be effective against *B. abortus* in cattle and *B. melitensis* and *B. ovis* in sheep and goats, respectively (Nicoletti, 1990; Elberg, 1996). Both vaccines have the disadvantages of causing abortion in a proportion of pregnant animals, and of being pathogenic for humans (Nicoletti, 1990). Similarly, AHA (2005) reported that *Brucella abortus* RB51 infection in humans is possible but has not been documented. *Brucella melitensis* Rev.1 has also been evaluated for the vaccination of cattle in countries where *B. melitensis* infection in sheep and goats is widespread (Banai, 2002). Experimental studies have shown that *B. melitensis* Rev.1 vaccine provided immunity to *B. melitensis* equal to or superior

than the immunity induced by *B. abortus* S19 with a lower vaccine dose in cattle. Despite these results, the use of *B. melitensis* Rev.1 in this species of animals has been very limited (Nicoletti, 1990).

It was reported that since 1971, a live attenuated smooth strain of *B. suis* biovar 1 strain 2 has been used as an oral vaccine to control brucellosis in cattle, sheep, goats and pigs in China. Further, it is safe when administered orally, and does not induce persistent antibody titres (Xin, 1986).

Although the attenuated S2 strain of *B. abortus* also gave a satisfactory protection rate in cattle (Di *et al.*, 2016), its efficacy against experimental *B. melitensis* infection in pregnant ewes or against *B. ovis* infection in rams was inferior to that of *B. melitensis* Rev.1 vaccine (Blasco, 1990; Verger *et al.*, 1995). It has also reported that currently, the live attenuated *B. melitensis* Rev.1 vaccine appears to be the most generally effective vaccine strain available for the prevention of animal brucellosis (Blasco, 1990; Verger *et al.*, 1995).

*Brucella abortus* strain 45/20 vaccine is normally administered as two doses, given 6 to 12 weeks apart, followed by an annual booster. In February 1996, a new attenuated vaccine, *Brucella* strain RB51, was licensed by the United States Department of Agriculture, Animal and Plant Health Inspection Service for use in cattle in the USA (AHA, 2005). This vaccine does not stimulate the production of antibodies detectable in standard diagnostic tests but does stimulate production of other antibodies that can be detected with special assays like competitive ELISA which indicates that the animal has been previously vaccinated (Adams, 1990). Corbel (2006) reported that vaccination against *B. ovis* is being practiced in New Zealand and some other countries, but not in the U.S.A. and also it was difficult to successfully develop vaccines for pigs.

### **2.9.5 Zoning and tracing**

If a disease is endemic in only a part of a country, it is possible to establish diseased and disease-free zones. Tight control on the movement of animals between the zones will therefore have to be enforced (Smits, 2003; AHA, 2005). When an infection is suspected or confirmed, trace-back and trace-forward of animal movements is essential to identify the index case and other potentially infected or exposed herds (AHA, 2005).

### **2.9.6 Depopulation of infected herds**

Animals that are confirmed to be infected and are close to parturition or having vaginal discharges pose a disease risk to personnel and are therefore preferably depopulated and disposed of immediately and should be burnt and/or buried (AHA, 2005). Parkinson (2009) reported that the infection level is at its peak 4 days before calving or abortion to 14 days afterwards in cattle. However, depopulation has serious economic impacts such as the institution of compensation from government which is an important incentive to ensure that owners promptly report any evidence of infection. Positive reactors should be sent to abattoirs for slaughter but this may pose a risk to handlers. Positive reactors must be identified and be transported in isolation from other animals. Cleaning of vehicles after unloading is highly recommended (AHA, 2005).

### **2.9.7 Decontamination of premises**

Decontamination can be achieved with the aid of sunlight, high temperatures and alkaline disinfectants like quaternary ammonium compounds, sodium dichloroisocyanurate and potassium monopersulphate/sodium dichloroisocyanurate (AHA, 2005; Jong-Hyun, 2009). Other measures to reduce the likelihood of environmental survival of infective bacteria include draining wet areas and ploughing of the soil to improve the rate of dessication of the organism. The spread of infection

can also be minimized by cleaning and disinfecting vehicles used to transport infected and suspect animals (AHA, 2005).

### **2.9.8 Handling of animal products and by-products**

The fact that raw milk and direct contact with infected animals and their materials and fluids are the major risks for transmission of brucellosis, precautions are usually taken in the handling and disposal of such materials (Smits, 2003). This involves the wearing of protective clothing when dealing with infected animals (e.g. when assisting with lambing or handling abortion materials). Milk for human consumption must be pasteurized (AHA, 2005).

### **2.9.9 Re-stocking of depopulated herd/flock**

Restocking of a decontaminated herd/flock is carried out after 30 days during the hot season and slightly longer in the cold season as this assures minimal risk of reinfection because *Brucella* organisms are rapidly inactivated by dessication and sunlight (AHA, 2005). Corbel (2006) suggested that animals for restocking or replacement should come from brucellosis-free areas or accredited herds. He further stated that animals from other sources should be isolated and screened before adding them to herds.

### **2.9.10 Control of brucellosis in wildlife**

Brucellosis in elk and bison in the early 20th Century was suspected to have originated from cattle (Meagher and Mayer, 1994). These wildlife species in the Greater Yellowstone Area of the Western United States are believed to be the remaining reservoirs for bovine brucellosis in the United States (White *et al.*, 2013). Infection with *Brucella* spp. have been documented worldwide

in a large number of terrestrial wildlife species and marine mammals. *Brucella abortus* has been isolated from African buffaloes (*Syncerus caffer*), eland (*Taurotragus oryx*), blue wildebeest (*Connochaetes taurinus*) hippos (*Hippopotamus amphibius*) and waterbuck (*Kobus ellipsiprymnus*) while *B. melitensis* rarely occurs in wildlife (it has been reported in Europe in chamois (*Rupicapra rupicapra*) and ibex (*Capra ibex*)) and recently in sable antelope (*Hippotragus niger*) in South Africa (Waghela and Karstad, 1986; Bengis et al., 2002). *Brucella ovis* and *B. canis* are seldom reported in wildlife. These reports highlights the need for establishment of specific wildlife free or restricted zones which may help to prevent contact with livestock and transmission of disease (Smits, 2013). Similarly, the control of brucellosis in livestock could result in a reduction of brucellosis in wildlife since some scavengers like crows and vultures, though may rarely get infected, can serve as mechanical vectors by moving abortion materials from an infected animal away from abortion sites (Serrano *et al.*, 2011).

## **2.10 Implications of brucellosis**

Brucellosis has been described as a disease with numerous implications which may be attributable to man, livestock and wildlife (McDermott *et al.*, 2013). The epidemiology of the disease and its effect on the economy may vary due to difference in geographical locations and the system of livestock management adopted (McDermott and Arimi, 2002; Perry *et al.*, 2002).

### **2.10.1 General economic implications of brucellosis**

Economic impacts due to brucellosis vary depending on the main livestock species affected, management systems, and on the capability of the country's Veterinary and Medical systems

(McDermott and Arimi, 2002; Perry *et al.*, 2002). In low-income countries, brucellosis is endemic and neglected, with large disease and livelihood burdens in animals and man and almost no effective control measures in place (McDermott and Arimi, 2002). The middle-income countries of tropical Africa and Asia tend to report the greatest number of outbreaks and animal losses. However, most data and evidence on the economic burden of brucellosis and the benefits of its control are from high-income and middle-income countries (McDermott *et al.*, 2013).

All data suggest that worldwide economic losses due to brucellosis are extensive not only in animal production (reduced milk, abortion and delayed conception), but also in public health (cost of treatment and productivity loss) (Acha and Szyfres 2003; McDermott *et al.*, 2013). For example, official estimates put annual losses due to bovine brucellosis in Latin America at approximately \$600 million (Acha and Szyfres 2003). Although brucellosis eradication programmes can be very expensive, they are estimated to save \$7 for each \$1 spent on eradication (Acha and Szyfres 2003).

### **2.10.2 Economic implications of brucellosis in animals**

Historically, official reports of estimates of brucellosis burden have been relatively rare from tropical Africa and Asia as compared to temperate regions of Asia and Europe (McDermott *et al.*, 2013). Most of the few available reports are those of cattle brucellosis probably due to the economic losses associated with low production in this species (Genc *et al.*, 2005; Buhari, 2014; Bertu, 2014). Generally, higher productivity losses are positively correlated with higher prevalence. Seropositive animals have higher rates of abortion, stillbirth, infertility and calf mortality, as well as reduced growth and longer calving intervals (McDermott *et al.*, 2013). A study by Matope *et al.* (2011) in Zimbabwe showed an association between seropositivity and abortions: around one fifth of cows may abort where seroprevalence is high (>30%) compared to less than 5% of cows in low-prevalence (<5%) areas or non-affected herds. Similarly, McDermott

*et al.* (2013) reported that in high-income countries aborting dairy cows produced 20% to 25% less milk for that season, while seropositive non-aborting cows produced 10% below potential. Economic production losses of bovine brucellosis are reasonably consistent across a range of production systems in Africa, with losses estimated at 6% to 10% of the income per animal (Mangen *et al.*, 2002). Economic losses recorded in Argentina at the end of the last century were estimated at US\$60 million per year or US\$1.20 per bovine when the prevalence was around 5% (Samartino, 2002), and in Nigeria losses were estimated at US\$575,605 per year or US\$3.16 per bovine (prevalence 7% to 12%) (Ajogi *et al.*, 1998). Similarly, local researchers estimated that brucellosis caused approximately 20% financial losses in traditional systems of cattle production in one Nigerian grazing reserves (Ajogi *et al.*, 1998). Though reports on productivity losses resulting from small ruminant brucellosis are less well documented in tropical Asia and Africa (McDermott *et al.*, 2013), one study in India estimated the annual economic loss at US\$21.1 and US\$ 37.9 per infected sheep and goat respectively (Sulima and Vencatarrhraman, 2010).

### **2.10.3 Public health implication of brucellosis**

Infection of livestock with *B. abortus*, *B. melitensis*, or *B. suis* poses a significant health risk for transmission to humans by direct contact with infected animals, animal materials and fluids or from consumption of unpasteurized milk and other dairy products (Olsen and Samartino, 2009). The disease in man is highly debilitating, though not considered to be fatal (Falade, 1974). Most human cases are occupational and occur in farmers, veterinarians, and butchers (Radostits *et al.*, 2003). Although there has been great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and, consequently, transmission to the human population frequently occurs (McDermott *et al.*, 2013). Economic losses caused by the disease in humans are a consequence of the cost of hospital treatment, cost of

drugs, patient out-of-pocket treatment expenses, and loss of work or income due to illness (McDermott and Arimi, 2002).

### **2.11 Participatory epidemiology (PE)**

Participatory epidemiology is a developing branch of Veterinary Epidemiology that involves a systematic use of participatory approaches and methods to solve epidemiological problems and provide options for animal disease control (Chambers, 1983; McCracken *et al.*, 1988). It is a practical approach to epidemiology that gives stakeholders a prodigious opportunity in shaping programmes for public health, animal health, disease surveillance and research (Loewenson, 2004). Participatory epidemiology recognises that local people have very rich and detailed knowledge about the animals they keep and the infectious and zoonotic diseases that can gravely affect their livelihoods and endanger human health. This body of knowledge has been termed ‘existing veterinary knowledge’ (EVK) (Mariner and Paskin, 2000). It is based on oral tradition, shared information and life experience of individuals (Catley and Mariner, 2002). Existing veterinary knowledge is specific to each community and utilizes local language terms, but are often able to describe common clinical presentations, epidemiological patterns of disease, principal pathological lesions, vectors and reservoirs using a vocabulary of specific disease terms in local languages that correspond to Western clinical case definitions in the developing world where laboratory data is limited, intelligence derived from participatory studies often provides a more accurate description of disease status (Mariner and Berhanu 2002). Similarly, when integrated with targeted laboratory studies, participatory data provide the context for realistic interpretation of laboratory results as reported by Mariner and Berhanu (2002). Participatory epidemiology learns from local knowledge, leading to disease control programmes that are both acceptable to the stakeholders and effective. The PE approach was developed to overcome the constraints in

applying conventional epidemiology and formal research in developing countries (Jost *et al.*, 2007). Conventional epidemiology can be expensive and logistically complex, producing large quantities of information from formal surveys that are often biased spatially, behaviourally and logistically and may only be confirmed by further research (Chambers, 1983). Jost *et al.* (2007) reported that researchers generally do not understand the local context, thus quantitative information is often misinterpreted.

### **2.11.1 History of participatory epidemiology**

In the early 1970s, it was realized by researchers that the formal system of inquiry was of limited value when working with rural communities in developing countries (Kearl, 1976). Later in the 1970s, they understood that quantitative data approach proved unrealistic in most pastoralist areas with relatively small and mobile human populations, limited infrastructure and frequent insecurity. In addition, most animal health data collection systems and research process have lacked the commitment to feedback information to pastoralist communities which consequently creates frustration among herders and unwillingness to participate in subsequent researches (Catley and Mariner, 2002). Experimentation began with less formal survey tools such as those used in social and medical anthropology, and agrosystem analysis. Experience with indigenous knowledge systems began to merge with field testing of informal interviewing, visualization and other testing methods (Chambers, 1994). This mixing of experiences and new learning involved many individuals and institutions, and culminated in a landmark conference on Rapid Rural Appraisal (RRA) at the University of Khon Kaen, Thailand in 1985 (Chambers, 1994).

As experience with Existing Veterinary Knowledge (EVK) and participatory methods increased, Veterinary field epidemiologists realized that there was tremendous potential in developing

participatory approaches in epidemiology. This was aimed at enhancing disease surveillance, outbreak investigations, and as research tools in a variety of rural and urban settings (Mariner and Paskin, 2000). In view of this, a system called the Rapid Rural Appraisal (RRA) evolved in the 1980s. Rather than collecting quantitative data on problems identified by researchers, RRA focused on farmers' perception of priority problems.

In the late 1980s, RRA evolved into Participatory Rural Appraisal (PRA) or Participatory Learning and Action (PLA). In PRA, data collection and analysis are undertaken by local people, with outsiders facilitating rather than controlling (Catley and Mariner, 2002). By the late 1990s there was increasing use of the methods and the term 'participatory epidemiology' (PE) became more commonly used to describe veterinary applications of PRA-type approaches and methods (Catley, 2000). However, whereas PRA was a multidisciplinary approach to various development problems in rural communities, PE evolved with a focus on livestock diseases (Jost *et al.*, 2007).

Since then, the approach has been extended to a diverse range of communities that include mixed livestock agriculture systems and even peri-urban and intra-urban livestock production systems (Jost *et al.*, 2007).

### **2.11.2 Participatory epidemiology methods**

Participatory approaches are based on open communication and transfer of knowledge, using a "toolkit" of methods guided by some key concepts and attitudes (Catley and Mariner, 2002; Jost *et al.*, 2007). The methods include semi-structured interviews, visualization and scoring and ranking.

### *2.11.2.1 Semi-structured interviews (SSI)*

The semi-structured interview is a fundamental method in the toolkit for PE (Catley and Mariner, 2002). It is more like a guided conversation which is done by the interviewers using checklists of topics to be covered rather than a structured questionnaire. The interviewer introduces a topic using an open-ended question. With a mental note of the key research themes, the interviewer could phrase and rephrase questions, and follow-up interesting and unexpected responses (Pretty, 1995). Semi-structured interviews can be used alone or in combination with other methods.

### *2.11.2.2 Visualization method*

This method was derived from approaches such as agro-ecosystem analysis (Conway, 1985). It is a second important group of participatory methods. This method recognises that certain types of information could not easily be expressed verbally or in writing (Catley and Mariner, 2002). Moreover, construction of diagrams does not necessarily require informants to be literate because objects and signs could be used to depict important features on the diagram. Diagrams could be constructed on the ground or on cardboards. In PE, participatory mapping, seasonal calendars, proportional piling and radar diagrams are examples of visualization methods. Proportional piling and some types of seasonal calendars use piles of counters (such as seeds or stones) and can thus result in numerical recordings. When standardized and repeated with individual informants or groups of informants, data can be summarized and analyzed using conventional statistical tests (Barasa *et al.*, 2008; Jost *et al.*, 2010).

### *2.11.2.3 Ranking and scoring*

Ranking and scoring methods are the third main group of participatory methods and usually required informants to compare different variables using either ranks or scores. Usually, piles of counters such as seeds or stones are used for this approach. Thus along with interviewing and visualization methods, illiterate informants could participate. Scoring methods were more sensitive than ranking, allowing a weighting of responses. The numerical nature of ranking and scoring methods made these methods easy to standardize and repeat, with the data being analyzed using conventional statistical tests (Catley *et al.*, 2009).

### *2.11.2.4 Triangulation*

Triangulation is the use of two or more methods, data sources, observers or investigators, or theories within the same study (Denzin, 2006). It is used as an approach for improving data validity. There are two types of triangulation which include ‘within-method’ and ‘across-method’ triangulation (Catley *et al.*, 2012).

Within-method triangulation is an interview method, in which the researcher crosschecks information provided by an informant during the interview itself. For example, a response during the early stage of an interview might be checked later on using a re-phrased question. This method to some extent, depends on the skill and experience of the researcher.

Across-method triangulation involves the use of two or more different methods to study the same research question. For example, a clinician can combine methods such as clinical examination, laboratory tests and post-mortem examination to reach a diagnosis (Catley *et al.*, 2012).

## 2.12 Geographical Information System (GIS)

New diseases and epidemics spread through the world's population every year. The discipline of medical geographic information systems (GIS) provides a strong framework for our increasing ability to monitor these diseases and identify their causes (Musa *et al.*, 2013). Geographic information system (GIS) is a computerised database management system that allows for the capture, storage, manipulation, analysis, display and reporting of spatial data (Walsh, 1988). Owing to its inherent ability to manage spatial information, it is a tool that can be employed by any discipline that handles data that can be linked with geographical locations, such as countries, regions, communities, or co-ordinates. Thus, the GIS is about becoming a tool for everyone (Norstom, 2001). It is believed that, advancement in molecular biology and genomics has given several sophisticated tools for rapid and confirmatory diagnosis of many diseases, yet disease surveillance, monitoring and the networking approaches are much more important for implementing effective prevention and control strategies (Deb and Chakraborty, 2012; Dhama *et al.*, 2013). A component of the GIS known as the Distance Inversed Weighted (DIW) rooted on first law of geography by the geographer Tobler (1930), which states that "Everything is related to everything else, but near things are more related than distant things". The DIW refers to the estimation of values at unsampled points based on known values of surrounding points in space (Li *et al.*, 2014).

The GIS can assist in increasing the speed of response in case of emergence of a disease outbreak. The availability of up-to-date data on the location of farms, poultry premises, roads among others prior to an emergency can help in implementing disease control measures faster, surveillance activities including movement control among others (Sanson *et al.*, 1994; Dhama *et al.*, 2013; Islam, 2014).The GIS technology works with four major functional components like input

(digitizing, scanning), storage (data structure), manipulation (analysis, modelling) and output (plot, print, display). Presently, numerous commercial software packages like Manifold, ArcGIS, ArcView, MapInfo among others offer GIS functionality within different computer operating systems.

### **2.12.1 Applications of GIS in epidemiology**

The novel history behind the concept of GIS can be traced to epidemiology. The father of modern epidemiology John Snow, in 1854 during the deadliest cholera outbreak in London that claimed about 600 lives, observed that the cholera cases were distributed in a tight cluster around a public water source located on Broad Street in London (Islam, 2014). He was able to map the occurrence of outbreak and public water sources, and he established a strong relationship between them. This was a major milestone towards the use of spatial data in epidemiology. Thus, traditionally, maps have been used by epidemiologists to present associations between location, environment, and disease (Gesler, 1986). GIS is particularly well suited for studying these associations because of its spatial analysis and display capabilities. Consequently, it can be used to produce maps of disease incidence, prevalence, mortality, morbidity on farm, region, or national levels. It makes the understanding of information easier when visualized on a map. The application of GIS in Veterinary Medicine was first described in 1994 for a Foot-and-Mouth Disease epidemic (Sanson *et al.*, 1994).

Ordinarily, information on diseases tend to be aggregated (from information on each individual herd to municipality or county level) making understanding of the situation difficult. However, with the use of GIS the information can be mapped at the farm level thereby allowing only small parts of the region to be visualised at a time. Also density area of the GIS can be used to create a

grid with a defined cell size which gives each cell in the area a density value of the infected farms (Norstrom, 2001). Therefore, in an epidemic emergency, GIS can provide an excellent tool for identifying the location of the infected farm and all farms at risk within a specified area of the outbreak. Also, buffer zones can be drawn around the infected farms and with a link to tables of the addresses of the farms at risk, the farms can be informed within a short time after a confirmed outbreak. More buffer zones can also be generated around other risk areas or point sources, such as roads where infected cattle have been driven or around market places (Norstrom, 2001). Further, the maps can assist the field veterinarians to plan their work in the current situation, and for the government authorities how to handle a potential outbreak of an infectious disease.

The GIS has been used in the surveillance and monitoring of several diseases and the analysis of disease policy and planning (Tempalski, 1994). It has been used to identify locations of high prevalence and to monitor intervention and control programs in areas of Guatemala for onchocerciasis (Richards, 1993), and also in Africa for trypanosomiasis (Roger and Williams, 1993). GIS was used in designing a national surveillance system for the monitoring and control of malaria in Israel (Kitron *et al.*, 1994). The regression analysis of the system can be used to locate areas where certain diseases are likely to occur (Norstrom, 2001). Thus GIS and Geographical Positioning System (GPS) provide powerful tool for the analysis and display of areas of high disease prevalence and the monitoring of on-going control efforts. The marrying of GIS and GPS enhances the quality of spatial and non-spatial data for analysis and decision making by providing an integrated approach to disease control and surveillance at the local, regional, and/or national level (Norstrom, 2001).

## CHAPTER THREE

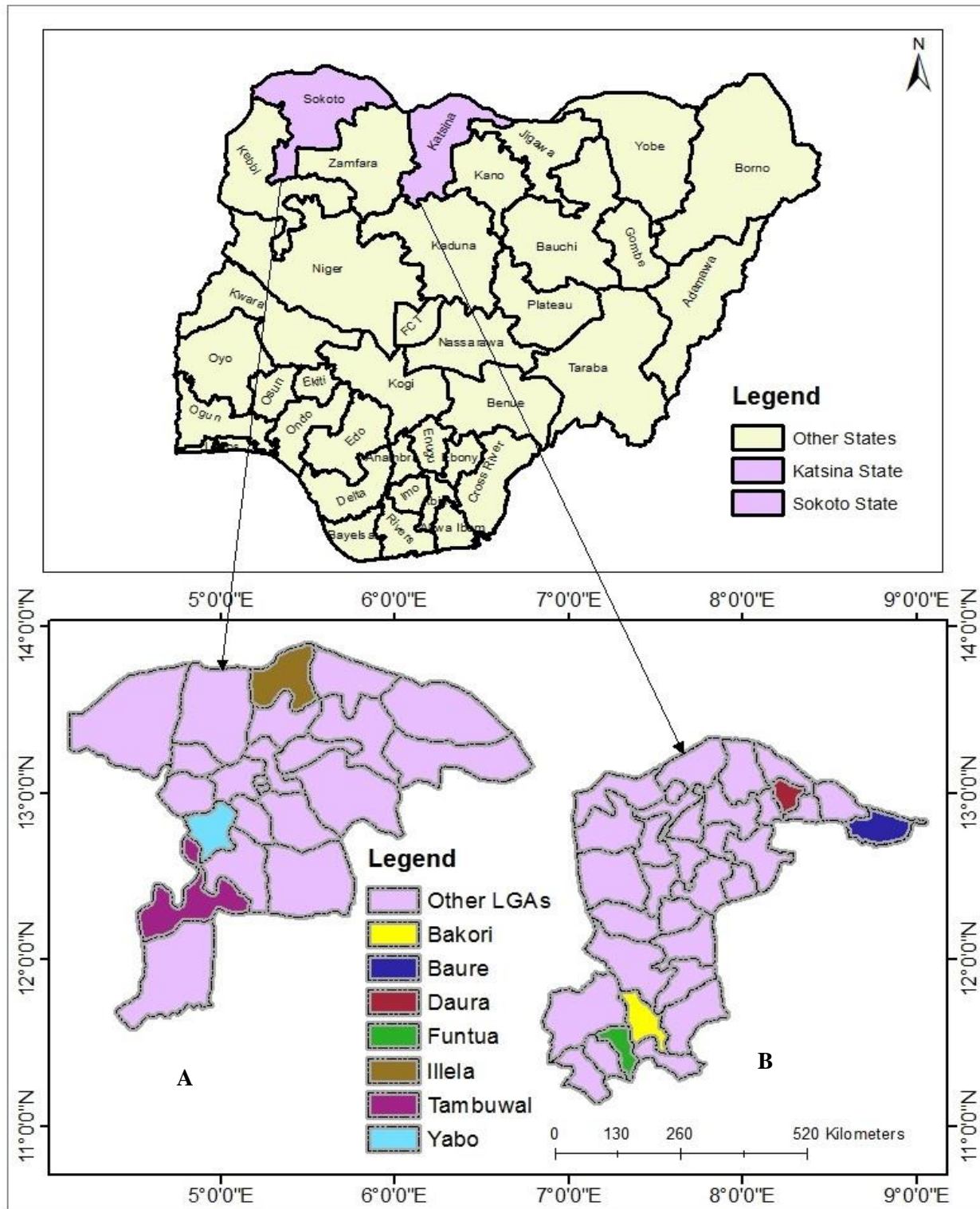
### 3.0 MATERIALS AND METHODS

#### 3.1 Study Area

This study was conducted in Katsina and Sokoto States that are located in the North-West geopolitical Zone of Nigeria. Katsina State is located between latitudes  $11^{\circ} 20'$  and  $13^{\circ} 20'$  N and longitudes  $6^{\circ} 45'$  and  $8^{\circ} 15'$  E. It shares its Northern border with Maradi province in the Niger Republic and Kaduna State to the South, Jigawa and Kano States to the East and with Zamfara State to the West (Mohammed and Danjuma, 2014) (Fig.3.1). The vegetation is the Sudan Savannah type in the Eastern part and Sahel in the Northern part. The State is made up of 34 Local Government Area (LGAs) and occupies a land mass of about 23,850 square kilometres with a human population of about 5,801,586 (NPC, 2006).

Sokoto State is located between longitudes  $11^{\circ} 30'$  and  $13^{\circ} 50'$  E and latitudes  $4^{\circ}$  and  $6^{\circ} 40'$  N (Figure 3.1). It shares common border with the Tahowa Province of Niger Republic to the north, Kebbi State to the west and South, Zamfara State to the east and south (SERC, 2005). The State also falls in the Sudan Savannah in the Southern part and Sahel in the Northern part. The State comprises of 23 LGAs and occupies a land mass of about 25,973 square kilometres with a human population of about 3, 696,999 (NPC, 2006).

A large percentage of the population in these two states are involved in arable farming and livestock rearing as full or part time occupation (NPC, 2006). Annual mean populations of goats and sheep in Katsina and Sokoto States are 2,079,178, 1,696,461, and 2,466,484, 2,566,245 respectively (RIM, 1992).



**Figure 3.1: Map of Nigeria highlighting Sokoto (A) and Katsina (B) States and the LGAs sampled in each of the two States**

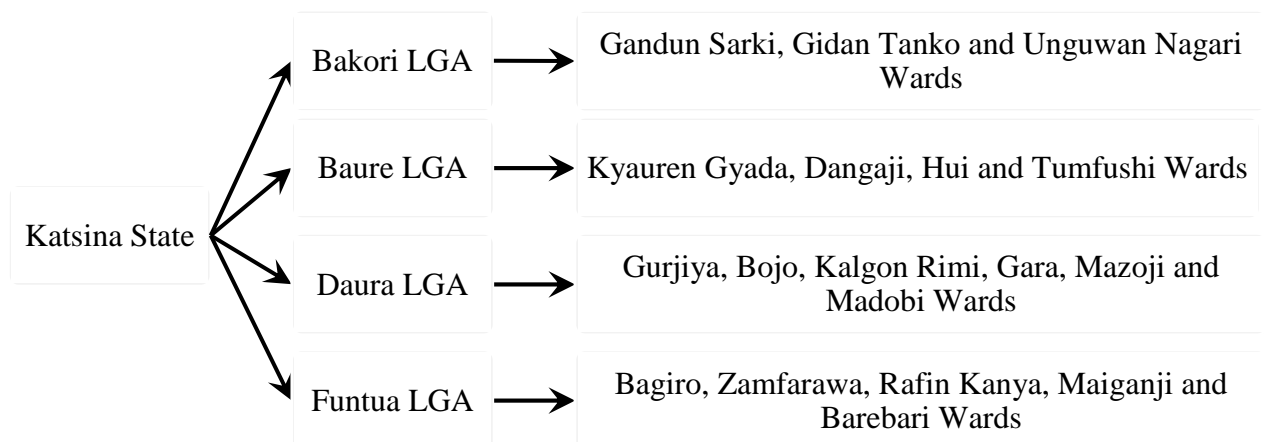
## 3.2 Methodology

### 3.2.1 Study design

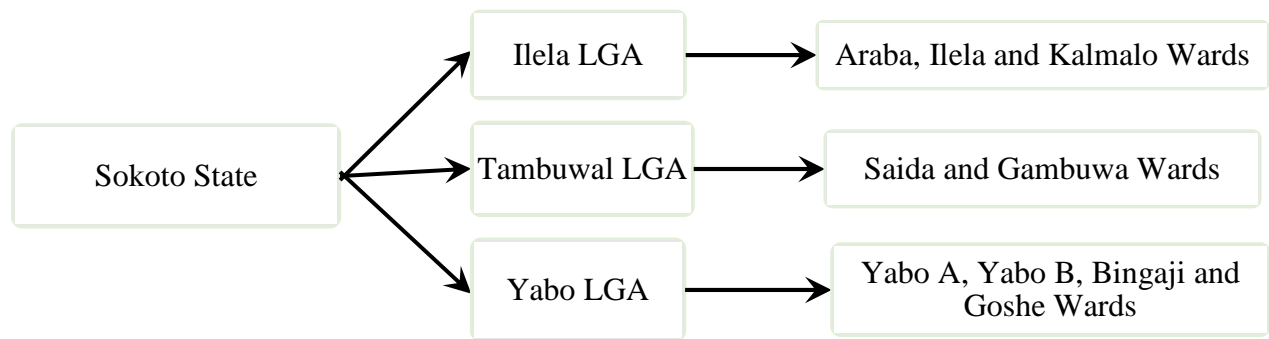
A cross-sectional study was carried out to determine the serological prevalence of *Brucella* infection in sheep and goats in Katsina and Sokoto States. Cluster sampling technique was used where each Local Government Area (LGA) was regarded as a cluster.

### 3.2.2 Study Location

The study was carried out in Katsina and Sokoto States located in the North-West Geopolitical Zone of Nigeria. Local Government Areas and Wards sampled were selected by random sampling without replacement. Ten per cent of the total number of LGAs and 50% of the Wards present in each LGA were selected for sampling. Therefore, 4 LGAs were used in Katsina State and 3 LGAs in Sokoto State, with 18 and 9 Wards being sampled from Katsina and Sokoto States, respectively. (Figs. 3.2 and 3.3).



**Figure 3.2:** Sampled LGAs and Wards in Katsina State



**Figure 3.3: Sampled LGAs and Wards in Sokoto State**

The locations of the flocks, ages (dentition based), breeds and sexes of the animals sampled were also noted and the geo-coordinates of the locations taken. The herds involved in the study were selected based on the willingness of the owners to participate in the study.

### 3.2.3 Sources of sample

Blood samples for sera were collected from sheep and goats from selected Wards and LGAs of Katsina and Sokoto States, Nigeria along with the geo-coordinates of such Wards. Information from focused group interviews were also obtained from selected Wards.

### 3.2.4 Sample size

The total population of sheep and goats in each state under study was obtained from RIM (1992). The number obtained for each small ruminant species was divided across the number of LGAs present in each state. Ten per cent of such population for both species was divided up to the Ward level. However, since the number of available animals for sampling were inadequate in some wards

and the distribution of the species across the Wards were not equal, convenience sampling technique was adopted.

**Katsina State:**

Goat and sheep population 2,079,178 and 1,696,461 respectively (RIM, 1992).

- i. **For goats:** Ten percent of 2,079,178 was divided by the number of LGAs (34) in the state

$$\frac{207,918}{34 \text{ LGAs}} \approx 6115$$

- ii. Population of goats per LGA = 10% of 6,115 = 612
- iii. Population of goats per Ward = 10% of 612  $\approx$  61
- iv. Total sample size for goats = (61  $\times$  18 wards) = 1,098

- i. **For sheep:** Ten percent of 1,696,461 was divided by the number of LGAs (23) in the state

$$\frac{1,696,461}{34 \text{ LGAs}} \approx 4990$$

- ii. Population of sheep per LGA = 10% of 4,990 = 499
- iii. Population of sheep per Ward = 10% of 499  $\approx$  50
- iv. Total sample size for sheep = (50  $\times$  18 wards) = 900

### **Sokoto State:**

Goat and sheep population 2,466,484 and 2,566,245 respectively (RIM, 1992).

- i. **For goats:** Ten percent of 2,466,484 was divided by the number of LGAs (23) in the state

$$\frac{246648}{23 \text{ LGAs}} \approx 10724$$

- ii. Population of goats per LGA = 10% of 10724 = 1072  
iii. Population of goats per Ward = 10% of 1,072  $\approx$  107  
iv. Total sample size for goats = (107  $\times$  8 wards) = 856

- i. **For sheep:** Ten percent of 2,566,245 was divided by the number of LGAs (23) in the state

$$\frac{256625}{23 \text{ LGAs}} \approx 11158$$

- ii. Population of sheep per LGA = 10% of 11158  $\approx$  1116  
iii. Population of sheep per Ward = 10% of 1116  $\approx$  112  
iv. Total sample size for sheep = (112  $\times$  8 wards) = 896

### **3.2.5 Evaluation of small ruminant diseases using Participatory Epidemiology (PE)**

Selection of districts was risk-based with those with the highest recorded sero-prevalence rates for *B. melitensis* as obtained by cELISA being involved. Thus, the PE interview was conducted after concluding the laboratory investigations of the serum samples. Participatory disease surveillance teams of 3 in each of the States under study were formed prior to the commencement of the study.

The teams consisted of an interviewer/facilitator, a livestock officer-in-charge of the LGA in question who was saddled with the responsibility of a key informant and recorder in each State under study and the Veterinarian in charge of the LGA in question. Locations and timing of the interviews were arranged prior to the visit by the key informants. Respondents/participants were selected among members of the Community where blood samples were collected. Such respondents comprised mostly of small ruminant keepers of different age groups. Interviews were conducted in one district from each LGA sampled in Katsina and Sokoto States. Sensitization of farmers was carried out prior to the interview. A semi-structured interview was carried out with the focus groups using a checklist of guided conversations (Appendix 2).

Relevant published and unpublished information regarding the sample sites, data regarding the socio-cultural background of the participants and the Geographical Positioning System (GPS) location of all study sites were taken. The participants were enlightened on the aim of the study prior to commencement of the discussions.

#### *3.2.5.1 Field work and use of participatory epidemiology tools*

Visits to villages were scheduled in line with the convenience of all participants. Each focus group comprised of an average of 10 participants. Of all the PE tools, those that best suited the information sort were used. A combination of tools were used in some villages with probing questions to ascertain consistency where necessary. Open ended interviews were conducted and its consistency was maintained with the aid of a check-list as described by Mariner and Roeder (2003).

### 3.2.5.2 *Semi structured interview (SSI)*

Interviews were conducted in ‘Hausa’ language which happened to be the language understood by all in the villages visited. Group discussion began with the introduction of the team to the participants after which they were asked to mention major constraint to livestock production in their villages. They were further requested to list the different types of livestock species kept with the economic importance of each. The prevailing diseases affecting small ruminants in their villages and description of the signs attributed to each disease were also investigated. They were further asked to describe local treatment and preventive measures against the diseases listed with emphasis on brucellosis where adequately described.

### 3.2.5.3 *Simple Ranking*

Participants in the villages visited were asked to list and rank:

- a. Livestock species they kept based on population
- b. Problems militating against livestock production
- c. Major diseases affecting livestock in general and small ruminants in particular

The listed items were written individually on pieces of cardboard paper (63.5×52.2 cm), after which a representative of the group was asked to rank them in order of importance, starting from the most important. Time was given for all the participants to come to a consensus before the information was recorded.

#### 3.2.5.4 *Proportional pilling*

In using this tool, participants were asked to list major animal species kept in the village. The method described by Catley *et al.* (2009) was used. This involved drawing of circles on a cardboard paper corresponding to the number of animal species mentioned, after which a sketch of each animal species was drawn in each of the circles. One hundred maize grains were provided as counters for which they were asked to pile relative population of each species. Time was given to allow for agreement among all participants on the distribution of counters before results were recorded.

#### 3.2.5.5 *Matrix scoring*

This tool was applied based on the method described by Catley *et al.* (2001). The tool was used to evaluate the perception of the participants on indicators (clinical signs) of major diseases of small ruminants. The diseases involved in this tool were those obtained in the SSI. A matrix was drawn on a cardboard paper (63.5×52.2 cm) with diseases written in their local names on the horizontal axis (x-axis) with their major clinical signs (indicators) written on the vertical axis (y-axis). Using a total of 30 maize grains as described by (AFENET, 2011), participants were asked to score the signs, on how common a particular sign is attributable to a disease.

#### 3.2.5.6 *Transect walks*

Transect walk was done across each village after the other activities. It was used to make direct observation of the village settlement and to make informal interviews with other rural dwellers not involved in the initial interviews. Information on the community life, management/production

systems and farming activities were observed and recorded. Clinical cases of diseases were observed and pictures were taken where necessary.

### **3.2.6 Sampling procedure**

#### *3.2.6.1 Blood sample collection and storage*

Five millilitres of blood were collected from each animal sampled through a jugular venipuncture using a 10 ml syringe and 21G needle after proper restraint by an assistant and the area around the jugular furrow was disinfected using 70% alcohol. The blood was then transferred into a 10 ml clean non-EDTA sampling bottle. The sampling bottles were labelled according to location, animal species and number while other details like; sex, age and breed of each animal sampled were recorded in a hard cover log book. The bottles were kept at 45° in slanting position under shade for 2 hours to allow for separation of serum from the cellular components of the blood. After separation, the sera were then transferred into 5 ml serum tubes and fully labelled corresponding to the bottle containing the blood as described above. The serum samples were transported in ice-packed containers to the Bacterial Zoonosis Laboratory of the Department of Veterinary Public Health, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria where they were stored in the freezer at -20°C until use (Bertu *et al.*, 2010).

### **3.2.7 Geographic information system (GIS)**

A global positioning system (GPS) receiver (*etrex*<sup>®</sup> Taiwan) was used to take geo-coordinates (latitude and longitude) of all sampling points. These coordinates were then arranged in a Microsoft<sup>®</sup> Excel Spreadsheet and saved as comma separated values (CSV). This was then

imported into the ArcGIS 10.3 (Ver.10.3, ESRI Inc., CA, USA) software and then overlaid on the shapefile of the LGAs which is the base map of the study area. The ‘symbolology tab’ in the ArcGIS was then used in order to map the distribution of *B. melitensis* prevalence by c-ELISA in the area.

### **3.2.8 Laboratory investigation of sera samples**

The stored sera were removed from the freezer and left on the laboratory bench to thaw at room temperature after which they were analyzed using modified Rose Bengal Plate Test (m-RBPT) using *B. abortus* and *B. melitensis* antigens, SAT-EDTA and 2-Mercaptoethanol test (2-MET). Positive samples from the SAT-EDTA were further subjected to Competitive Enzyme Linked Immunosorbent Assay (c-ELISA) for *B. melitensis*. All samples were analysed in batches according to their LGAs and States to ensure proper data collation and analysis.

#### *3.2.8.1 Modified Rose Bengal plate test (m-RBPT)*

The procedure as described by Bale (2008) and modified by Bertu (2014) was carried out using m-RBPT (*B. abortus*) antigen and m-RBPT (*B. melitensis*) antigen sourced from Onderstepoort Biological Products Ltd, South Africa and Central Veterinary Laboratory (CVL), Weybridge, Surrey, U.K. respectively. Briefly, 75 µl of serum were placed on a white ceramic tile and 25 µl of m-RBPT antigen were placed beside it after which they were mixed in a circular manner using an applicator stick and then rocked gently for 4 minutes. A separate applicator stick was used for each sample.

The results of the rocking of the tile were interpreted as follows:

- a) Absence of agglutination as Negative (-).

- b) Presence of any degree of agglutination as Positive (+).

#### 3.2.8.2 Serum Agglutination Test with ethylenediaminetetraacetic acid (SAT-EDTA)

The test was performed using the microtitre technique as described by Brown *et al.* (1981). The SAT antigen was obtained from Onderstepoort Biological Products Ltd, South Africa.

All the 3,777 sera from sheep and goats in Katsina and Sokoto States previously analysed using m-RBPT were further tested using SAT-EDTA to determine the titre levels of *Brucella* antibodies for the purpose of ascertaining the severity of the infection.

#### Preparation of stock solution for SAT-EDTA:

The procedure as described by Brown *et al.* (1981) was used. Phenol saline with EDTA buffer solution containing 5 g phenol crystals, 8.5 g sodium chloride, and 1.8612 g disodium EDTA, dissolved in 100 ml of warm distilled water was prepared. A 1:10 dilution of the concentrated SAT antigen with the prepared buffer was made for each day's work. A 96-well "U bottom" microtitre plate was set up on the bench. Serum vials were labelled and placed on the bench to correspond to the positions of the wells already labelled. A representative entry of the sample details were made in the laboratory record book. Using automatic micropipette 40 µl of the buffer solution were dispensed into the first well and 25 µl into each of the remaining 4 microtitre wells. This was followed by the addition of 10 µl of test serum into the first microtitre well using a fresh disposable pipette plastic tip for each test sample. A twofold serial dilution was done by transferring 25 µl aliquots from the first well up to the fifth well after which 25 µl of the aliquot were discarded from the last well. The content of the working dilution of the SAT antigen were mixed gently and 25 µl

added to each well. Finally, the contents in the microtitre plate were gently mixed by tapping the edges of the plate for 20 seconds. The microtitre plates were then covered using foil paper to prevent evaporation of the contents in the wells and the plates incubated for 20 hours at 37°C in an incubator after which the results were read.

A positive reaction (+) was indicated by a mat of stained cells covering the bottom of the well surrounded by slightly opaque diluents while a negative reaction (-) was indicated by a large button of red stained particles in the centre of the well (button-like), surrounded by clear pinkish diluents. Any well with agglutination titre of 1:40 (equivalent to 67.1 International Unit; I.U.) or greater was regarded as a positive sample.

#### 3.2.8.3 2-Mercaptoethanol test (2-MET)

The procedure described by Bale (2008) was used determine acute and chronic infections due to *Brucella* in the study animals.

Of the 480 serum samples that were positive at titre 1:80 and above (severe infection or strong reactors) from SAT-EDTA, 459 were tested for status of *B. melitensis* from sheep and goats in Katsina and Sokoto States while the remaining 21 were not analysed due to insufficient quantity of the serum samples.

#### Test procedure

A stock solution of 1 molar 2-ME was prepared by adding 6.81 ml 2-ME to 93.19 ml of distilled water. The solution was stored in an amber coloured bottle at 4°C until used. A 0.2 molar solution of 2-ME was made by diluting the stock solution 1:5 with distilled water before use.

A 96-well “U bottom” microtitre plate was set up on the bench. Labelled serum vials were placed on the bench to correspond to the positions of the wells already labelled. A representative entry of the sample details was made in the laboratory record book. Using automatic micropipette, 10 µl of the serum sample was dispensed into the first well using a fresh disposable pipette plastic tip for each test sample, this was followed by addition of 40 µl of 0.85% saline and 50 µl of 0.2 molar stock solution was added (dilution of serum is thus 1:10). Fifty (50 µl) of the 0.85% saline were dispensed in the remaining microtitre wells. The microtitre plate was then incubated at 37°C for 1 hour to ensure homogeneity. A twofold serial dilution was done by transferring 50 µl aliquots from the first well up to the fifth well after which 50 µl of the aliquot were discarded from the last well. This was followed by the addition of 50 µl of *B. abortus* SAT antigen. The microtitre plates were then covered using a foil paper to prevent evaporation of the contents in the wells and the plates incubated for 20 hours at 37°C in an incubator after which the results were read.

#### Interpretation of results

Unchanged or increased titre from SAT-EDTA after 2-mercaptoethanol (2-ME) treatment is indicative of presence of IgG which was indicative of a chronic infection. However, decreases in titre after addition of 2-ME is indicative of presence of IgM which was indicative of an acute infection.

#### 3.2.8.4 *Competitive enzyme linked immunosorbent assay (c-ELISA)*

The c-ELISA test kits were sourced from Central Veterinary Laboratory (CVL), Weybridge, Surrey, United Kingdom (U.K.). For the purpose of analysis for *B. melitensis* antibodies using the c-ELISA, 400 serum samples were selected from the 459 serum samples used for the 2-ME Test by random sampling technique without replacement. The c-ELISA analysis was carried out

according to the manufacturers' instructions and results were read using an ELISA reader (UNIEQUIP® Germany).

Diluting buffer was prepared by adding 5 tablets of phosphate buffered saline (PBS), 500µl phenol red indicator and 250µl of Tween 20 to 500ml distilled water. The pH was maintained between 7.2 and 7.6. (Phenol red will turn yellow below pH of 7.2 and violet above pH of 7.6). The preparation was stored at 4°C. The wash solution was prepared by adding the content of the ampoule of Na<sub>2</sub>HPO<sub>4</sub> and 1ml of Tween 20 to 10 litres of distilled water and stored at room temperature. Conjugate was prepared by adding the content of the ampoule kit (BM40) into 11ml of diluting buffer. The stopping solution was prepared by diluting the content of the ampoule of citric acid with 38ml of distilled water and stored at 4°C. Both positive and negative control samples in the kit were reconstituted with 1ml sterile distilled water. They were then allowed to stand until an even suspension was obtained and then stored at 4°C. The substrate and chromogen Optical Density (OPD) solution was prepared by dissolving one tablet of urea H<sub>2</sub>O<sub>2</sub> in 12ml distilled water after which the OPD tablet was added and mixed thoroughly.

The c-ELISA procedure: The microtitre plate has 96 wells coated with *B. melitensis* antigen. Twelve of the wells are arranged in rows (1-12) while 8 are arranged columns (A-H) (Appendix 1). The diluting buffer was brought to room temperature by warming at 23°C in a water bath. The conjugate concentrate (BM40) was removed from the freezer, hand warmed, mixed thoroughly and diluted to working strength in the warmed diluting buffer according to the instructions on the conjugate ampoule. 20µl of each test serum were added to all wells except those on columns 11 and 12. Twenty microlitres (20µl) of the positive control were added to wells F11, F12, G11, G12, H11 and H2. The same 20µl of the negative control were added to wells A11, A12, B11, B12, C11 and C12. Wells D11, D12, E11 and E12 served as conjugate control. One hundred microlitres

(100µl) of the prepared conjugate solution were immediately dispensed into all wells giving a serum dilution of 1/6. The microtitre plate was then gently hand-shaken for 2 minutes in order to mix the serum and conjugate solution. It was then covered with an aluminium foil and shaken for another 30 seconds, pause, then 10 seconds and subsequently after every 10 minutes for 1 hour. The content of the microtitre plate was then discarded by shaking out and then rinsed 5 times with the wash solution and then dried by gently tapping on an absorbent tissue paper until no more liquid was present. One hundred microlitres (100µl) of the OPD solution were added to all wells and incubated for 15 minutes at room temperature. The plate was gently tapped at intervals of 5 minutes during this incubation. The ELISA reader was switched on and allowed to stabilize for 10 minutes. The reaction was then slowed down by adding 100µl of the stopping solution to all wells. The plate was then read at 450nm using an ELISA reader (UNIEQUIP® Germany).

Interpretation of results: Sample was considered

- a) Positive if optical density (OD)  $\leq$  60% of the mean OD of the 4 conjugate control wells in the plate
- b) Negative if the optical density (OD)  $>$  60% of the mean OD of the 4 conjugate control wells in the plate.

### **3.2.9 Statistical analysis of data**

Data generated were analysed with SPSS version 17.0 (2009) where Chi-square ( $\chi^2$ ) and Fishers exact test (FET) were used to test for association among categorical variables. A multi-variable adjusted logistic regression was carried out using all the variables that were statistically significant at the uni-variable analysis. Statistical significance was set at  $p \leq 0.05$ . Data generated from serological aspects of the study were presented as tables.

Descriptive statistical analysis was used to describe basic features of the data from participatory epidemiology using 3 main statistical approaches that included distribution of data, Central tendency and Dispersion. Also, data were exported to SPSS version 17.0 (2009) where Friedman's Test was used to score responses of the pastoralists and Kendall's coefficient of concordance (W) used to measure levels of agreement among different informant groups. Data were presented as tables, plates and charts. All geographical analyses were done using the ArcGIS software version 10.3 and its extension package ArcView Spatial Analyst (Environmental System Research Institute, Inc. Redlands, USA).

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Study population

Blood samples for serum were collected from a total of 3,843 sheep and goats from both Katsina and Sokoto States. Out of these, 66 (44 sheep and 22 goats) samples were damaged and therefore not analysed. Consequently, a total of 3,777 serum samples were analysed, out of which 2,066 (54.7%) and 1,711 (45.3%) were collected from Katsina and Sokoto States respectively (Tables 4.1). Regarding the species, 1,988 (52.6%) were sheep while 1,789 (47.6%) were goats. One thousand one hundred and ninety five (66.8%) of the goats sampled were of the Kano Brown breed. Similarly, 1311 (56.9%) of the sheep sampled were of the Yankasa breed. Three thousand one hundred and seventy five (84.1%) of the sampled animals were females. With regards to age, animals of all age groups were sampled with those of three years and above 1768 (46.8%) making up bulk of the study population. Details on the characteristics of the study population are presented in Tables 4.1 and 4.2.

#### 4.2 Sero-prevalence of *Brucella* in sheep and goats based on m-RBPT

##### 4.2.1 Sero-prevalence of *B. melitensis* in sheep and goats

Out of the 3,777 sera tested from sheep and goats in Katsina and Sokoto States and analysed for *B. melitensis* antibodies, sero-prevalence rate of 13.5% were obtained for both Katsina and Sokoto States. There was no statistically significant difference ( $p > 0.05$ ) in the sero-prevalence between the two states under study. Various sero-prevalence levels were recorded in all the LGAs sampled, with the highest sero-prevalence 20.0% recorded in Baure LGA and the lowest 8.0% recorded in Funtua LGA all of Katsina State. There was statistically significant difference ( $p < 0.05$ ) in the sero-prevalence distribution by LGA.

Similarly, the sero-prevalence for the two animal species under study were 13.6% for sheep and 13.4% obtained for goats and the difference was not statistically significant ( $p > 0.05$ ) (Table 4.1). With reference to breed, sero-positive results were obtained for all the breeds of animals tested except for the West African Dwarf breed of goats (WADG) where 0.0% was recorded. The highest sero-prevalence of 14.3% was obtained for the Yankasa breed of sheep. This difference was, however, not significant ( $p > 0.05$ ) (Table 4.1).

Regarding the sex of the sampled animals, the sero-prevalence obtained was slightly higher (13.7%) in the females than (12.5%) in their male counterparts. The difference between them was not significant ( $p > 0.05$ ) (Table 4.1).

Based on age, the highest sero-prevalence of 15.5% was obtained for animals aged three years and above while the lowest (11.5%) was obtained in animals less than one year old. This difference between them was found to be significant ( $p < 0.05$ ) (Table 4.1).

The specific sero-prevalence for *B. melitensis* antibodies based on wards was highest (25.2%) in Tumfushi and lowest (4.6%) in Rafin Kanya. There was statistical significant difference ( $p < 0.05$ ) in the distribution of *B. melitensis* sero-prevalence among all the wards sampled (Table 4.2).

#### *4.2.1.1 Risk factor analysis for B. melitensis*

The result of the univariable analysis for exposure risks for *B. melitensis* are summarized in Table 4.3. The risk factors revealed for *B. melitensis* seropositivity were LGA, age and Ward. These same factors still remain significantly associated with infection with *B. melitensis* seropositivity in the final regression model (multivariable analysis) (Table 4.4).

**Table 4.1: Sero-prevalence of *Brucella melitensis* using mordified Rose Bengal Plate Test in sheep and goats based on State, LGA, species, breed, sex and age in Katsina and Sokoto States, Nigeria**

Variable	Level	No. of sera Tested	No. <i>B. melitensis</i> positive (%)	95% C.I. on sero-prevalence	Pearson's Chi-square ( $\chi^2$ )	P-value
State	Katsina	2066	278 (13.5)	12.0 – 15.0	0.002	0.968
	Sokoto	1711	231 (13.5)	11.9 – 15.2		
LGA	Bakori	445	65 (14.6)	11.5 – 18.2	37.713	<0.001
	Baure	531	106 (20.0)	16.6 – 23.6		
	Daura	487	59 (12.1)	9.4 – 15.4		
	Funtua	603	48 (8.0)	5.9 – 10.4		
	Illela	375	50 (13.3)	10.1 – 17.2		
	Tambuwal	405	62 (15.3)	11.9 – 19.2		
	Yabo	931	119 (12.8)	10.7 – 15.1		
	Species	Sheep	1988	270 (13.6)		
Goat	1789	239 (13.4)	11.8 -15.0			
Breed	Balami	20	2 (10.0)	1.2 – 31.7	2.723	0.843
	Kano Brown	1195	164 (13.7)	11.8 - 15.8		
	Sahel (goat)	95	10 (10.5)	5.2 - 18.5		
	Sokoto Red	620	77 (12.4)	9.9 - 15.3		
	Uda	532	69 (12.9)	10.2 – 16.1		
	WAD (goat)	2	0 (0.0)	-		
	Yankasa	1311	187 (14.3)	12.4 – 16.3		
Sex	Female	3175	434 (13.7)	12.5 -14.9	0.636	0.425
	Male	602	75 (12.5)	9.9 -15.4		
Age Group (yrs.)*	Young	829	95 (11.5)	9.4-13.8	6.920	0.031
	Mature	1180	232 (13.1)	11.6 - 14.8		
	Old	1768	182 (15.5)	13.4 -17.6		

\* <1 yr. = Young; 1-3.0 yr. = Mature; >3.0 yr. = Old. ( $p \leq 0.05$ ) regarded as significant

**Table 4.2: Sero-prevalence of *Brucella melitensis* using modified Rose Bengal Plate Test in sheep and goats based on Ward in Katsina and Sokoto States, Nigeria**

Variable	Level	No. of sera Tested	No. <i>B. melitensis</i> positive (%)	95% C.I. on sero-prevalence	Pearson's Chi-square ( $\chi^2$ )	P-value
Ward	Araba	163	20 (12.3)	7.7 - 18.3	75.861	<0.001
	Bagiro	93	6 (6.45)	2.4 - 13.5		
	Barebari	129	9 (6.98)	3.2 - 12.8		
	Bingaji	86	8 (9.30)	4.1-17.5		
	Bojo	81	7 (8.64)	3.6-17.0		
	Dangaji	122	20 (16.39)	10.3-24.2		
	Gambuwa	222	32 (14.4)	10.1-19.7		
	Gandun Sarki	149	32 (21.5)	15.2-28.9		
	Gara	62	6 (9.68)	3.6-19.9		
	Gidan Tanko	145	14 (9.66)	5.4-15.7		
	Goshe	203	27 (13.30)	9.0-18.9		
	Gurjiya	63	7 (11.11)	4.6-21.6		
	Hui	118	28 (23.7)	16.4-32.4		
	Illela	212	30 (14.15)	9.8-19.6		
	Kalgon Rimi	59	7 (11.86)	4.9-22.9		
	Kalmalo	214	21 (9.81)	6.2-14.6		
	Kyaren Gyada	136	19 (13.97)	8.6-21.0		
	Madobi	100	12 (12.0)	6.4-20.0		
	Maiganji	94	16 (17.02)	10.1-16.2		
	Mazoji	122	20 (16.39)	10.3-24.2		
	Rafin Kanya	152	7 (4.60)	1.9-9.2		
	Saida	183	30 (16.39)	11.3-22.6		
	Tumfushi	155	39 (25.16)	18.6-32.8		
	Unguwani Nagari	151	19 (12.58)	7.8-19.0		
	Yabo A	230	29 (12.60)	8.6-17.6		
	Yabo B	198	34 (17.17)	12.2-23.2		
Zamfarawa	135	10 (7.41)	3.6-13.2			

( $p \leq 0.05$ ) regarded as significant

**Table 4.3: Summary of significant exposure variables related to *Brucella melitensis* infection in sheep and goats sampled in Katsina and Sokoto States, Nigeria in the univariable analysis**

Variable	Level	Sero-prevalence	Crude OR	95% C.I. on Crude OR	P-value
LGA	Bakori	14.6	1.17	0.84 – 1.62	<0.001
	Baure	20.0	1.70	1.28 – 2.27	
	Daura	12.1	0.94	0.67 – 1.39	
	Funtua	8.0	0.59	0.42 – 0.84	
	Illela	13.3	1.05	0.74 – 1.50	
	Tambuwal	15.3	1.23	0.89 – 1.72	
	Yabo	12.8			
Age	Young	11.5			0.032
	Mature	13.1	1.167	0.91-1.51	
	Old	15.5	1.409	1.08-1.84	
Ward	Araba	12.3	1.748	0.79-3.88	<0.001
	Bagiro	6.45	0.862	0.30-2.46	
	Barebari	6.98	0.938	0.37-2.39	
	Bingaji	9.30	1.282	0.49-3.39	
	Bojo	8.64	1.182	0.43-3.23	
	Dangaji	16.39	2.451	1.10-5.47	
	Gambuwa	14.4	2.105	0.99-4.44	
	Gandun Sarki	21.5	3.419	1.61-7.26	
	Gara	9.68	1.339	0.46 -3.87	
	Gidan Tanko	9.66	1.336	0.57-3.12	
	Goshe	13.30	1.918	0.810 - 4.10	
	Gurjiya	11.11	1.562	0.57 - 4.32	
	Hui	23.7	3.889	1.710 - 8.41	
	Illela	14.15	2.060	0.97 - 4.37	
	Kalgon Rimi	11.86	1.683	0.61 - 4.66	
	Kalmalo	9.81	1.360	0.62 - 2.98	
	Kyauren Gyada	13.97	2.030	0.91 - 4.55	
	Madobi	12.0	1.705	0.71 - 4.12	
	Maiganji	17.02	2.564	1.11-5.94	
	Mazoji	16.39	2.451	1.098-5.47	
	Rafin Kanya	4.60	0.603	0.22-1.63	
	Saida	16.39	2.451	1.15-5.21	
	Tumfushi	25.16	4.203	2.01-8.80	
	Unguan Nagari	12.58	1.799	0.81-4.02	
	Yabo A	12.60	1.803	0.85-3.83	
	Yabo B	17.17	2.591	1.23-5.45	
Zamfarawa	7.41				

( $p \leq 0.05$ ) regarded as significant

**Table 4.4: Significant factors associated with sero-positivity to *Brucella melitensis* infection in sheep and goats sampled in Katsina and Sokoto States, Nigeria**

Variable	Level	Sero-prevalence	Adjusted OR	95% C.I. on adjusted OR	P-value
LGA	Bakori	14.6	0.617	0.34-1.14	0.001
	Baure	20.0	1.648	0.98-2.77	
	Daura	12.1	0.906	0.49-1.66	
	Funtua	8.0	0.347	0.17-0.73	
	Illela	13.3	0.767	0.45-1.31	
	Tambuwal	15.3	0.859	0.50-1.48	
	Yabo	12.8			
Age	Young	11.5			0.005
	Mature	13.1	1.365	0.97-1.92	
	Old	15.5	1.801	1.26-2.57	
Ward	Araba	12.3			0.005
	Bagiro	6.45	0.916	0.32-2.62	
	Barebari	6.98	0.999	0.39-2.55	
	Bingaji	9.30	0.479	0.21-1.09	
	Bojo	8.64	0.525	0.21-1.31	
	Dangaji	16.39	0.549	0.30-1.00	
	Gambuwa	14.4	0.920	0.53-1.57	
	Gandun Sarki	21.5	2.013	1.08-3.75	
	Gara	9.68	0.547	0.21-1.44	
	Gidan Tanko	9.66	0.773	0.37-1.61	
	Goshe	13.30	0.737	0.43-1.28	
	Gurjiya	11.11	0.673	0.27-1.61	
	Hui	23.7	0.890	0.51-1.56	
	Ilela	14.15	0.844	0.48-1.48	
	Kalgon Rimi	11.86	0.701	0.28-1.77	
	Kalmalo	9.81	0.497	0.28-0.89	
	Kyauren Gyada	13.97	0.495	0.27-0.91	
	Madobi	12.0	0.716	0.33-1.55	
	Maiganji	4.60	2.744	1.18-6.36	
	Mazoji	16.39	0.548	0.12-2.60	
	Rafin Kanya	4.60	0.548	0.12-2.60	
	Saida	16.39	2.222	1.05-4.72	
	Tumfushi	25.16	4.839	2.30-10.19	
	Unguan Nagari	12.58	1.775	0.79-3.97	
	Yabo A	12.60	1.958	0.92-4.17	
	Yabo B	17.17	2.873	1.36-6.06	
	Zamfarawa	7.41			

( $p \leq 0.05$ ) regarded as significant

#### 4.2.2 Sero-prevalence of *Brucella abortus* in sheep and goats

The same 3,777 sera used for m-RBPT analysis were also used to investigate for the presence of *Brucella abortus* antibodies out of which 326 (15.8%) of the 2,066 from Katsina State and 302 (17.7%) of the 1711 sera from Sokoto State were positive. There was no significant difference ( $p > 0.05$ ) in the prevalences between the states (Table 4.5).

Observed sero-prevalence were highest (21.7%) in Baure LGA of Katsina State and lowest (10.3%) in Funtua LGA of the same State. The difference in the sero-prevalence values in the LGAs under study was statistically significant ( $p < 0.05$ ) (Table 4.5).

Sero-prevalence in sheep was 16.7% compared to (16.6%) obtained in goats and the difference was not significant difference ( $p > 0.05$ ) in the sero-positivity based on the species of animals sampled (Table 4.5).

Similarly, the difference in sero-positivity across the breeds in the two states was not statistically significant ( $p > 0.05$ ) with the highest value being 25.3% in the Sahel breed of goats and the lowest 0.0% in the WAD breed of goats (Table 4.5).

The sero-prevalence was slightly higher (16.9%) in the males than in the females (16.6%). The difference was, however, not statistically significant ( $p > 0.05$ ).

As regards to sero-prevalence by age, animals less than 1 year of age had the lowest sero-prevalence of 15.9%, while those within the age bracket of 1 – 3 years old had a sero-prevalence of 16.4% and animals older than 3 years old had a sero-prevalence 17.1%. There was no statistical significant difference ( $p > 0.05$ ) between the sero-prevalence across the age groups (Table 4.5).

With regards to wards, the sero-prevalence of *B. abortus* was highest (29.0%) in Tumfushi Ward of Katsina State and lowest (5.3%) in Maiganji ward of the same State (Table 4.6). There was

statistically significant difference ( $p < 0.05$ ) in the sero-positivity of *B. abortus* antibodies by Ward in the areas sampled.

#### *4.2.2.1 Risk factor analysis for B. abortus*

The result of the univariable analysis for exposure risks for *B. abortus* are summarised in Table 4.7. The risk factors revealed for *B. abortus* seropositivity were LGA and Ward. These same factors still remained significantly associated with infection with *B. abortus* seropositivity in the final regression model. (Table 4.8).

**Table 4.5: Sero-prevalence of *Brucella abortus* using modified Rose Bengal Plate Test in sheep and goats based on State, LGA, species, breed, sex and age in Katsina and Sokoto States, Nigeria**

Variable	Level	No. of sera Tested	No. <i>B. abortus</i> positive (%)	95% C.I. on sero-prevalence	Pearson's Chi-square ( $\chi^2$ )	P-value
State	Katsina	2066	326 (15.8)	14.2 - 17.2	2.364	0.124
	Sokoto	1711	302 (17.7)	15.9 - 19.5		
LGA	Bakori	445	48 (10.8)	8.1 - 14.1	52.540	0.000
	Baure	531	115 (21.7)	18.2 - 25.4		
	Daura	487	101 (20.7)	17.2 - 24.6		
	Funtua	603	62 (10.3)	8.0 - 13.0		
	Illela	375	72 (19.2)	15.3 - 23.6		
	Tambuwal	405	54 (13.3)	10.2 - 17.0		
	Yabo	931	176 (18.9)	16.4 - 21.6		
	Species	Goat	1789	297 (16.6)		
	Sheep	1988	331 (16.7)	15.0 - 18.4		
Breed	Balami	20	1 (5.0)	0.1 - 24.8	10.119	0.120
	Kano Brown	1195	207 (17.3)	15.2 - 19.6		
	Sahel (goat)	95	24 (25.3)	16.9 - 35.2		
	Sokoto Red	620	91 (14.8)	12.0 - 17.7		
	Uda	532	83 (15.6)	12.6 - 19.0		
	WAD (goat)	2	0 (0.0)			
	Yankasa	1311	222 (16.9)	14.9 - 19.1		
Sex	Female	3175	526 (16.6)	15.3 - 17.9	0.052	0.820
	Male	602	102 (16.9)	14.0 - 20.2		
Age Group (yrs.)*	Young	829	132 (15.9)	13.5 - 18.6	0.590	0.745
	Mature	1180	194 (16.4)	14.4 - 18.7		
	Old	1768	302 (17.1)	15.4 - 18.9		

\* <1 yr. = Young; 1-3.0 yr. = Mature; >3.0 yr. = Old. ( $p \leq 0.05$ ) regarded as significant

**Table 4.6: Sero-prevalence of *Brucella abortus* using modified Rose Bengal Plate Test in sheep and goats based on Ward in Katsina and Sokoto States, Nigeria**

Variable	Level	No. of sera Tested	No. <i>B. abortus</i> positive (%)	95% C.I. on sero-prevalence	Pearson's Chi-( $\chi^2$ )	P-value
Ward	Araba	163	23 (14.1)	9.6 – 20.4	91.33	0.000
	Bagiro	93	12 (12.9)	6.9 – 21.5		
	Barebari	129	16 (12.4)	7.3 – 19.4		
	Bingaji	86	13 (15.1)	8.3 – 24.5		
	Bojo	81	23 (28.4)	18.9 – 39.5		
	Dangaji	122	23 (18.9)	12.3 – 26.9		
	Gambuwa	222	30 (13.5)	9.3 – 18.7		
	Gandun Sarki	149	13 (8.7)	4.7 – 14.5		
	Gara	62	9 (14.5)	6.9 – 25.8		
	Gidan Tanko	145	16 (11.0)	6.4 – 117.3		
	Goshe	203	29 (14.3)	9.8 – 19.9		
	Gurjiya	63	12 (19.0)	10.3 – 30.9		
	Hui	118	23 (19.5)	12.8 – 27.8		
	Illela	212	49 (23.1)	17.6 – 29.4		
	Kalgon Rimi	59	17 (28.8)	17.8 – 42.1		
	Kalmalo	214	37 (17.3)	12.5 – 23.0		
	Kyauren Gyada	136	24 (17.5)	11.7 – 25.1		
	Madobi	100	18 (18.0)	11.0 – 27.0		
	Maiganji	94	5 (5.3)	1.8 – 12.0		
	Mazoji	122	22 (18.0)	11.7 – 26.0		
	Rafin Kanya	152	16 (10.5)	6.1 – 16.5		
	Saida	183	24 (13.1)	8.6 – 18.9		
	Tumfushi	155	45 (29.0)	22.0 – 36.9		
	Unguan Nagari	151	19 (12.9)	7.8 – 19.0		
	Yabo A	230	59 (25.7)	20.1 – 31.8		
	Yabo B	198	38 (19.2)	14.0 – 25.4		
	Zamfarawa	135	13 (9.6)	5.2 – 15.9		

( $p \leq 0.05$ ) regarded as significant

**Table 4.7: Summary of exposure variables related *Brucella abortus* infection in sheep and goats sampled in Katsina and Sokoto States, Nigeria in the Univariable analysis**

Variable	Level	Sero-prevalence	Crude OR	95% C.I. on Crude OR	P-value
LGA	Bakori	10.8	0.519	0.369 – 0.730	0.000
	Baure	21.7	1.186	0.911 – 1.544	
	Daura	20.7	1.122	0.854 – 1.476	
	Funtua	10.3	0.492	0.361 -0.670	
	Illela	19.2	1.019	0.751 – 1.383	
	Tambuwal	13.3	0.660	0.474 – 0.918	
	Yabo	18.9			
Ward	Araba	14.1	1.542	0.749 - 3.174	0.000
	Bagiro	12.9	1.390	0.604 -3.199	
	Barebari	12.4	1.329	0.612 – 2.885	
	Bingaji	15.1	1.671	0.735 – 3.801	
	Bojo	28.4	3.721	1.761 – 7.866	
	Dangaji	18.9	2.180	1.051 – 4.524	
	Gambuwa	13.5	1.466	0.736 – 2.921	
	Gandun Sarki	8.7	0.897	0.400 – 2.010	
	Gara	11.0	1.594	0.642 – 3.955	
	Gidan Tanko	14.3	1.164	0.538 – 2.520	
	Goshe	19.0	1.564	0.781 – 3.131	
	Gurjiya	19.5	2.208	0.944 – 5.166	
	Hui	23.1	2.272	1.094 – 4.720	
	Illela	28.8	2.821	1.466 – 5.431	
	Kalgon Rimi	17.3	3.799	1.702 – 8.477	
	Kalmalo	17.5	1.962	1.001 – 3.844	
	Kyauren Gyada	18.0	2.011	0.977 – 4.140	
	Madobi	5.5	2.060	0.957 – 4.433	
	Maiganji	18.0	0.527	0.181 – 1.532	
	Mazoji	10.5	2.065	0.990-4.305	
	Rafin Kanya	13.1	1.104	0.510-2.388	
	Saida	29.0	1.417	0.693-2.896	
	Tumfushi	12.9	3.839	1.967-7.494	
Unguan Nagari	25.7	1.351	0.640-2.852		
Yabo A	19.2	3.238	1.701-6.165		
Yabo B	9.6	2.229	1.138-4.366		
Zamfarawa					

( $p \leq 0.05$ ) regarded as significant

**Table 4.8: Summary of significant exposure variables related to *B. abortus* infection in sheep and goats sampled in Katsina and Sokoto States, Nigeria in the multivariable analysis**

Variable	Level	Sero-prevalence	Adjusted OR	95% C.I. on adjusted OR	P-value	
LGA	Bakori	10.8	0.606	0.334 – 1.101	0.000	
	Baure	21.7	1.722	1.050 – 2.827		
	Daura	20.7	0.926	0.518 – 1.657		
	Funtua	10.3	0.449	0.229 – 0.879		
	Illela	19.2	1.266	0.786 – 2.038		
	Tambuwal	13.3	0.636	0.364 – 1.108		
	Yabo	18.9	1.054	0.835 – 1.752		
Ward	Araba	14.1	0.547	0.317 – 0.942	0.020	
	Bagiro	12.9	1.390	0.604 – 3.199		
	Barebari	12.4	1.329	0.612 – 2.885		
	Bingaji	15.1	0.750	0.377 – 1.492		
	Bojo	28.4	1.803	0.924 – 3.516		
	Dangaji	18.9	0.568	0.321 – 1.005		
	Gambuwa	13.5	1.035	0.582 – 1.842		
	Gandun Sarki	8.7	0.664	0.315 – 1.399		
	Gara	11.0	0.772	0.332 – 1.795		
	Gidan Tanko	14.3	0.862	0.425 – 1.749		
	Goshe	19.0	0.702	0.414 – 1.191		
	Gurjiya	19.5	1.070	0.490 – 2.333		
	Hui	23.1	0.502	0.334 – 1.049		
	Kalgon Rimi	17.3	1.840	0.888 – 3.811		
	Kalmalo	17.5	0.880	0.534 – 1.452		
	Kyauren	18.0	0.524	0.299 – 0.918		
	Gyada					
	Madobi	5.5	0.998	0.501 – 1.985		
	Maiganji	18.0	0.527	0.181 – 1.532		
	Rafin Kanya	10.5	1.104	0.510 – 2.388		
	Yabo A	19.2	1.453	0.916 – 2.304		
	Zamfarawa					

( $p \leq 0.05$ ) regarded as significant

### 4.2.3 Sero-prevalence of single and co-infection with *Brucella* spp in sheep and goats

The sero-prevalence of *B. melitensis* or *B. abortus* (single infection) among sheep and goats at State level analysis indicated 21.1% and 20.6% for Katsina and Sokoto States respectively, while the corresponding co-infection sero-prevalence were 4.1% and 5.0% for Katsina and Sokoto States respectively (Table 4.9). There was no statistical significant difference ( $p > 0.05$ ) in the single and co-infections among the two states.

Similarly, the single infection sero-prevalence for LGAs ranged from 12.9% in Funtua LGA of Katsina State to 29.0% in Baure LGA of the same State. The co-infection sero-prevalence ranged from 2.2% in Tambuwal LGA to 7.7% in Illela LGA all in the same Sokoto State. There was statistical significance difference ( $p < 0.05$ ) in the single and co-infection sero-prevalence between the LGAs sampled (Table 4.9).

The single infection (*B. melitensis* or *B. abortus*) sero-prevalence by animal species indicated 20.6% in sheep and 21.1% in goats. In terms of co-infection (*B. melitensis* and *B. abortus*) sero-prevalence of 4.7% and 4.2% were recorded for sheep and goats respectively. There was no statistical significant difference ( $p > 0.05$ ) in the sero-prevalence among the two animal species by way of single and co-infection (Table 4.9).

With regard to breed single and co-infection with *B. melitensis* and *B. abortus*, the lowest sero-prevalence in animals with single (*B. melitensis* or *B. abortus*) infection was 15.0% in the Balami breed of sheep while the highest sero-prevalence rate of 23.2% was obtained in the Sahel breed of goats. Similarly, the breed co-infection (*B. melitensis* and *B. abortus*) ranged between 0.0% in the Balami breed of sheep to 5.6% in the Uda breed of sheep. There was no statistical significant difference ( $p > 0.05$ ) in the sero-prevalence among the breeds of animals under study based on single and co-infections (Table 4.9).

The results of single and co-infection with *B. melitensis* and *B. abortus* based on sex showed that the sero-prevalence by single infection with either *B. melitensis* or *B. abortus* was 20.9% in the female animals and 20.8% in the males. Also, the sero-prevalence for co-infection with both *B. melitensis* and *B. abortus* was 4.5% in the females and 4.1% in the males. The difference in the sero-prevalence between the sexes was not statistically significant ( $p > 0.05$ ) (Table 4.9).

Regarding single infection (*B. melitensis* or *B. abortus*) with respect to age group, animals less than 1 year old had a sero-prevalence of 20.9% those within the age bracket of 1 and 3 years and above 3 years had a sero-prevalence of 31.4% and 13.7% respectively. Also, for co-infection category (*B. melitensis* and *B. abortus*), a sero-prevalence of 3.0% was obtained in animals less than 1 year of age, while 6.78% and 2.62% were obtained in animals between 1 – 3 years and above 3 years of age respectively. The difference across the different age groups was statistically significant ( $p < 0.05$ ) (Table 4.9).

The sero-prevalence of single infection (*B. melitensis* or *B. abortus*) at Ward level was seen to range from 9.9% in Rafin Kanya ward of Funtua LGA and 35.5% in Tumfushi ward in Baure LGA all in Katsina State.

The sero-prevalence for co-infection (*B. melitensis* and *B. abortus*) in sheep and goats in the study wards was lowest (1.6%) in Barebari Ward of Funtua LGA and Gara Ward of Daura LGA and highest (9.0%) in Tumfushi ward of Baure LGA all in Katsina State. There was no statistically significant difference among the Wards in terms of single and co-infection ( $p > 0.05$ ) (Table 4.10).

#### *4.2.3.1 Risk factor analysis for single and co-infection with *Brucella spp* in sheep and goats*

Regarding the univariable analysis for exposure risks for single and co-infection with *Brucella spp*, the risk factors revealed were LGA, Ward and age (Table 4.11). However, only LGA and age remain significantly associated with single and co-infections in the final regression model (Table 4.12).

**Table 4.9: Sero-prevalence of single and co-infection due to *Brucella melitensis* and *Brucella abortus* using modified Rose Bengal Plate Test in sheep and goats based on State, LGA, species, breed, sex and age in Katsina and Sokoto States, Nigeria**

Variable	Level	No. of sera Tested	Single Infection ( <i>B. melitensis</i> or <i>B. abortus</i> ) (%)	95% C.I. on sero-prevalence	Co – infection ( <i>B. melitensis</i> and <i>B. abortus</i> ) (%)	95% C.I. on sero-prevalence	Pearson's Chi-square ( $\chi^2$ )	P-value
State	Katsina	2066	435 (21.1%)	19.3 – 22.9	84 (4.1%)	3.3 – 5.0	1.716*	0.132*
	Sokoto	1711	352 (20.6%)	18.7 – 22.6	85 (5.0%)	4.0 – 6.1	1.782**	0.182**
LGA	Bakori	445	82 (18.4%)	14.9 – 22.4	16 (3.6%)	2.1 – 5.8	59.594*	0.000*
	Baure	531	154 (29.0%)	25.2 – 33.1	33 (6.2%)	4.3 – 8.6	24.461**	0.000**
	Daura	487	121 (24.8%)	21.1 – 28.9	19 (3.9%)	2.4 – 6.0		
	Funtua	603	78 (12.9%)	10.4 – 15.9	16 (2.7%)	1.5 – 4.3		
	Illela	375	58 (15.5%)	12.0 – 19.5	29 (7.7%)	5.2 – 10.9		
	Tambuwal	405	97 (23.9%)	19.9 - 28.4	9 (2.2%)	1.0 – 4.2		
	Yabo	931	197 (21.1%)	18.6 – 23.9	47 (5.0%)	3.7 – 6.7		
	Species	Sheep	1988	410 (20.6%)	18.9 - 22.5	93 (4.7%)	3.8 – 5.7	0.115*
Goat		1789	377 (21.1%)	19.2 - 23.0	76 (4.2%)	3.4 -5.3	0.734**	0.523**
Breed	Balami	20	3 (15.0%)	3.2 – 37.9	0 (0.0%)	0.0 – 16.8	6.132	0.294
	Kano Brown	1195	259 (21.7%)	19.4 – 24.1	55 (4.6%)	3.5 – 5.9		
	Sahel (goat)	95	22 (23.2%)	15.1 – 33.0	4 (4.2%)	1.2 – 10.4		
	Sokoto Red	620	120 (19.4%)	16.3 – 22.7	23 (3.7%)	2.4 – 5.5		
	Uda	532	89 (16.7%)	13.7 – 20.2	30 (5.6%)	3.8 – 8.0		
	Yankasa	1311	294 (22.4%)	20.2 – 24.8	57 (4.3%)	3.3 – 5.6		
Sex	Female	3175	662 (20.9%)	19.5 – 22.3	144 (4.5%)	3.8 – 5.3	1.647*	0.199**
	Male	602	125 (20.8%)	17.6 – 24.2	25 (4.1%)	2.7 – 6.1	0.213*	0.644**
Age Group (yrs.)*	Young	829	174 (20.9%)	18.3 – 24.0	25 (3.0%)	2.0 – 4.4	2.718*	0.058*
	Mature	1180	370 (31.4%)	28.7 – 34.1	80 (6.78%)	5.4 – 8.4	2.543*	0.280**
	Old	1768	243 (13.7%)	12.2 – 15.4	64 (2.62%)	2.8 - 4.6		

\* <1 yr. = Young; 1-3.0 yr. = Mature; >3.0 yr. = Old. \* = Single Infection; \*\* = co-infection ( $p \leq 0.05$ ) regarded as significant

**Table 4.10: Sero-prevalence of single and co-infection due to *Brucella melitensis* and *Brucella abortus* using modified Rose Bengal Plate Test in sheep and goats based on Wards in Katsina and Sokoto States, Nigeria**

Variable	Level	No. of sera Tested	Single Infection ( <i>B. melitensis</i> or <i>B. abortus</i> ) (%)	95% C.I. on sero-prevalence	Co – infection ( <i>B. melitensis</i> and <i>B. abortus</i> ) (%)	95% C.I. on sero-prevalence	Pearson’s Chi-square ( $\chi^2$ )	P-value
Ward	Araba	163	20 (12.3%)	7.7 – 18.3	11 (6.7%)	3.4 – 11.8	37.355	0.069
	Bagiro	93	12 (12.9%)	6.8 – 21.5	3 (3.22%)	0.7 – 9.1		
	Barebari	129	21 (16.3%)	10.3 – 23.8	2 (1.6%)	0.1 – 5.5		
	Bingaji	86	15 (17.4%)	10.1 – 27.1	3 (3.4%)	0.7 – 9.9		
	Bojo	81	23 (28.4%)	18.9 - 36.5	3 (3.7%)	0.8 – 10.4		
	Dangaji	122	31 (25.4%)	18.0 -34.1	6 (4.9%)	1.8 – 10.4		
	Gambuwa	222	52 (23.4%)	18.0 – 30.0	5 (2.3%)	0.7 – 5.2		
	Gandun Sarki	149	37 (24.8%)	18.1 -32.7	4 (2.7%)	0.7 – 6.7		
	Gara	62	13 (20.9%)	11.7 – 33.2	1 (1.6%)	0.0 – 8.7		
	Gidan Tanko	145	20 (13.8%)	8.6 – 20.5	5 (3.4%)	1.1 – 7.9		
	Goshe	203	40 (19.7%)	14.5 – 25.9	8 (3.9%)	1.7 – 7.6		
	Gurjiya	63	15 (23.8%)	14.0 – 36.2	2 (3.2%)	0.4 – 11.0		
	Hui	118	33 (27.9%)	20.1 – 37.0	9 (7.6%)	3.5 – 34.0		
	Illela	212	38 (17.9%)	13.0 – 23.8	18 (8.5%)	5.1 – 13.1		
	Kalgon Rimi	59	16 (27.1%)	16.4 – 40.3	4 (6.8%)	1.9 – 16.5		
	Kalmalo	214	31 (14.5%)	10.1 – 20.0	12 (5.6%)	3.0 – 10.0		
	Kyauren Gyada	136	35 (25.7%)	18.6 – 33.9	4 (2.9%)	0.8 – 7.4		
	Madobi	100	18 (18.0%)	11.0 – 26.9	6 (6.0%)	2.2 – 12.6		
	Maiganji	94	13 (13.8%)	7.6 – 22.5	4 (4.3%)	1.2 – 10.5		
	Mazoji	122	36 (29.5%)	21.6 – 38.4	3 (2.5%)	0.5 – 7.0		
	Rafin Kanya	152	15 (9.9%)	0.6 – 15.8	4 (2.6%)	0.7 – 6.6		
	Saida	183	45 (24.5%)	18.5 – 31.5	4 (2.2%)	0.6 – 5.5		
	Tumfushi	155	55 (35.5%)	28.0 – 43.6	14 (9.0%)	5.0 – 14.7		
	Unguan Nagari	151	25 (16.6%)	11.0 – 23.5	7 (4.6%)	1.9 – 9.3		
Yabo A	230	57 (24.7%)	19.3 – 30.9	15 (6.5%)	3.7 – 10.5			
Yabo B	198	54 (27.3%)	21.2 – 34.0	9 (4.5%)	2.1 – 8.5			
Zamfarawa	135	17 (12.6%)	7.5 – 19.4	3 (2.2%)	0.5 – 6.4			

( $p \leq 0.05$ ) regarded as significant

**Table 4.11: Summary of exposure variables related to single and co-infection with *Brucella melitensis* and *Brucella abortus* in sheep and goats in Katsina and Sokoto States, Nigeria in the univariable analysis**

Variable	Level	No. of sera Tested	Single Infection ( <i>B. melitensis</i> or <i>B. abortus</i> ) (%)	Co – infection ( <i>B. melitensis</i> and <i>B. abortus</i> ) (%)	Crude OR	95% C.I. on Crude OR	P-value
LGA	Bakori	445	82 (18.4%)	16 (3.6%)	1.223	0.656 – 2.280	0.001
	Baure	531	154 (29.0%)	33 (6.2%)	1.113	0.680 – 1.822	
	Daura	487	121 (24.8%)	19 (3.9%)	1.519	0.852 – 2.711	
	Funtua	603	78 (12.9%)	16 (2.7%)	1.163	0.623 – 2.173	
	Illela	375	58 (15.5%)	29 (7.7%)	0.477	0.276 – 0.825	
	Tambuwal	405	97 (23.9%)	9 (2.2%)	2.571	1.210 – 5.462	
	Yabo	931	197 (21.1%)	47 (5.0%)			
Age (yrs.)*	Young	829	174 (20.9%)	25 (3.0%)			0.050
	Mature	1180	370 (31.4%)	80 (6.78%)	0.665	0.410 – 1.078	
	Old	1768	243 (13.7%)	64 (2.62%)	0.546	0.330 – 0.901	
Ward	Araba	163	20 (12.3%)	11 (6.7%)	0.321	0.77 – 1.342	0.123
	Bagiro	93	12 (12.9%)	3 (3.22%)	0.706	0.121 – 4.114	
	Barebari	129	21 (16.3%)	2 (1.6%)	1.853	0.277 – 12.389	
	Bingaji	86	15 (17.4%)	3 (3.4%)	0.882	0.154 – 5.049	
	Bojo	81	23 (28.4%)	3 (3.7%)	1.353	0.243 -7.546	
	Dangaji	122	31 (25.4%)	6 (4.9%)	0.912	0.202 – 4.114	
	Gambuwa	222	52 (23.4%)	5 (2.3%)	1.835	0.396 – 8.497	
	Gandun Sarki	149	37 (24.8%)	4 (2.7%)	1.632	0.328 – 8.112	
	Gara	62	13 (20.9%)	1 (1.6%)	2.294	0.213 – 24.679	
	Gidan Tanko	145	20 (13.8%)	5 (3.4%)	0.706	0.147 – 3.395	
	Goshe	203	40 (19.7%)	8 (3.9%)	0.882	0.208 – 3.736	
	Gurjiya	63	15 (23.8%)	2 (3.2%)	1.324	0.194 – 9.020	
	Hui	118	33 (27.9%)	9 (7.6%)	0.647	0.155 – 2.708	
	Illela	212	38 (17.9%)	18 (8.5%)	0.373	0.097 – 1.436	
	Kalgon Rimi	59	16 (27.1%)	4 (6.8%)	0.706	0.136 – 3.658	
	Kalmalo	214	31 (14.5%)	12 (5.6%)	0.456	0.113 – 1.842	
	Kyauren Gyada	136	35 (25.7%)	4 (2.9%)	1.544	0.310 – 7.688	
	Madobi	100	18 (18.0%)	6 (6.0%)	0.529	0.114 – 2.460	
	Maiganji	94	13 (13.8%)	4 (4.3%)	0.574	0.109 – 3.022	
	Mazoji	122	36 (29.5%)	3 (2.5%)	2.118	0.386 – 11.604	
	Rafin Kanya	152	15 (9.9%)	4 (2.6%)	0.662	0.127 – 3.446	
	Saida	183	45 (24.5%)	4 (2.2%)	1.985	0.402 – 9.809	
	Tumfushi	155	55 (35.5%)	14 (9.0%)	0.693	0.178 – 2.702	
Unguwan Nagari	151	25 (16.6%)	7 (4.6%)	0.630	0.143 – 2.786		
Yabo A	230	57 (24.7%)	15 (6.5%)	0.671	0.173 – 2.594		
Yabo B	198	54 (27.3%)	9 (4.5%)	1.059	0.257 – 4.362		
Zamfarawa	135	17 (12.6%)	3 (2.2%)				

\* <1 yr. = Young; 1-3.0 yr. = Mature; >3.0 yr. = Old. ( $p \leq 0.05$ ) regarded as significant.

**Table 4.12: Significant Variables associated with single and co-infection with *Brucella melitensis* and *Brucella abortus* in sheep and goats sampled in Katsina and Sokoto States in the multivariable analysis**

Variable	Level	No. of sera Tested	Single Infection ( <i>B. melitensis</i> or <i>B. abortus</i> ) (%)	Co – infection ( <i>B. melitensis</i> and <i>B. abortus</i> ) (%)	Adjusted OR	95% CI on Adjusted OR	P-value
LGA	Bakori	445	82 (18.4%)	16 (3.6%)	1.357	0.721 – 2.553	0.001
	Baure	531	154 (29.0%)	33 (6.2%)	1.06.1	0.646 – 1.743	
	Daura	487	121 (24.8%)	19 (3.9%)	1.538	0.860 – 2.750	
	Funtua	603	78 (12.9%)	16 (2.7%)	1.251	0.666 – 2.349	
	Illela	375	58 (15.5%)	29 (7.7%)	0.496	0.286 - 0.860	
	Tambuwal	405	97 (23.9%)	9 (2.2%)	2.777	1.302 – 5.923	
Age (yrs.)*	Young	829	174 (20.9%)	25 (3.0%)			0.036
	Mature	1180	370 (31.4%)	80 (6.78%)	0.655	0.401 – 1.071	
	Old	1768	243 (13.7%)	64 (2.62%)	0.509	0.303 – 0.852	

\* <1 yr. = Young; 1-3.0 yr. = Mature; >3.0 yr. = Old. ( $p \leq 0.05$ ) regarded as significant

### **4.3 Status (mild or severe) of *Brucella* infection in sheep and goats based on Serum**

#### **Agglutination Test with Ethylenediaminetetracetic acid**

Of the 2066 and 1711 serum samples tested from Katsina and Sokoto States respectively for status of *Brucella* infection, sero-prevalence of 85 (4.11%) and 56 (3.3%) were obtained for Katsina and Sokoto States respectively for mild infections while 232 (11.3%) and 107 (6.3%) were obtained for severe infections from the same states respectively (Table 4.13). The difference in the status of infection between the two states was not statistically significant ( $p > 0.05$ ) but was significant for severe infection ( $p < 0.05$ ).

Based on LGAs, the highest sero-prevalence rate (5.8%) for mild infections was obtained in Daura LGA of Katsina State while the lowest (2.2%) was obtained in Tambuwal LGA of Sokoto State. Similarly, for severe infections, the highest (16.6%) was obtained in Funtua, while the lowest (3.5%) was obtained in Tambuwal LGA (Table 4.13). There was no statistically significant difference in the sero-prevalence across the LGAs tested ( $p > 0.05$ ) but was so for severe infections ( $p < 0.05$ ) (Table 4.13).

With regards to status of *Brucella* infection based on animal species, the sero-prevalence of mild infection in sheep was 3.5% compared to the 4.0% obtained in goats and for severe infections, sero-prevalence of 7.5% and 10.3% were obtained for sheep and goats respectively (Table 4.13). However, these differences were not statistically significant ( $p > 0.05$ ) for mild infections but was significant for severe infection ( $p < 0.05$ ).

The highest sero-prevalence for mild infections based on breed was 5.3% and was obtained in the Sahel breed of goats while the lowest a sero-prevalence of 0.0% was obtained for Balami breed of sheep and WAD breed of goats (Table 4.13). In the case of severe infections, the highest sero-prevalence (12.5%) was obtained in the Kano Brown breed of goats while 0.0% sero-prevalence

was recorded for Balami breed of sheep and WAD breed of goats (Table 4.13). The difference was not statistically significant ( $p > 0.05$ ) for mild infections but was significant for severe infections ( $p < 0.05$ ).

With regards to sex, sero-prevalence for mild infections were 3.9% for female animals and 2.8% for male counterparts (Table 4.13). Similarly, sero-prevalence of 8.9% was recorded in female animals for severe infections and 9.5% was obtained in males (Table 4.13). There was no statistically significant difference in the between both sexes tested ( $p > 0.05$ ) for both mild and severe infections.

Based on age and mild infections, animals of 1 – 3 years and above recorded a prevalence of 4.0% while those below 1 year of age recorded a sero-prevalence of 2.8%. For severe infection and age group, a sero-prevalence of 9.8% was obtained in animals above 3 years of age, 8.2% for animals withing the age bracket of 1 – 3 years of age and 9.4% for animals below 1 year of age. However the differences were not statistically significant ( $p > 0.05$ ) for both mild and severe infections (Table 4.13).

In the Wards sampled, the highest sero-prevalence rate of 9.0% for mild infections was obtained from Tumfushi Ward of Baure LGA and the lowest sero-prevalence rate of 0.0% was recorded in Gandun Sarki Ward of Bakori LGA all in Katsina State. Also, the highest sero-prevalence (30.3%) for severe infections was obtained in Rafin Kanya Ward of Funtua LGA while the lowest (0.8%) was obtained from Hui Ward in Baure LGA, all in Katsina State. There differences were statistically significant ( $p < 0.05$ ) for both mild and severe infections (Table 4.14).

**Table 4.13: Status of *Brucella* infection using Serum Agglutination Test with Ethylenediaminetetracetic acid in sheep and goats sampled in Katsina and Sokoto States, Nigeria based on State, LGA, species, breed, sex and age**

Variable	Level	No. of sera Tested	No. of Mild Infections (%) (1:40 = 67 I.U)	95% C.I on Sero-prevalence	No. of Severe Infections (%) ( $\geq 1:80 = \geq 134$ I.U)	95% C.I on Sero-prevalence	Pearson's Chi-square ( $\chi^2$ )	p-value
State	Katsina	2066	85 (4.11)	3.2 – 5.1	232 (11.3)	9.8– 12.7	1.843**	0.175**
	Sokoto	1711	56 (3.3)	2.5 – 4.2	107 (6.3)	5.1 – 7.6	28.362***	0.000***
LGA	Bakori	445	15 (3.4)	1.9 – 5.5	57 (12.8)	9.8 – 16.3	10.396**	0.109**
	Baure	531	24 (4.5)	2.9 – 6.7	39 (7.3)	5.2 – 9.9	0.109***	0.000***
	Daura	487	28 (5.8)	3.9 – 8.2	36 (7.4)	5.2 – 10.1		
	Funtua	603	18 (3.0)	1.8 – 4.7	100 (16.6)	13.7 – 19.8		
	Illela	375	12 (3.2)	1.7 – 5.5	29 (7.3)	5.2 – 10.9		
	Tambuwal	405	9 (2.2)	1.0 – 4.2	14 (3.5)	1.9 – 5.8		
	Yabo	931	35 (3.8)	2.6 – 5.2	64 (6.9)	5.3 – 8.7		
Species	Sheep	1988	69 (3.5)	2.7– 4.4	154 (7.5)	6.6 – 9.0	0.804**	0.370**
	Goat	1789	72 (4.0)	3.2 – 5.0	185 (10.3)	8.9– 11.8	7.758***	0.005***
Breed	Balami	20	0 (0.0)	0.0-1.7	0 (0.0)	-	11.283**	0.080**
	Kano Brown	1195	53 (4.4)	3.3– 5.8	149 (12.5)	10.6 – 14.5	37.889***	0.000***
	Sahel (goat)	95	5 (5.3)	1.7 – 11.9	7 (7.4)	3.0 – 14.6		
	Sokoto Red	620	21 (3.4)	2.1 – 5.1	35 (5.7)	3.9 – 7.8		
	Uda	534	8 (1.5)	10.1 – 39.2	28 (5.2)	3.5 – 7.5		
	WAD (goat)	2	0 (0.0)	0.0-84.1	0 (0.0)	-		
	Yankasa	1311	54 (4.1)	3.1 – 5.3	120 (9.2)	7.6 – 10.8		
Sex	Female	3175	124 (3.9)	3.3– 4.6	282 (8.9)	7.9 – 9.9	1.647**	0.213**
	Male	602	17 (2.8)	1.6– 4.5	57 (9.5)	7.2 – 12.0	0.199***	0.644***
Age Group (yrs.)*	Young	829	23 (2.8)	1.8 – 4.1	78 (9.4)	7.5 – 11.6	2.718**	0.257**
	Mature	1768	71 (4.0)	3.1 – 5.0	145 (8.2)	6.9 – 9.6	2.543***	0.280***
	Old	1180	47 (4.0)	2.9 – 5.3	116 (9.8)	8.2 – 11.7		

\* <1 yr. = Young; 1-3.0 yr. = Mature; >3.0 yr. = Old. \*\*Mild Infection; \*\*\* Severe Infection ( $p \leq 0.05$ ) regarded as significant

**Table 4.14: Status of *Brucella* infection using Serum Agglutination Test with Ethylenediaminetetracetic acid in sheep and goats sampled in Katsina and Sokoto States, Nigeria based on Wards**

Variable	Level	No. of sera Tested	No. of Mild Infections (%) (1:40 = 67 I.U)	95% C.I on Sero-prevalence	No. of Severe Infections (%) ( $\geq 1:80 = \geq 134$ I.U)	95% C.I on Sero-prevalence	Pearson's Chi-square ( $\chi^2$ )	p-value
Ward	Araba	163	3 (1.8)	0.3 – 5.3	12 (7.4)	3.8 – 12.5	46.888*	0.007*
	Bagiro	93	3 (3.2)	0.6 – 9.1	5 (5.4)	1.7 – 12.1	200.745**	0.000**
	Barebari	129	2 (1.6)	0.1 – 5.4	19 (14.7)	9.1 – 22.0		
	Bingaji	86	4 (4.7)	1.2 – 11.5	14 (16.3)	9.1 – 25.8		
	Bojo	81	9 (11.1)	5.2 – 20.0	5 (6.2)	2.0 – 13.8		
	Dangaji	122	2 (1.6)	0.1 – 5.8	15 (12.3)	7.0 – 19.4		
	Gambuwa	222	5 (2.3)	0.7 – 5.2	6 (2.7)	0.9 – 5.8		
	Gandun Sarki	149	0 (0.0)	0.0 – 2.4	14 (9.4)	5.2 – 15.3		
	Gara	62	4 (6.5)	1.8 – 15.7	2 (3.2)	0.3 – 11.22		
	Gidan Tanko	145	7 (4.8)	1.9 – 9.6	33 (22.7)	16.2 – 30.5		
	Goshe	203	5 (2.5)	0.8 – 5.7	7 (3.4)	1.4 – 6.9		
	Gurjiya	63	4 (6.3)	1.8 – 15.5	4 (6.3)	1.8 – 15.5		
	Hui	118	5 (4.2)	1.4 – 9.6	1 (0.8)	0.0 – 4.6		
	Illela	212	9 (4.2)	1.9 – 7.9	17 (8.0)	4.7 – 12.5		
	Kalgon Rimi	59	2 (3.4)	0.4 – 11.7	4 (6.8)	1.8 – 16.4		
	Kalmalo	214	7 (3.3)	1.3 – 6.6	14 (6.5)	3.6 – 10.7		
	Kyauren Gyada	136	3 (2.2)	0.4 – 6.3	8 (5.9)	2.6 – 11.3		
	Madobi	100	6 (6.0)	2.2 – 12.6	13 (13.0)	7.1 – 21.2		
	Maiganji	94	3 (3.2)	0.6 – 9.0	5 (5.3)	1.7 – 11.9		
	Mazoji	122	3 (2.5)	0.5 – 7.0	8 (6.6)	2.8 – 12.5		
	Rafin Kanya	152	5 (3.3)	1.1 – 7.5	46 (30.3)	23.1 – 38.2		
	Saida	183	4 (2.2)	0.5 – 5.5	8 (4.4)	1.9 – 8.4		
	Tumfushi	155	14 (9.0)	5.0 – 14.7	15 (9.7)	5.5 – 15.5		
	Unguan Nagari	151	8 (5.3)	2.3 – 10.2	10 (6.6)	3.2 – 11.8		
	Yabo A	230	11 (4.8)	2.4 – 8.4	9 (3.9)	1.8 – 7.3		
	Yabo B	198	8 (4.0)	1.7 – 7.8	20 (10.1)	6.3 – 15.2		
	Zamfarawa	135	5 (3.7)	1.2 – 8.4	25 (18.5)	12.3 – 26.1		

\*Mild Infection; \*\* Severe Infection. Serum Agglutination Test with Ethylenediaminetetracetic acid. Infection ( $p \leq 0.05$ ) regarded as significant

#### 4.4 Forms of *Brucella* infection in sheep and goats based on 2-Mercaptoethanol Test (2MET)

Out of the 459 samples tested, 228 (76.5%) and 124 (77.0%) were found to be acutely infected from Katsina and Sokoto States respectively while 70 (23.5%) and 37 (23.0%) were chronically infected in the same States respectively. However, these differences were not statistically significant ( $P > 0.05$ ) (Table 4.15).

Based on LGAs, the highest sero-prevalence of acutely infected animals 51(85.0%) was recorded in Baure LGA while the lowest value of 39 (66.1%) was obtained in Bakori LGA, all in Katsina State (Table 4.16). For the chronically infected animals, the highest prevalence 20 (33.9%) was recorded in Bakori LGA while the lowest prevalence 9 (15.0%) obtained for this category was recorded in Baure LGA, all of Katsina State. The difference in sero-prevalence between LGAs studied was not statistically significant ( $p > 0.05$ ) (Table 4.15).

The form of *Brucella* infection by animal species has shown that 160 (73.3%) of the sheep were acutely infected while 58 (26.6%) were chronically infected. As for goats, 192 (76.6%) were acutely infected while 49 (20.3%) were chronically infected. There was no statistically significant difference ( $p > 0.05$ ) in the form of infection between sheep and goats (Table 4.15).

As for acutely infected animals based on breed, the Sahel breed of goat had the highest sero-prevalence of 11 (91.6%) while the lowest prevalence 113 (71.5%) was recorded in the Yankasa breed of sheep. Also, for chronically infected animals based on breed, the highest prevalence of 1 (33.3%) was obtained for Balami breed of sheep while the lowest prevalence 1 (8.3%) was obtained for Sahel breed of goats. There was no statistical difference ( $p > 0.05$ ) among the breeds of animals studied (Table 4.15).

Female animals had a sero-prevalence of 304 (77.7%) and 87 (22.2%) for acute and chronic infections respectively, while the males had 48 (70.5%) and 20 (29.4%) for acute and chronic

infections respectively. The difference in the prevalence between the sexes was not statistically significant ( $p > 0.05$ ) (Table 4.15).

Based on age group, the animals less than 1 year of age had the highest sero-prevalence of 73 (79.4%) for acute infection followed by those above 3 years of age 143 (76.9%) and least 172 (76.8%) by those between 1 and 3 years of age. With regards to chronic *Brucella* infection, animals 1 to 3 years had a sero-prevalence of 52 (23.2%) and those above 3 years old had a sero-prevalence of 22 (23.1%). Animals less than 1 year old had the least 19 (13.0%) sero-prevalence. The difference across the various age groups was not statistically significant ( $p > 0.05$ ) (Table 4.15).

With respect to forms of *Brucella* infection at Ward level, animals in Dangaji Ward of Baure LGA of Katsina State had the highest acute infection of 15 (100%) while animals in Hui Ward of the same Baure LGA had the lowest acute infection 0 (0.0%). Similarly, animals in Gandun Sarki Ward of Bakori LGA of Katsina State had the highest chronic infection of 5 (45.5%) while animals in Dangaji Ward in Baure LGA of Katsina State recorded the lowest chronic infection 0 (0.0%). The difference between the Wards as regards form of *Brucella* infection was not statistically significant ( $p > 0.05$ ) (Table 4.16).

**Table 4.15: Forms (acute/chronic) of *Brucella* positive sheep and goats using 2-Mercaptoethanol Test based on State, LGA, species, breed, sex and age in Katsina and Sokoto States, Nigeria**

Variable	Level	No. of sera Tested	No. of Acute Infections (%)	95% C.I on Sero-prevalence	No. of Chronic Infections (%)	95% C.I on Sero-prevalence	Pearson's Chi-square ( $\chi^2$ )	p-value
State	Katsina	298	228 (76.5)	71.3 – 81.2	70 (23.5)	19.0 – 28.7	0.015	0.902
	Sokoto	161	124 (77.0)	69.7 – 83.3	37 (23.0)	16.7 – 30.3		
LGA	Bakori	59	39 (66.1)	52.6 – 77.9	20 (33.9)	22.1 – 47.4	8.354	0.213
	Baure	60	51 (85.0)	73.4 – 92.9	9 (15.0)	7.1 -26.6		
	Daura	60	46 (76.7)	64.0 – 86.6	14 (23.3)	13.4 – 36.0		
	Funtua	119	92 (77.3)	68.7 – 84.5	27 (22.7)	15.5 – 31.3		
	Illela	39	29 (74.4)	57.9 – 87.0	10 (25.6)	14.2 – 45.2		
	Tambuwal	24	16 (66.7)	44.7 – 84.4	8 (33.3)	15.6 – 55.3		
	Yabo	98	79 (80.6)	71.4 – 87.9	19 (19.4)	12.1 – 28.6		
Species	Sheep	218	160 (73.3)	67.0-79.1	58 (26.6)	20.8-32.9	2.520	0.112
	Goat	241	192 (76.6)	74.0-84.5	49 (20.3)	15.5-25.9		
Breed	Balami	3	2 (66.6)	9.4-99.2	1 (33.3)	0.8-90.5	6.127	0.294
	Kano Brown	181	146 (80.6)	74.1-86.1	35 (19.3)	13.8-25.8		
	Sahel (goat)	12	11 (91.6)	61.5-99.8	1 (8.3)	0.2-38.4		
	Sokoto Red	61	45 (74.0)	60.9-84.2	16(26.2)	15.7-39.0		
	Uda	44	35 (79.5)	64.7-90.2	9 (20.4)	9.8-35.3		
	Yankasa	158	113 (71.5)	63.8-78.4	45 (28.4)	21.5-36.1		
Sex	Female	391	304 (77.7)	73.2-81.7	87 (22.2)	18.2-27.0	1.662	0.197
	Male	68	48 (70.5)	58.3-81.0	20 (29.4)	18.9-41.7		
Age Group (Yrs.)*	Young	92	73 (79.4)	69.6 – 87.1	19 (13.0)	15.6 – 33.9	0.024	0.988
	Mature	224	172 (76.8)	70.6 – 82.1	52 (23.2)	17.9 – 29.3		
	Old	143	110 (76.9)	69.1 – 83.6	22 (23.1)	9.9 – 22.4		

\* <1 yr. = Young; 1-3.0 yr. = Mature; >3.0 yr. = Old. ( $p \leq 0.05$ ) regarded as significant

**Table 4. 16: Forms of *Brucella* positivity using 2-Mercaptoethanol Test based on wards in Katsina and Sokoto States, Nigeria.**

Variable	Level	No. of sera Tested	No of Acute Infections (%)	95% C.I on Sero-prevalence	No of Chronic Infections (%)	95% C.I on Sero-prevalence	Pearson's Chi-square ( $\chi^2$ )	p-value
Ward	Araba	12	8 (66.7)	34.9 – 90.1	4 (33.3)	9.2 – 65.1	25.621	0.484
	Bagiro	9	6 (66.7)	29.9 – 92.5	3 (33.3)	7.5 – 70.1		
	Barebari	20	17 (85.0)	62.1 – 96.7	3 (15.0)	3.2 – 37.9		
	Bingaji	11	7 (63.6)	30.7 – 89.1	4 (36.4)	10.9 – 69.2		
	Bojo	14	11 (78.6)	49.2 – 95.3	3 (21.4)	4.7 – 50.8		
	Dangaji	15	15 (100.0)	78.2 – 100.0	0 (0.0)	0.0 – 21.8		
	Gambuwa	10	8 (80.0)	44.4 -97.5	2 (20.0)	2.5 – 55.6		
	Gandun Sarki	11	6 (54.6)	23.4 – 83.3	5 (45.5)	16.7 – 76.6		
	Gara	7	4 (57.2)	18.4 – 90.1	3 (42.9)	10.0 – 81.6		
	Gidan Tanko	30	19 (63.3)	43.9 – 80.1	11 (40.0)	19.9 -56.1		
	Goshe	20	18 (90.0)	68.3-99.0	2 (1.0)	1.2-31.6		
	Gurjiya	7	6 (85.7)	42.1-99.6	1 (14.2)	0.3-58.0		
	Hui	6	0 (0.0)	0.0 - 46.0	6 (100.0)	54.1-1*		
	Illela	27	21 (77.8)	57.8-91.3	6 (22.2)	8.6-42.2		
	Kalgon Rimi	3	2 (66.7)	9.4-99.1	1 (33.3)	0.8-90.5		
	Kalmalo	17	13 (76.5)	50.1-93.1	4 (24.0)	6.8-49.8		
	Kyauren Gyada	10	8 (80.0)	44.3-97.4	2 (2.0)	2.5-56.0		
	Madobi	17	14 (82.4)	56.5-96.2	3 (17.6)	3.7-43.4		
	Maiganji	20	14 (70.0)	46.0-88.1	6 (3.0)	11.8-54.2		
	Mazoji	12	9 (75.0)	42.8-94.5	3 (25.0)	5.4-57.1		
	Rafin Kanya	40	31 (77.5)	61.5-89.1	9 (22.5)	10.8-38.4		
	Saida	14	8 (57.1)	29.0-82.3	6 (43.0)	17.6-71.1		
	Tumfushi	29	22 (75.9)	56.4-88.0	7 (24.1)	10.2-43.5		
	Unguwan Nagari	18	14 (77.8)	52.3-93.5	4 (22.2)	6.4-48.0		
	Yabo A	24	18 (75.0)	53.2-90.2	6 (25.0)	9.7-46.7		
	Yabo B	26	23 (88.5)	69.8-97.5	3 (11.5)	2.4-30.1		
	Zamfarawa	30	24 (80.0)	61.4-92.2	6 (2.0)	7.7-39.0		

( $p \leq 0.05$ ) regarded as significant

#### **4.5 Sero-prevalence and geo-spatial distribution of *B. melitensis* in sheep and goats based on c-ELISA**

A sero-prevalence of 4.0% (51/269) and 13.0% (17/131) were obtained from Katsina and Sokoto States respectively. Though, the difference was not statistically significant ( $p > 0.05$ ) (Table 4.17). The colour map imagery showing the geospatial burden of *B. melitensis* by c-ELISA in sheep and goats in LGAs under study in Katsina State is shown in Fig 4.1. The colour map for Katsina State showed that the prevalence of *B. melitensis* was distributed through most of the LGAs sampled (Fig. 4.1). The highest prevalence displayed (red) was located in the southern part of Funtua LGA while the lowest prevalence was in majority of Daura LGA, except for a very tiny area in the southern and western parts of Daura LGA (dark green) which had a relatively high prevalence displayed. Also, a colour map showing the predicted sero-prevalence by Inverse Distance Weighted (IDW) in other LGAs not sampled in Katsina State is presented in Fig 4.2.

Similarly, in Sokoto State, the colour map imagery showing the geospatial burden of *B. melitensis* by c-ELISA in sheep and goats in LGAs under study is shown in Fig 4.3. The colour map showed that the prevalence of *B. melitensis* was distributed through most of the LGAs/Wards sampled. The largest prevalence (red) was obtained from the north-eastern part of Illela LGA and majority of Yabo LGA while the least was observed in eastern part of Tambuwal LGA. The map showing the predicted sero-prevalence by IDW in other LGAs not sampled in Sokoto State is shown in Fig 4.4.

Using c-ELISA, the highest sero-prevalence rate of 23 (23.0%) was obtained in Funtua LGA while the lowest 11 (2.0%) was obtained in Daura LGA. The difference in the prevalence across the LGAs involved in the study was however not statistically significant ( $p > 0.05$ ) (Table 4.17).

The sero-prevalence of 40 (23.7%) obtained for *B. melitensis* antibodies in sheep was almost double of that obtained in goats 12.1 (12.1%). Here again, the difference was not statistically significant ( $p > 0.05$ ) (Table 4.17).

In the case of breed, the highest sero-prevalence of 37 (20.4%) was obtained in Kano Brown goats while the lowest value of 0 (0.0%) was obtained in the Sahel breed of goats. Similarly, there was no statistical significant difference ( $p > 0.05$ ) between the breeds of animals for c-ELISA (Table 4.17).

With regards to the sero-prevalence by sex, value of 59 (17.5%) obtained for female animals was slightly higher than the 9 (14.3%) obtained in the males. The difference was also not statistically significant ( $p > 0.05$ ) (Table 4.17).

Based on age, the highest sero-prevalence 28 (20.7%) was obtained for animals aged three years or older while the lowest sero-prevalence 12 (13.3%) was obtained in animals less than one year old. The difference between the age groups was also not statistically significant ( $p > 0.05$ ) (Table 4.17).

Ward specific sero-prevalence was highest 2 (29.0%) in Araba Ward and lowest 0 (0.0%) in Bingaji, Gandun Sarki, Hui, Kalgon Rimi and Saida Wards. Similarly, the difference in the prevalence between the Wards under study was not statistically significant ( $p > 0.05$ ) (Table 4.18).

**Table 4.17: Sero-prevalence of *B. melitensis* positive sheep and goats using cELISA based on State, LGA, species, breed, sex and age in Katsina and Sokoto States, Nigeria**

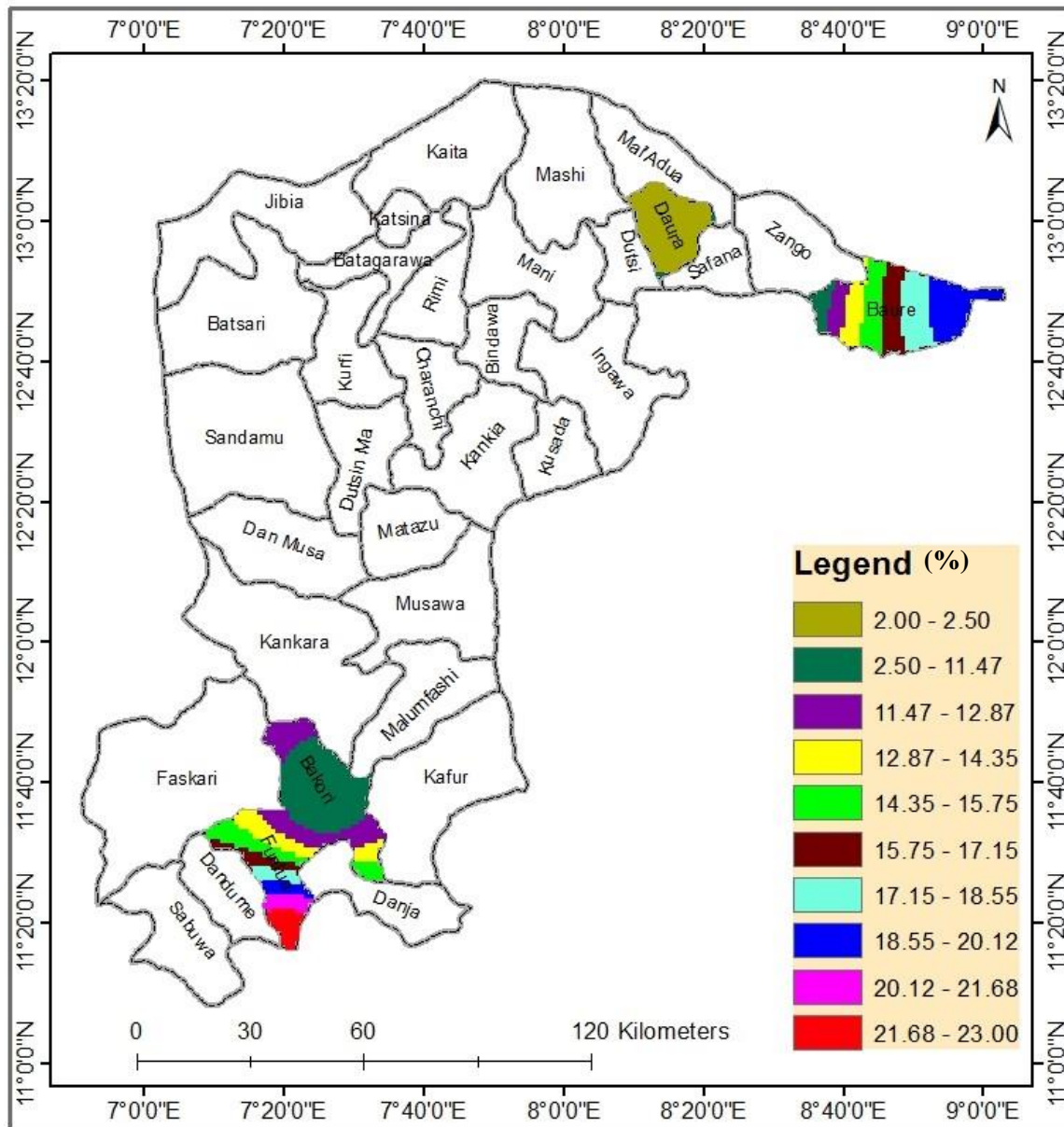
Variable	Level	No. of sera Tested	No. positive (%)	95% C.I on Sero-prevalence	Pearson's Chi-square ( $\chi^2$ )	P-value
State	Katsina	269	51 (4.0)	3.0 – 5.3	2.239	0.135
	Sokoto	131	17 (13.0)	7.7 – 20.0		
LGA	Bakori	57	6(10.52)	3.9 - 21.5	6.679	0.323
	Baure	56	11 (20.0)	10.2 - 32.4		
	Daura	55	11(2.0)	10.4 - 33.0		
	Funtua	101	23(23.0)	15.0 - 32.1		
	Illela	22	3(14.0)	29.0 - 35.0		
	Tambuwal	19	1(5.2)	0.1 - 26.0		
	Yabo	90	13 (14.4)	8.0 - 23.4		
Species	Sheep	169	40 (23.7)	17.5 – 30.8	0.061	0.804
	Goat	231	28 (12.1)	8.2 – 17.0		
Breed	Kano Brown	181	37 (20.4)	14.8 – 27.1	5.457	0.244
	Sahel (goat)	12	0 (0.0)	0.0 – 26.5		
	Sokoto Red	50	5 (10.0)	3.3 – 21.8		
	Uda	27	4 (14.8)	4.2 – 33.7		
	Yankasa	130	22 (16.9)	10.9- 24.5		
Sex	Female	337	59 (17.5)	13.6 – 22.0	0.385	0.535
	Male	63	9 (14.3)	6.7 – 25.4		
Age Group (Yrs.)*	Young	90	12 (13.3)	7.1 – 22.1	2.248	0.325
	Mature	175	28 (16.0)	10.9 – 22.3		
	Old	135	28 (20.7)	14.3 – 28.6		

\* <1 yr. = Young; 1-3.0 yr. = Mature; >3.0 yr. = Old. ( $p \leq 0.05$ ) regarded as significant

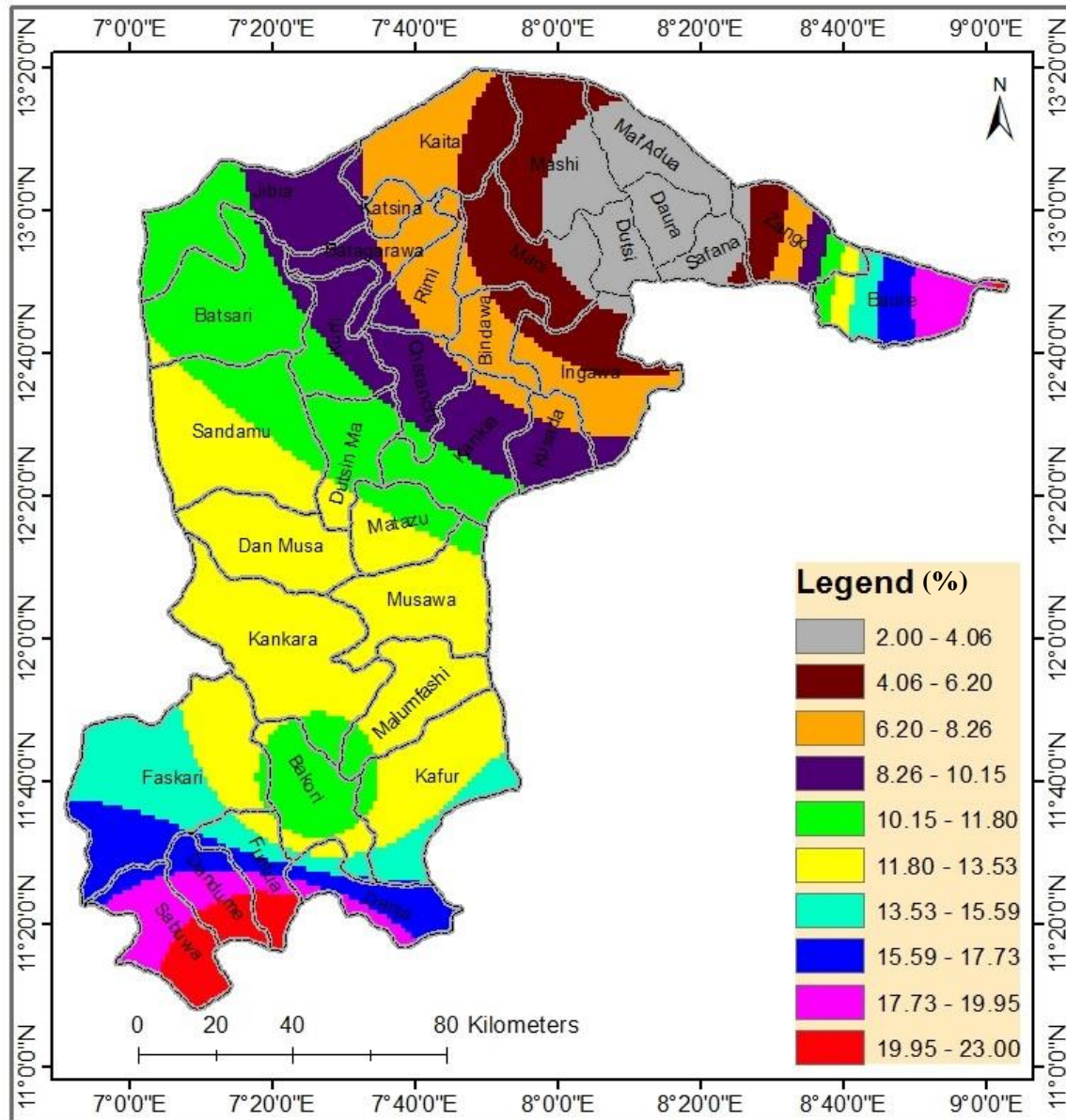
**Table 4.18: Sero-prevalence of *B. melitensis* positive sheep and goats using cELISA based on Wards in Katsina and Sokoto States, Nigeria**

Variable	Level	No. of sera Tested	No positive for cELISA (%)	95% C.I on Sero-prevalence	Pearson's Chi-square ( $\chi^2$ )	P-value
Ward	Araba	7	2 (29.0)	4.0 - 81.0	25.756	0.477
	Bagiro	2	1 (5.0)	1.2 - 98.7		
	Barebari	18	2 (11.1)	1.3 - 35.0		
	Bingaji	15	0 (0.0)	0.0 - 21.8		
	Bojo	13	3 (23.0)	5.0-54.0		
	Dangaji	17	1 (5.8)	0.1-27.0		
	Gambuwa	10	1 (1.0)	0.2-44.5		
	Gandun Sarki	4	0 (0.0)	0.0-60.2		
	Gara	4	1(25.0)	0.6-80.5		
	Gidan Tanko	37	3 (8.1)	1.7-22.0		
	Goshe	10	1 (1.0)	0.2-46.0		
	Gurjiya	7	1 (14.2)	0.3-58.0		
	Hui	5	0 (0.0)	0.0-52.1		
	Illela	15	1 (6.6)	0.1-31.0		
	Kalgon Rimi	3	0 (0.0)	0.0-71.0		
	Kalmalo	21	4 (19.0)	5.4-41.9		
	Kyauren Gyada	8	1 (12.5)	0.3-52.6		
	Madobi	18	4 (22.2)	6.4-48.0		
	Maiganji	4	1 (25.0)	0.6-80.5		
	Mazoji	10	2 (2.0)	2.5-57.0		
	Rafin Kanya	49	12 (24.4)	13.3-38.8		
	Saida	9	0 (0.0)	0.0-34.0		
	Tumfushi	26	9 (34.6)	17.2-55.6		
	Unguan Nagari	15	3 (2.0)	4.3-48.0		
	Yabo A	21	4 (19.0)	5.4-41.9		
	Yabo B	23	4 (17.3)	5.0-38.7		
	Zamfarawa	29	7 (24.1)	10.2-43.5		

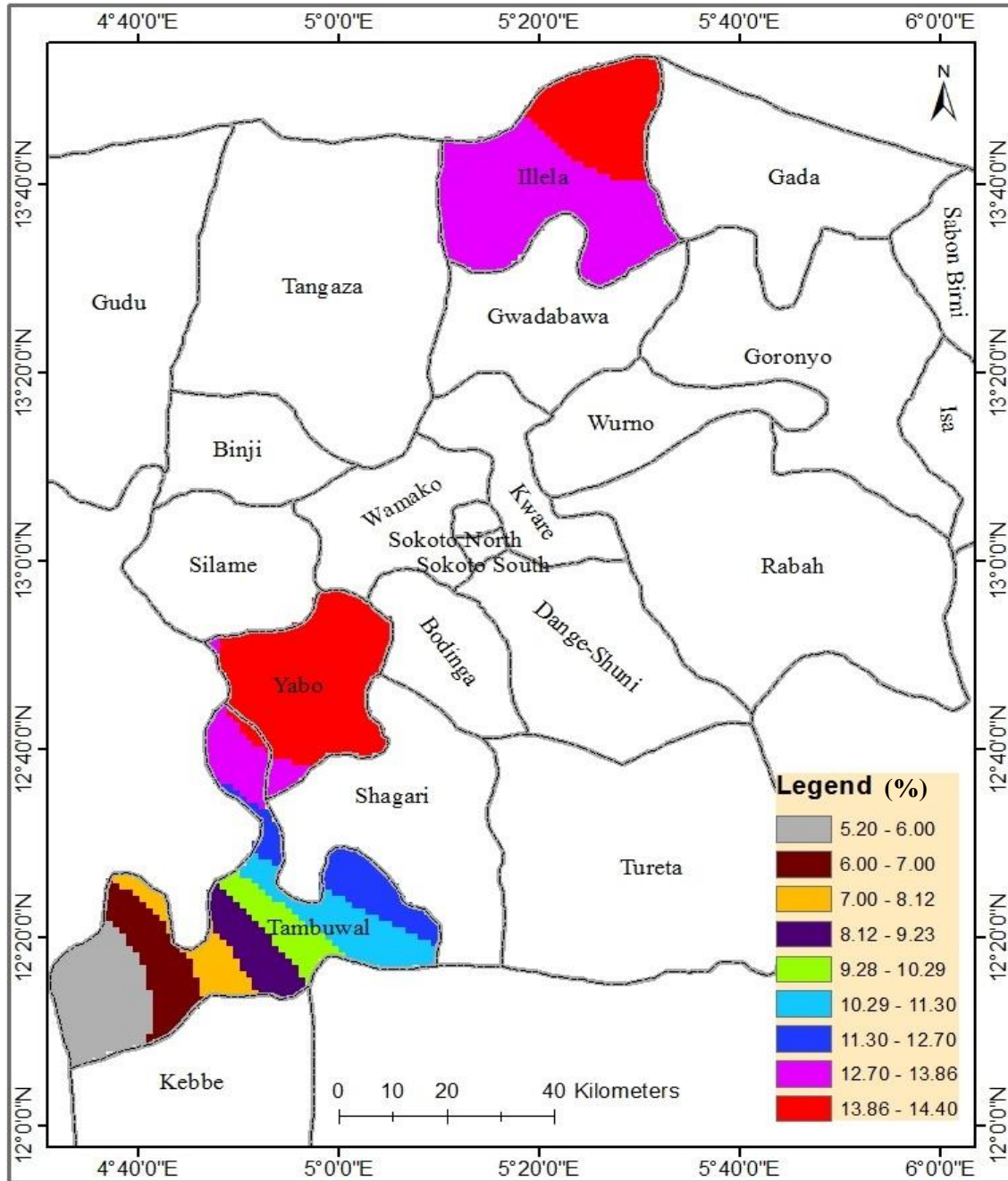
( $p \leq 0.05$ ) regarded as significant



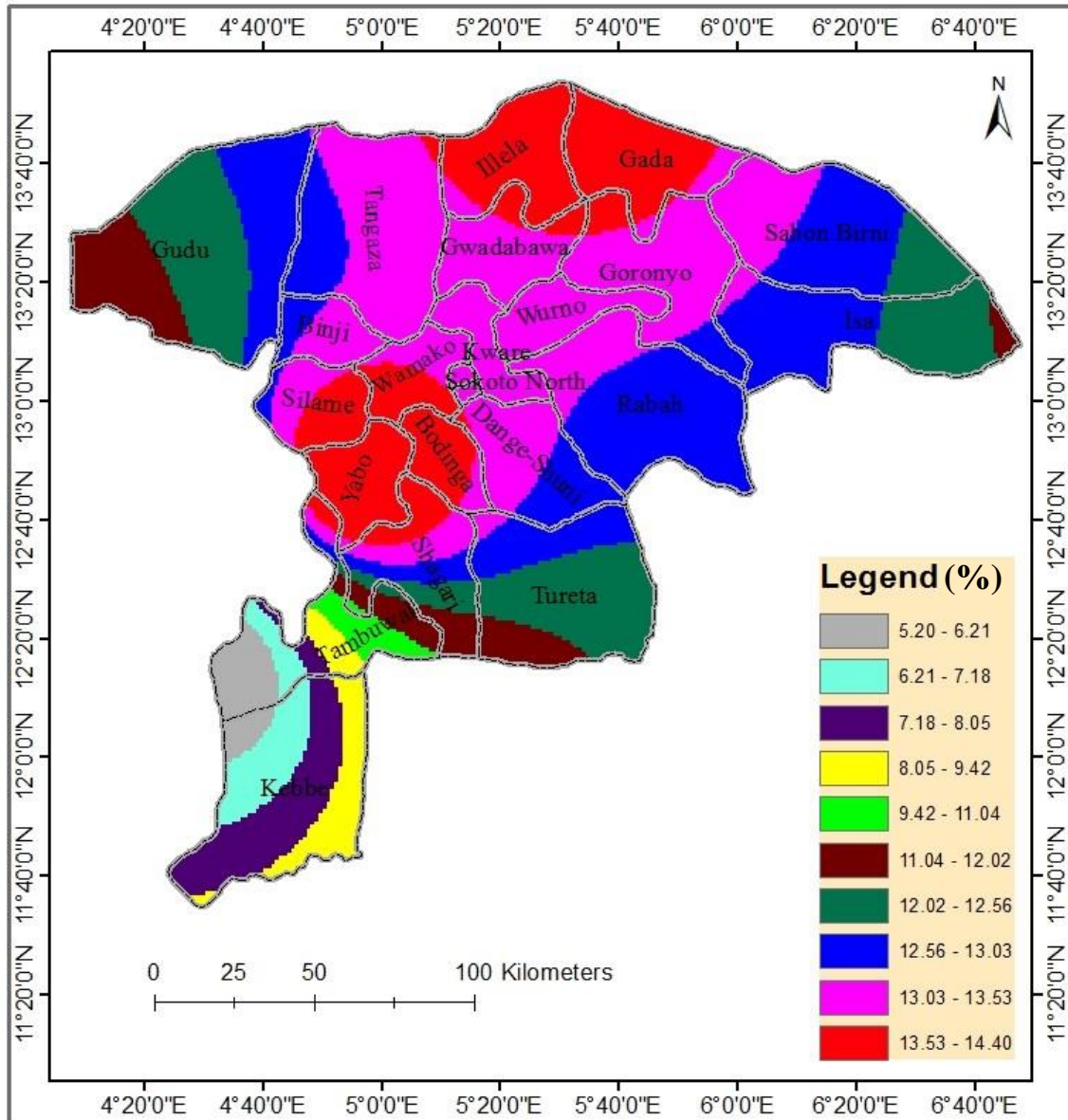
**Figure 4.1: Spatial map imagery showing *Brucella melitensis* density distribution in 4LGAs in Katsina State**



**Figure 4.2: Spatial Interpolation map imagery showing *Brucella melitensis* density distribution in all LGAs in Katsina State**



**Figure 4.3: Spatial map imagery showing *Brucella melitensis* density distribution in 4LGAs in Sokoto State**



**Figure 4.4: Spatial Interpolation map imagery showing *Brucella melitensis* density distribution in all LGAs in Sokoto State**

#### **4.6 Participatory Epidemiological study on brucellosis**

From this study, it was observed that inhabitants of villages in the selected LGAs of Katsina and Sokoto States were predominantly Hausa and Fulani. Their socio-economic activities were similar in both states. They were largely involved in crop farming and livestock rearing as major source of their livelihood. They kept virtually similar species of animals except for 2 LGAs in the southern part of Katsina State (Bakori and Funtua) where camels were rarely seen.

#### **4.7 Species of animals kept, their estimated population and risk of brucellosis spread**

As revealed by simple ranking and probed by proportional piling, cattle constituted the largest proportion (ranked 1<sup>st</sup>) of livestock specie kept by pastoralists in Katsina and Sokoto States. This was followed by goats (ranked 2<sup>nd</sup>), sheep (ranked 3<sup>rd</sup>), poultry (ranked 4<sup>th</sup>), camels (ranked 5<sup>th</sup>) and donkeys (ranked 6<sup>th</sup>) (Plates I and II). These animals were largely on extensive system of management with only few herds managed on semi-intensive system.



**Plate I: Proportional piling of animals kept by pastoralists in Katsina State**

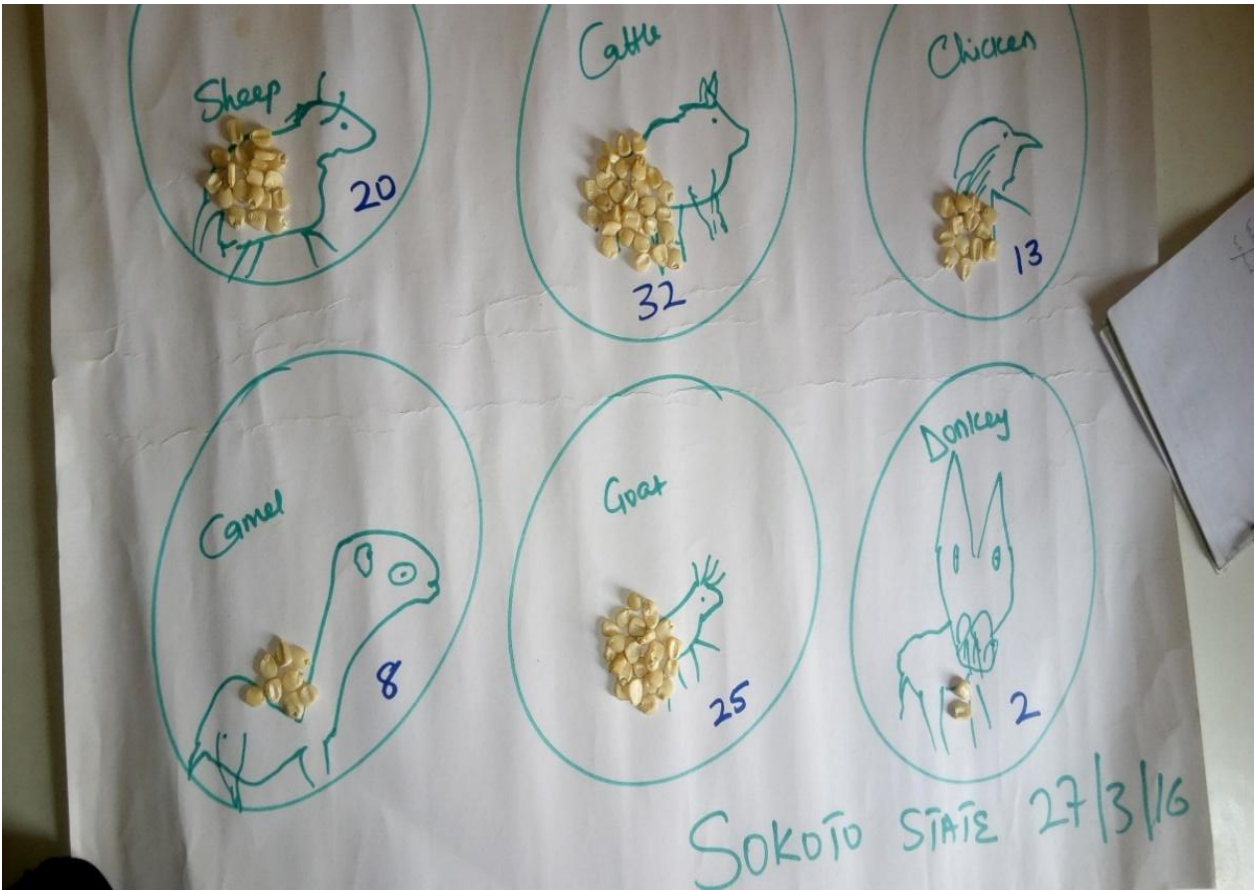


Plate II: Proportional piling of animals kept by pastoralists in Sokoto State

#### **4.8 Economic importance of animal species**

The economic benefits derived from the animal species kept by pastoralists in Katsina and Sokoto States as deduced from a Semi Structured Interview (SSI) included fertilizer (manure), means of livelihood, transport, Farm traction, source of emergency funds, festivities, meat and milk/milk products.

In Katsina State, with regards to cattle, matrix scoring showed that fertilizer, means of livelihood and transport scored 6 each while Farm traction scored 5, milk/milk products scored 3, festivities and meat scored 2 and emergency funds scored 0. For sheep, the highest score of 11 was allocated to festivities, emergency funds scored 8, meat scored 5, means of livelihood and fertilizer scored 4 and 2 respectively, transport, Farm traction, milk/milk products all scored 0. For goats, emergency funds scored 11, means of livelihood and meat scored 5, festivities scored 4, milk/milk products and fertilizer scored 3 and 2 respectively while 0 score was allocated to transport and Farm traction. With regards to poultry, emergency funds was allocated 15 scores, festivities scored 7, meat and means of livelihood scored 5 and 3 respectively, while fertilizer, transport, Farm traction, milk/milk products all scored 0. As for donkeys, all the variables had scores of 0 except for transport with a score of 30. For camels, transport scored 6, means of livelihood, Farm traction, festivities, milk and milk products all scored 5, meat scored 4 while 0 score was allocated to fertilizer and emergency funds (Table 4.19).

Similarly, in Sokoto State, regarding cattle, fertilizer, Farm traction and means of livelihood all scored 6, milk/milk products and transport scored 4, meat scored 3, festivities scored 1 and emergency funds scored 0. As for sheep, means of livelihood and emergency funds transport scored 8, meat and festivities scored 6, fertilizer scored 2, Farm traction, milk/milk products and transport all scored 0. For goats, meat and emergency funds scored 8, means of livelihood scored

5, milk/milk products scored 4, festivities and fertilizer scored 3 and 2 respectively while Farm traction and transport scored 0. Regarding camels, Farm traction scored 7, milk/milk products scored 6, meat, means of livelihood and transport all scored 5, fertilizer, and festivities both scored 1 while emergency funds scored 0. As for poultry, emergency fund scored 11, festivities scored 8, means of livelihood and meat scored 7 and 4 respectively while fertilizer, Farm traction, milk/milk products and transport all scored 0. All scores were 0 for donkey except transport which had a score of 30 (Table 4.20).

#### **4.9 Factors militating against livestock production in Katsina and Sokoto States, Nigeria**

Based on the SSI, pastoralists in Katsina State identified 7 factors as the major husbandry problems militating against livestock production in their villages (Table 4.21). These included; cattle rustling which was ranked 1 ( $Z = 2.17$ ) followed by effects of transhumance (Rank = 2;  $Z = 2.07$ ), lack of watering points (Rank = 3;  $Z = 0.79$ ), lack/inadequate shelter (Rank = 4;  $Z = -0.3$ ), diseases (Rank = 5;  $Z = -1.05$ ), cost of medication (Rank = 6;  $Z = -1.58$ ) and feeding (Rank = 7;  $Z = -2.1$ ). The median score for husbandry problems that affect livestock production was significantly different ( $p < 0.05$ ).

Similarly, in Sokoto State, the factors identified by pastoralists as major husbandry problems militating against livestock production were; disease (Rank 1 =;  $Z = 1.73$ ), lack/inadequate shelter (Rank 2 =;  $Z = 1.46$ ), effects of transhumance (Rank 3 =;  $Z = 1.1$ ), conversion of cattle routes to cropland (Rank 4 =;  $Z = -0.46$ ), cost of medication (Rank 5 =;  $Z = 1.28$ ), feeding (Rank = 6;  $Z = -1.28$ ) and lack of watering points (Rank 7 = ;  $Z = -1.28$ ) (Table 4.22). There was no significant difference in the median scores for livestock husbandry problems ( $p > 0.05$ ).

**Table 4.19: Matrix scoring for uses of animal species kept by pastoralists in Katsina State, Nigeria**

Economic Benefit	<u>Species of Animals Kept</u>					
	Cattle	Sheep	Goat	Poultry	Donkey	Camel
Fertilizer	6	2	2	0	0	0
Means of livelihood	6	4	5	3	0	5
Transport	6	0	0	0	30	6
Farm traction	5	0	0	0	0	5
Emergency fund	0	8	11	15	0	0
Festivities	2	11	4	7	0	5
Meat	2	5	5	5	0	4
Milk/milk products	3	0	3	0	0	5

**Table 4.20: Matrix scoring for economic uses of animal species kept by pastoralists in Sokoto State, Nigeria**

Economic Benefit	<u>Species of Animals Kept</u>					
	Cattle	Sheep	Goat	Poultry	Camel	Donkey
Meat	3	6	8	4	4	0
Fertilizer	6	2	2	0	0	0
Farm traction	6	0	0	0	9	0
Milk/ milk products	4	0	4	0	7	0
Means of livelihood	6	8	5	7	4	0
Festivities	1	6	3	8	0	0
Emergency fund	0	8	8	11	0	0
Transport	4	0	0	0	6	30

**Table 4.21: Scores for estimated husbandry problems that affect livestock production as reported by pastoralists in Katsina State, Nigeria**

Problem	Mean (%)	Median (%)	Range (%)	Z-Score	Rank
Cattle Rustling	3.3	3	3	2.17	1
Effects of Transhumance	3	3	2	2.07	2
Lack of watering Points	2	2	4	0.79	3
Lack/Inadequate Shelter	1	1	2	-0.3	4
Diseases	0.8	0	3	-1.05	5
Cost of Medication	0.3	0	1	-1.58	6
Feeding	0	0	0	-2.1	7

H = 16.24; df = 6; p = 0.0125

**Table 4.22: Scores for estimated husbandry problems that affect production as reported by pastoralists in Sokoto State, Nigeria**

Problem	Mean (%)	Median (%)	Range (%)	Z-Score	Rank
Diseases	3	3	2	1.73	1
Lack/Inadequate shelter	2.5	3	1	1.46	2
Effects of transhumance	2	2	2	1.1	3
Diversion of cattle routes to cropland	0.5	0	1	-0.46	4
Cost of medication	0	0	0	-1.28	5
Feeding	0	0	0	-1.28	6
Lack of watering points	0	0	0	-1.28	7

H = 10.63; df = 6; p = 0.1004

#### 4.10 Common small ruminant diseases as reported by pastoralists in Katsina and Sokoto States, Nigeria

Semi-structured interviews of participants in the selected villages in Katsina State revealed 9 diseases/conditions as commonly affecting small ruminants. These were pestes de petits ruminante (PPR) (*gishu/gurda*) (Rank 1 = ; Z = 2.78), anaplasmosis (*saifa*) (Rank = 2; Z = 1.86), fasciolosis (*balku/hanta*) (Rank = 3; Z = 0.41), helminthosis (*matsattsaku*) (Rank 4 = ; Z = 0.24), Contageous Caprine Pleuropneumonia (CCPP) (*huhu*) (Rank = 5; Z = 0.02), brucellosis (*bakkale/bari*) (Rank = 6; Z = -0.29), tick infestation (*kaska/koti*) (Rank 7 = ; Z = -0.45), foot rot (*ciwon kofoto*) (Rank = 8; Z = -1.14) and arthritis (*kumburin guiwa*) (Rank = 8; Z = -1.14) as determined by pairwise ranking. The median scores for these small ruminant diseases were significantly different ( $p < 0.05$ ) (Table 4.23).

Similarly, in Sokoto State using the SSI, participants identified 7 diseases/conditions as commonly affecting small ruminants in the villages visited. The diseases/conditions were; Foot and Mouth Disease (FMD) (*chabo*) (Rank = 1; Z = 1.97), pox (*ado/gaye*) (Rank = 2 ; Z = 1.82), PPR (*dan kap*) (Rank = 3; Z = 1.63), helminthosis (*matsattsaku*) (Rank = 4; Z = 1.16), fasciolosis (*balku*) (Rank = 5; Z = -0.94), CCPP (*huhu*) (Rank = 6; Z = -0.94), catarrh (*mura*) (Rank = 6; Z = -0.94), brucellosis (*bakkale*) (Rank = 6; Z = -0.94), fot rot (*ciwon kofoto*) (Rank = 6; Z = -0.94) as determined by pairwise ranking. The median scores for these small ruminant diseases were significantly different ( $p < 0.05$ ) (Table 4.24).

**Table 4.23: Scores for estimated prevalence of small ruminant diseases as reported by pastoralists in Katsina State, Nigeria**

Disease	Local Names	Mean (%)	Median (%)	Range (%)	Z-score	Rank
PPR	Gishu/Gurda	2.8	3	1	2.78	1
Anaplasmosis	Saifa	2.3	3	4	1.86	2
Fasciolosis	Balku/Hanta	0.8	0	2	0.41	3
Helminthosis	Matsattsaku	0.5	0	1	0.24	4
CCPP	Huhu	1.3	0	5	0.02	5
Brucellosis	Bakkale/Bari	0.5	0	2	-0.29	6
Tick Infestation	Kaska/koti	0.3	0	1	-0.45	7
Footrot	Ciwon kofoto	0	0	0	-1.14	8
Arthritis	Kumburin Guiwa	0	0	0	-1.14	8

H = 21.54; df = 8; p = 0.0176

**Table 4.24: Scores for estimated importance of small ruminant diseases as reported by pastoralists in Sokoto State, Nigeria**

Disease	Local Names	Mean (%)	Median (%)	Range (%)	Z-score	Rank
FMD	Chabo	2	2	2	1.97	1
Pox	Gaye/Ado	1	1	2	1.82	2
PPR	Iska/Dan kap	1.7	4	4	1.63	3
Helminthosis	Matsattsaku	2.7	2	3	1.16	4
Fasciolosis	Balku	1.7	0	0	-0.94	5
CCPP	Huhu	0	0	0	-0.94	6
Catarrh	Mura	0	0	0	-0.94	6
Brucellosis	Bakkale/Bari	0	0	0	-0.94	6
Footrot	Ciwon kofoto	0	0	0	-0.94	6

H = 22.43, df = 8, p = 0.0131

#### 4.11 Pastoralists' diagnosis of small ruminant diseases

From the study, the interviewees associated signs of fasciolosis (*Balku/Hanta*) to be high morbidity 3 (0-6), high mortality 9 (0-18), seasonal occurrence 3 (0-6), inappetance 8.5 (3-15), abortion 2 (0-3), fever 6 (0-12), diarrhoea 7 (5-8), liver involvement 20 (10-30) and oculonasal discharges 3 (0-7) (Table 4.26). They also identified signs of brucellosis (*bakkale*) to be high morbidity 3 (0-6), high mortality 2.5 (0-5), inappetance 0 (0-6), abortion 20 (10-30) and retained placenta 4 (0-8). With regards to footrot (*ciwon kofoto*), they associated it with high morbidity 4 (0-8), high mortality 3 (0-5), seasonal occurrence 17 (15-20), inappetance 1.5 (0-3), limping 22.5 (15-30) and fever 3.5 (0-7). Signs they associated to PPR (*gishu/gurda*) included high morbidity 8 (10-25), high mortality 20 (15-25), seasonal occurrence 10 (0-20), inappetance 20 (15-25), abortion 6 (0-12), respiratory distress 18 (5-30), mouth erosion/ discharges 20 (15-25) salivation 5.5 (0-12), fever 17.5 (10 -25), diarrhoea 23 (15-30) and oculonasal discharges 20 (15-25). As for CCPP (*huhu*), its indicators were high morbidity 4 (0-6), high mortality 1.5 (0-3), seasonal occurrence 18 (15-20), inappetance 10 (5-15), abortion 1.5 (0-3), respiratory distress 15.5 (5-25) mouth erosion/salivation 12.5 (5-20) and fever 15 (10-20). Anaplasmosis (*saiifa*) was linked to high morbidity 7.5 (0-15), high mortality 17.5 (15-30), seasonal occurrence 4 (0-10), inappetance 13 (5-20), respiratory distress 5.5 (0-10), fever 6.5 (0-12), diarrhoea 8 (5-10) and oculonasal discharges 2.5 (0-5). High morbidity 18.5 (6-25), high mortality 4 (2-6), seasonal occurrence 18 (15-20), inappetance 2 (0-4), diarrhoea 20 (15-25) and oculonasal discharges 9 (0-18) were signs associated with helminthosis (*matsattaku*) (Table 4.24). Kendall's coefficient ( $W$ ) of concordance showed strong agreements for these indicators between the focus groups in Katsina State (Table 4.25).

Similarly, in Sokoto State pastoralists interviewed associated signs of pox (*ado*) to be high morbidity 16 (12-20), high mortality 2 (0-4), seasonal occurrence 3 (0-6), abortion 2 (2-3),

respiratory distress 10 (5-15), mouth erosion/salivation 2 (0-4), limping 1 (0-2), inappetence 12 (10-15), fever 5 (4-7) and skin involvement 30 (30-30) (Table 4.27). For fasciolosis (*balku*), the indicators identified were high morbidity 11 (12-20), high mortality 3 (0-5), seasonal occurrence 7 (5-8), inappetence 15(10-20), fever 8 (5-10), liver involvement 25 (20-30) and diarrhoea 5.5 (3-8). As for brucellosis (*bakkale*), the signs associated were high morbidity 5 (0-8), high mortality 2 (0-4), seasonal occurrence 1 (0-2), abortion 25 (20-30), inappetence 1 (0-2) and fever 1 (0-2). With regards to FMD (*chabo*), the indicators were high morbidity 18 (10-25), high mortality 6.5 (3-10), seasonal occurrence 4 (0-8), mouth erosion/salivation 25 (20-30), limping 27.5 (25-30), inappetence 6 (15-20) and fever 12.5 (15-20). As for PPR (*dan kap*), the signs were high morbidity 20 (15-25), high mortality 27.5 (25-30), seasonal occurrence 10 (5-15), abortion 1.5 (0-3), respiratory distress 18 (12-25), mouth erosion/salivation 11 (4-18), inappetence 17.5 (15-20), fever 22.5 (20-25) and diarrhoea 25 (20-30). High morbidity 15 (5-25), high mortality 3 (0-6), seasonal occurrence 5 (0-10), abortion 1 (0-2), inappetence 1.5(0-3), fever 0.5 (0-1) and diarrhoea 15 (10-20) were signs associated with helminthosis (*matsattaku*). As for catarrh (*mura*), high morbidity 3 (0-5), high mortality 2 (0-4), seasonal occurrence 10 (5-15), abortion 2 (0-3), respiratory distress 15 (10-20), inappetence 5 (2-8) and fever 4 (2-6) were the indicators identified. Kendall's coefficient (*W*) of concordance also showed strong agreements for these indicators between the focus groups as shown in Table 4.26.

**Table 4.25: Matrix scores of clinical indicators for characterization of small ruminant diseases by pastoralists in Katsina State, Nigeria**

Clinical Indicator ( <i>W</i> )	<u>Diseases</u>						
	Fasciolosis ( <i>Balku/Hanta</i> )	Brucellosis ( <i>Bakkale</i> )	Foot rot ( <i>Ciwon kofoto</i> )	PPR ( <i>Gishu/Gurda</i> )	CCPP ( <i>Huhu</i> )	Anaplasmosis ( <i>Saifa</i> )	Helminthosis ( <i>Matsattsaku</i> )
High morbidity (0.641***)	3 (0-6)	3 (0-6)	4 (0-8)	18 (10-25)	4 (0-6)	7.5 (0-15)	18.5 (6-25)
High mortality (0.723***)	9 (0-18)	2.5 (0-5)	3 (0-5)	20 (15-25)	1.5 (0-3)	17.5 (15-30)	4 (2-6)
Seasonal Occurence (0.722***)	3 (0-6)	0 (0-0)	17 (15-20)	10 (0-20)	18 (15-20)	4 (0-10)	18 (15-20)
Inappetance (0.604**)	8.5 (3-15)	0 (0-6)	1.5 (0-3)	20 (15-25)	10 (5-15)	13 (5-20)	2 (0-4)
Abortion (0.721***)	2(0-3)	20 (10-30)	0 (0-0)	6 (0-12)	1.5 (0-3)	0 (0-0)	0 (0-0)
Retained Placenta (0.250*)	0 (0-0)	4 (0-8)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Respiratory Distress (0.670**)	0 (0-0)	0 (0-0)	0 (0-0)	18 (5-30)	15.5 (5-25)	5.5 (0-10)	0 (0-0)
Mouth Erosion/Salivation (0.563**)	0 (0-0)	0 (0-0)	0 (0-0)	5.5 (0-12)	12.5 (5-20)	0 (0-0)	0 (0-0)
Limping (0.800***)	0 (0-0)	0 (0-0)	22.5 (15-30)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Fever (0.544**)	6 (0-12)	0 (0-0)	3.5 (0-7)	17.5 (10 -25)	15 (10-20)	6.5 (0-12)	0 (0-0)
Diarrhoea (0.846***)	7 (5-8)	0 (0-0)	0 (0-0)	23 (15-30)	0 (0-0)	8 (5-10)	20 (15-25)
Liver Involvement (0.8564***)	20 (10-30)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Oculonasal Discharges (0.789**)	3 (0-7)	0 (0-0)	0 (0-0)	20 (15-25)	0 (0-0)	2.5 (0-5)	9 (0-18)

Cells showed median scores (range) for clinical sign against a corresponding disease; maximum obtainable score = 30; *W* = Kendall's coefficient of concordance (\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001). PPR= Pestes des petits ruminants

**Table 4.23: Matrix scores of clinical indicators for characterization of small ruminant diseases by pastoralists in Sokoto State, Nigeria**

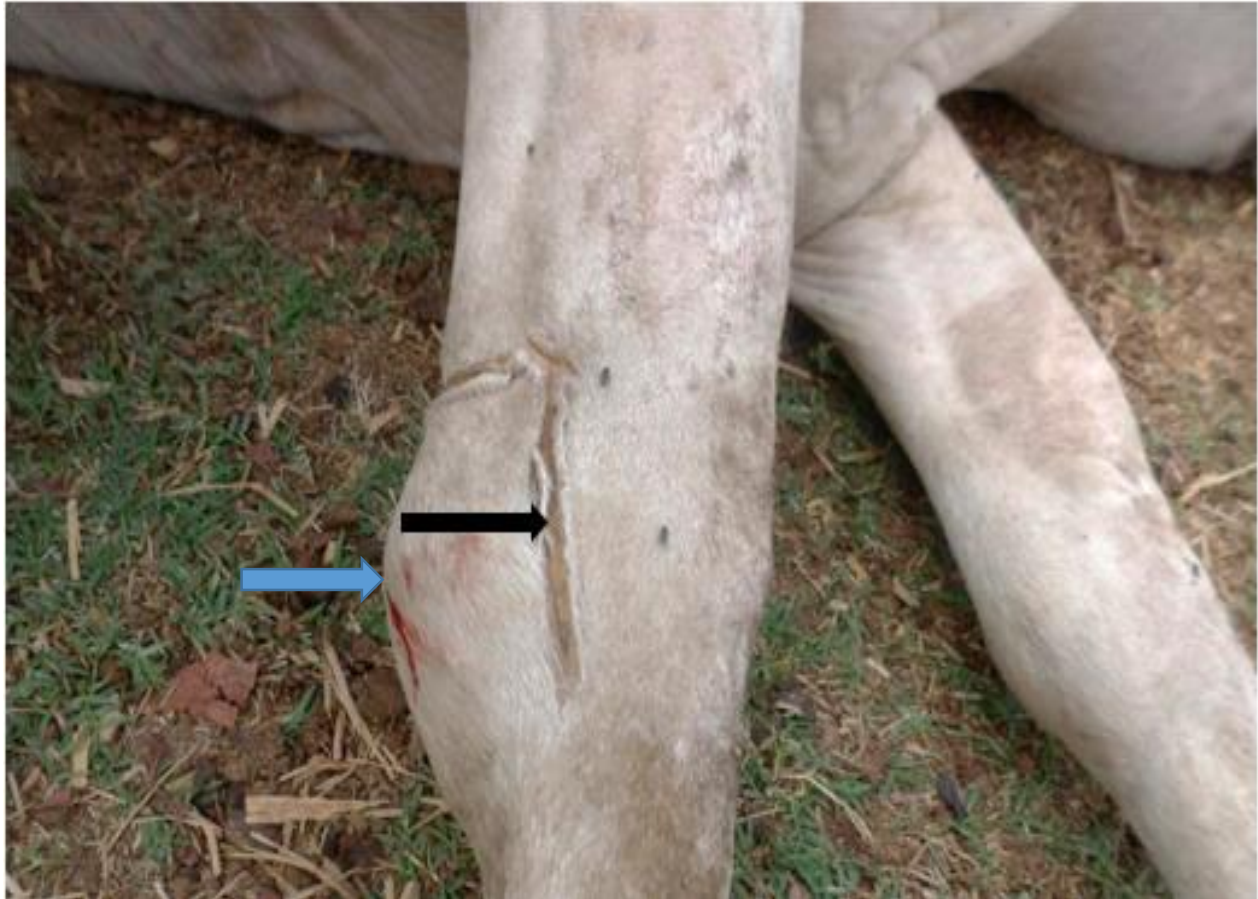
Clinical Indicators ( <i>W</i> )	<u>Diseases</u>						
	Pox ( <i>Ado</i> )	Fasciolosis ( <i>Balku</i> )	Brucellosis ( <i>Bakkale</i> )	FMD ( <i>Chabo</i> )	PPR ( <i>Dan kap</i> )	Helminthosis ( <i>Matsattsaku</i> )	catarrh ( <i>Mura</i> )
High morbidity (0.863***)	16 (12-20)	11 (12-20)	5 (0-8)	18 (10-25)	20 (15-25)	15 (5-25)	3 (0-5)
High mortality (0.920***)	2 (0-4)	3 (0-5)	2 (0-4)	6.5 (3-10)	27.5 (25-30)	3 (0-6)	2 (0-4)
Seasonal (0.333*)	3 (0-6)	7 (5-8)	1 (0-2)	4 (0-8)	10 (5-15)	5 (0-10)	10 (5-15)
Abortion (0.925***)	2 (2-3)	0 (0-0)	25 (20-30)	0 (0-0)	1.5 (0-3)	1 (0-2)	2 (0-3)
Respiratory Distress (0.647**)	10 (5-15)	0 (0-0)	0 (0-0)	0 (0-0)	18 (12-25)	0 (0-0)	15 (10-20)
Mouth Erosion/Salivation (0.862**)	2 (0-4)	0 (0-0)	0 (0-0)	25 (20-30)	11 (4-18)	0 (0-0)	0 (0-0)
Limping (0.933***)	1 (0-2)	0 (0-0)	0 (0-0)	27.5 (25-30)	0 (0-0)	0 (0-0)	0 (0-0)
Inappetence (0.825***)	12 (10-15)	15(10-20)	1 (0-2)	16 (15-20)	17.5 (15-20)	1.5(0-3)	5 (2-8)
Fever (0.604**)	5 (4-7)	8 (5-10)	1 (0-2)	12.5 (15-20)	22.5 (20-25)	0.5 (0-1)	4 (2-6)
Skin Involvement (1.000***)	30 (30-30)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Liver involvement (0.934***)	0 (0-0)	25 (20-30)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Diarrhoea (0.719**)	0 (0-0)	5.5 (3-8)	0 (0-0)	0 (0-0)	25 (20-30)	15 (10-20)	0 (0-0)

Cells showed median scores (range) for clinical sign against a corresponding disease; maximum obtainable score = 30; *W* = Kendall's coefficient of concordance (\**P* < 0. 5; \*\**P* < 0.01; \*\*\**P* < 0.001). PPR= Pestes de petit Ruminante; FMD = Foot and Mouth Disease

#### 4.12 Pastoralists' Existing Veterinary Knowledge

Using the SSI as a PE tool, pastoralists in the villages visited reported that, sequel to lack/inadequate veterinary care in most of the villages in Katsina and Sokoto States, they resorted to using herbs and other traditional means in treating livestock ailments. They also strongly believed that these traditional treatment methods were more effective, readily available and quite cheaper than the orthodox medicine. For traditional treatment of brucellosis in Bakori and Funtua LGAs located in the southern part of Katsina State, firing of the knee in case of hygroma was the practice (Plate III), after which a powder of 'Rai dore' (*Senna occidentalis*) (Plate IV) was applied on the wound site and left to heal naturally. Some other pastoralists in the same area reported drenching affected animals with a solution of the same powder. However, in Baure and Daura LGAs also in Katsina State, pastoralists believed that the cure for brucellosis was achieved by recitation of some verses of the Holy Qur'an on the afflicted animal. Pastoralists in both states under study had traditional remedies for other livestock ailments (Appendix 3).

Also, in Yabo and Tambuwal LGAs of Sokoto State, traditional treatment of brucellosis was done by drenching afflicted animal with a medicinal plant, 'Loda' (Plate V) in its powdered form.



**Plate III: Hygroma (blue arrow) and mark due to firing on the left knee (black arrow) as a method of treatment of brucellosis in cattle in Zamfarawa village in Bakori LGA of Katsina State**



**Plate IV: 'Rai dore' (*Senna occidentalis*), plant with the leaf (arrow) which is used for traditional treatment of brucellosis in Rafin Kanya Ward of Funtua LGA of Katsina State**



**Plate V: 'Loda' plant with leaf (arrow) which is used for traditional treatment of brucellosis in Tambuwal and Yabo LGAs of Sokoto State.**

#### **4.13 Transect Walk**

A walk through some of the villages visited verified most of what were revealed during the SSI by the pastoralists. These included lack of feed, inadequate housing facilities as both small ruminants and dogs co-habituate with dogs (Appendix IV), animals were spending several hours grazing in the sahelian vegetation without water. Most importantly, several practices in such villages which could be potential dangers to spread of brucellosis between animals and even to humans were noticed. These included amongst others, the communal grazing practice (Plate VI), presence of animals with hygroma (sign of brucellosis) in the herd (Plate VII), construction of pastoralists abode very close to where their animals were kept (Plate VIII) and keeping animals close to wells where both humans and animals took their drinking water (Plate IX).



**Plate VI: Collation of different livestock species (cattle (A), sheep (B), goat (C)) ready to go for communal grazing in Illela, Sokoto State.**



**Plate VII: Hygroma in the left knee of a bull (arrow) at Zamfarawa village in Bakori LGA of Katsina State**



**Plate VIII: Pastoralists abode (A) alongside cattle (B) and sheep (C) in Gidan Tanko Ward of Bakori Local Government Area of Katsina State**



**Plate IVIII:** Behind a focus group interview session are animals (A) located close to grain storage facilities (B) and a well (C)

## CHAPTER FIVE

### 5.0 DISCUSSION

The findings in this study demonstrate the existence of *Brucella* organisms in small ruminants in the study areas with a sero-prevalence of 13.5% obtained for each of Katsina and Sokoto States. The sero-prevalence of 13.5% of *Brucella* infection using *B. melitensis* antigen in each of the two states under study was quite higher than the overall sero-prevalence of 2.83% reported by Ogugua *et al.* (2015) in a study they carried out in Benue, Borno, Oyo and Sokoto States. However, comparing this study with individual state prevalence from the study by Ogugua *et al.* (2015), the finding in this study was lower than the 17.30 % reported for Benue State and quite higher than the 0.00% they reported for Sokoto State.

Similar research conducted in Sokoto State by Junaidu *et al.* (2010) using *B. abortus* antigen revealed a sero-prevalence of 10.9% for goats. Other reports on sero-prevalence of small ruminants' brucellosis in northern Nigeria include those of Okoh (1980), Bale *et al.* (1982) and Bale *et al.* (2003) who reported sero-prevalence of 14.5%, 14.1% and 15.9% from studies conducted in Kano, northern Nigeria and seven government farms in northern Nigeria respectively. These reports are comparable to the 15.8% and 17.7% obtained for sheep and goats in Katsina and Sokoto States respectively in the present study. However, higher sero-prevalence (25.8%) was reported by Kaltungo *et al.* (2013) for small ruminants in the North Senatorial District of Kaduna State while 35.2% was reported by Ya'u (2014) for small ruminants in Bauchi State. Evidence of *Brucella* antibodies in small ruminants as obtained in this study is not surprising as these animals under the holdings of pastoralists are normally herded together with cattle. In addition, the husbandry and management practices by these pastoralists that allowed different animal species to mix freely could easily facilitate disease transmission and spread among livestock species (Saidu

*et al.*, 1991; Kaltungo *et al.*, 2013; Buhari, 2014, Baba, 2016). As for the small ruminants in rural settings, the flocking system, especially during the dry season whereby animals are allowed to roam about freely in and around the villages could lead to these animals coming in contact with infected animals within the community (Saidu *et al.*, 1991). Besides, the fact that different livestock species were seen to be gathered for the purpose of communal grazing in this study could further facilitate the transmission and even spread brucellosis among these animals. During such contacts, bodily discharges like vaginal discharges from recently aborting animals, urine or other discharges from infected animals which are very rich in *Brucella* organisms could contaminate grazing fields and expose susceptible animals to risk of infection in the light of report of Bercovich (1998) that the organisms could survive for long periods in manure. Also, some practices by pastoralists whereby they hang aborted foetuses and placentae on trees or leave them unattended to may lead to contamination of the environment thereby favouring the dissemination of the organism in the environment (Kaltungo *et al.*, 2013). This may also promote the spread of infection to susceptible animals.

The variations in sero-prevalence rates obtained in the study could be attributed to differences in geographical locations, sample size, sources of animals sampled, sampling techniques and individual differences in interpretation of tests results.

This study similarly demonstrated co-infections with *B. abortus* and *B. melitensis* in some of the sampled sheep and goats in Katsina and Sokoto States, indicating the possibility for animals to harbour both pathogens concurrently. It also indicates that there could be a cross-infection between different animal species by herding different species of animals together. Not only that, it could indicate the potentials for reduced livestock production and productivity, especially that brucellosis is characterized by abortion, stillbirth, neo-natal death and orchitis (EC, 2001).

The LGAs where the flocks/herds were located and the ages of the animals were found to be significant for co-infection. This may have far reaching implications, not only on livestock development, but also on human health and productivity. This is particularly important due to involvement of *B. melitensis* which has been reported to be the most pathogenic species for humans (Nasinyama *et al.*, 2014). The reports of *Brucella* infections in other parts of Nigeria have similarly indicated that *Brucella* spp. are widespread in livestock in Nigeria and have been long standing (Bale *et al.*, 1982; Bale *et al.*, 2003; Ocholi *et al.*, 2004; Kaltungo *et al.*, 2013; Buhari, 2014).

The study has further established the highest sero-positive rates for co-infection in sheep and goats at Ward levels in the two states under study at 29.0% and 25.16% all from Tumfushi ward in Baure LGA using *B. abortus* and *B. melitensis* antigens respectively. The fact that small ruminants are a major source of animal protein in rural and semi-urban dwellings in Nigeria could further sound a threat to human health and productivity. This may be true in view of the fact that less is known about small ruminant brucellosis by livestock owners in Nigeria (Kaltungo, 2012). Also, the sociocultural belief of some rural populace whereby raw goat milk is being consumed for medicinal purposes which may sometimes be taken directly from the udder of the animal. This, along with the habit of consumption of millet dough with soured milk ('fura da nono') could be a potential zoonotic risk (Kaltungo *et al.*, 2013; Buhari, 2014; Ibrahim, 2016). In addition to these, the common habit/practice of these people in taking delicacy in roasted meat ('Balangu/agashe') or dried meat ('kilishi') which in most cases are partially done may expose such consumers to *Brucella* infection.

The study has similarly confirmed sero-positivity of both *B. abortus* and *B. melitensis* infections in all the breeds of sheep and goats found in the study areas. This could be understandable as the animals usually meet during grazing and watering, especially those under rural settings as they

were allowed to roam about in the villages and even those in towns since there is no livestock movement control in Nigeria (FAO, 2011), and this makes all the breeds of animals to have similar exposure potentials. The fact that livestock owners, pastoralists inclusive, add new animals into their herds without the necessary quarantine procedures could also result in the level of infection seen in all the breeds of animals used in this study (Kaltungo *et al.*, 2013, Buhari, 2014; Ibrahim, 2016).

Higher sero-prevalence recorded for co-infection in the females in this study is similar to the reports of like Bertu *et al.* (2010), Kaltungo *et al.* (2013) and Buhari (2014) who also reported a higher prevalence of *Brucella* infection in the females than males. This is expected as it is the males that are usually slaughtered during festivities like “Sallah” and naming ceremonies in the northern part of Nigeria which consequently reduce their population and makes them less available for sampling and also antibody detection. Moreover, female animals are usually retained longer in herds/flocks than males as they contribute in increasing herd or flock size therefore have more chances of acquiring the infection. Even though small ruminants are commonly used as poor man’s bank, females are rarely sold unless if they are less productive (Kaltungo *et al.*, 2013). Not only that, the organisms have more affinity for the female reproductive tract, especially when pregnant, due to the presence of erythritol which facilitates the growth of the organisms in the pregnant uterus which makes female animals particularly susceptible to the disease (Meador and Deyoe, 1989).

This study showed that older animals were more sero-positive for *Brucella* spp. than the younger ones. This finding agrees with the reports of earlier workers like Abubakar *et al.* (2010), Kaltungo *et al.* (2013) and Buhari (2014) who reported that susceptibility to *Brucella* infection increases with age. The younger animals especially the females may not be susceptible to infection with the

organisms as they are not reproductively mature as to begin reproductive life since the organisms require the sugar alcohol erythritol present in pregnant uterus of ruminants for multiplication (Meador and Deyoe, 1989). The findings in this study may translate to serious economic implications by way of losses due to reproductive wastages such as abortions, stillbirths, infertility, sterility, and reduced milk production since the older animals are more actively involved in breeding. Also, because of their involvement in breeding activities, they may help in the spread of the infection to susceptible animals within the same herd/flock or beyond since they mix freely among themselves from different flocks and even across villages. It is important to note also that there is the likelihood to sell animals from this age group for slaughter or for other economic or social reasons thereby serving as sources for human infections during processing.

There seems to be great public health risk in working closely with small ruminants in the study area because this study has revealed that a higher percentage of the sampled animals have severe infections with *Brucella* spp with titres of 1:80 and above. The danger lies especially in handling aborted fetuses and placentae in cases of abortions as these materials are very rich sources of *Brucella* organisms. The fact that, it is the sick/ unproductive animals that are sent for emergency slaughter further exposes the handler to risk of infection with this dreadful pathogen particularly if the animal is severely infected with *Brucella* (Shima *et al.*, 2015). Reports indicate that human infection and spread of the disease could be real as *Brucella* organisms have been reported to penetrate intact mucous membranes and skin (Mathew *et al.*, 2004; Addis, 2015). Therefore, it may not be surprising to find some evidence of *Brucella* infection in these livestock owners if investigated. It is therefore imperative for handlers especially of severely infected animals to take precautionary measures in handling animals of unknown status by wearing protective clothing.

This study reduced the possibility of doubtful results by the addition of EDTA which increases the specificity of agglutination by altering the pH of the serum to the isoelectric point of IgM to prevent its agglutination (Trap *et al.*, 1985; Radostits *et al.*, 2003). Consequently, EDTA modified SAT showed less false positive reactions than those obtained with the ordinary SAT as reported by Basyony *et al.* (2012). The fact that most of the small ruminants sampled were severely infected with *Brucella* organisms without overt clinical signs highlights danger to other animals and man. This is true because, these animals are capable of disseminating massive amounts of the organism in their vaginal discharges thereby contaminating the environment and endangering other susceptible animals. Therefore, these group of animals ideally should be slaughtered and processed in a designated abattoir with stringent precautionary measures put in place.

Determination of acute and chronic infection the IgG titre using the 2-MET technique in this study has shown that there were animals among those studied that had acute and chronic infections with *Brucella* organisms. This test has been identified as a confirmatory test that allows selective quantification of IgG anti-*Brucella* due to inactivation of IgM in the test sample. Production of IgG is usually associated with chronic infection, and therefore, a positive result with this test is a strong indicator of brucellosis. However, this test has some drawbacks including the toxicity of 2-mercaptoethanol, which requires a fume hood for its manipulation, and the possibility of IgG degradation caused by the 2-mercaptoethanol, which may result in false negative results (Poiester *et al.*, 2010). In the presence of acute and chronic infections with *Brucella* organisms, the economics of brucellosis in the study areas could be imagined. This is further buttressed by the fact that most of the animals involved were apparently healthy and do not give room for suspicion by the farmer. There is also the risk of these animals shedding the organism and contaminating the environment in case these animals abort or parturate naturally. The economic impact could be in

the form of loss of foetuses through abortion, neonatal death, stillbirth, and loss of milk for the suckling young where there was normal parturition (Addis, 2015).

Competitive ELISA (cELISA) was used as the confirmatory test in this study as it is an accepted test for serological confirmation of animal brucellosis by the OIE and also regarded as a 'gold standard' for confirmation of human brucellosis and an adequate test for the diagnosis of small ruminant brucellosis (OIE, 2009; Magwedere *et al.*, 2011). Also, when compared to the conventional agglutination methods, ELISA is more sensitive in acute and chronic cases of brucellosis and it offers a significant diagnostic advantage for brucellosis in endemic areas (Gerasu and Kassa, 2016).

A sero-prevalence of 4.0% and 13.0% was obtained for Katsina and Sokoto States respectively based on c-ELISA. These findings are quite lower than the overall sero-prevalence of 23.8% obtained by Ya'u (2014) from small ruminants in Bauchi State but comparable to the 13.5% obtained in goats in the same Bauchi State by the same person using the same test. However, Dogo *et al.* (2016) reported a lower sero-prevalence (2.5%) obtained in goats sampled from Giwa LGA of Kaduna State. It is generally observed that the level of sero-prevalence obtained using cELISA were quite lower than those obtained using m-RBPT. This may be due to the fact that RBPT being a screening test is more sensitive and less specific than the cELISA. The seeming differences in sero-prevalence in these locations could have something to do with variations in climatic condition and vegetation in the different locations by way of supporting the *Brucella* organisms off the host. This is supported by the reports of Davis and Casey (1773), Bale (1982) and Ogugua *et al.* (2015) that *Brucella* organisms can survive longer in cool environments along with other factors.

Geographical Information System technique has been found to be a valuable method that can be used to provide descriptive colour maps of disease burden in various areas and thus can serve as a visualized descriptive augmentations to estimated sero-positivity of diseases in pastoralists' herds/villages. The colour maps describing *B. melitensis* infected areas in Katsina and Sokoto States as shown in this study can undoubtedly support the surveillance/monitoring and possibly control/eradication programmes for brucellosis in these states. The IDW explicitly makes the assumption that, things that are close to one another, are more alike than those that are farther apart. Therefore, measured point has a local influence that diminishes with distance (Li *et al.*, 2014). This can go a long way in epidemiological surveillance and control of diseases. Also the IDW used for prediction can be very handy, especially in low income countries where sampling of a very large area may be very difficult due to lack/limited resources and also in due to inaccessibility of some areas of the country as experienced in the recent past in the North-Eastern part of Nigeria due to the insurgence of "Boko haram". Furthermore, the technology can be used when dealing with highly infectious agents where the researcher may be exposed to the agent by frequent sampling. This tool seems to be very handy as it is able to predict sero-prevalence rates in unsampled areas based on data obtained from some other areas. The results obtained in this study using GIS agrees with Rinaldi *et al.* (2009) and Haghdoost *et al.* (2007) who reported that the GIS is capable of integrating several spatial databases into a single environment for possibilities of improving surveillance and control programmes for infectious diseases and zoonoses. Musa *et al.* (2013) also reported that the most important application of GIS in the field is in the identification of disease clusters. Despite the fact that the technique has been adopted in the study of various zoonoses in the world (Kshirsagar *et al.*, 2013) its application is still in its infancy stage, especially in the northern part of Nigeria (Alhaji *et al.*, 2016). Except for the likes

of Alhaji *et al.* (2016) who used the technique to map out the burden of contagious bovine pleuropneumonia (CBPP) other pieces of work have been done using this technique in Ibadan, Nigeria, there does not seem to be any serious consideration of its use in disease surveillance and monitoring programmes in Nigeria (Babalobi, 2007).

From the study, it has been established that cattle constituted the largest proportion of livestock species kept by pastoralists in the study areas. This finding may be so because pastoralists attach more prestige and economic importance to cattle than small ruminants. Other livestock kept by these pastoralists included camels, poultry and donkeys and that these livestock species were seen to be mixed both at home and during communal grazing. The implication of this finding in relation to the result obtained from the serological analysis is that there is a risk of cross infection of *Brucella* organisms between different livestock species if it is so present. Also, these other species can further spread the disease to susceptible hosts in neighbouring villages and even across the country. This may be true, especially as the sero-prevalence of brucellosis has been reported to be on the increase in cattle population (Ocholi *et al.*, 2004). The fact that Salisu *et al* (2016) reported sero-prevalence of *Brucella* in camels in Katsina State supports this argument. Furthermore, the report of brucellosis in chicken in poultry in Nigeria by Adesiyun and Abdu (1984) can support the fact mixing of different animal species could facilitate the spread of the disease, brucellosis in a given community. Although, there is no available literature on brucellosis in donkeys in Nigeria, evidence of the disease in horses was reported by Baba (2016). Therefore, should *Brucella* organisms be present in these donkeys, there is the possibility of spread of the infection to southern part of Nigeria where these donkeys are delicacies for man and could result in serious consequences. Among the small ruminant species, goats were found to have a higher population

than sheep. Though, reasons for the discrepancy have not been fully elucidated (Dossa *et al.*, 2008), but logically, it may not be unconnected to the fact that goats have more multiple births than sheep. Also, the ovine species are more involved in festivities like ‘Sallah’ and naming ceremonies in the northern part of Nigeria which may consequently reduce their population. Keeping more of goats could also be explained by the fact that sheep have been reported to be more adversely affected by feed shortages than goats and unlike sheep, goats mostly feed near the homestead and can easily be fed with household wastes especially in times of feed scarcity (Okali and Sumberg, 1986).

The study has verified that the pastoralists derive several economic benefits from different livestock species they kept. These livestock are generally a means of survival in most villages visited. During the dry season, cattle, sheep and goats settle on their farmlands and consequently fertilize such farmlands thereby reducing the cost and risk of using inorganic fertilizers (Jigme *et al.*, 2015). The study also showed that work bulls were used for transport and draught on farms and also served as sources of income for the owners as they are being lent to other farmers. This practice may be beneficial to the farmer, but from the health perspective to other animals, it may be a potential risk for the spread of diseases like brucellosis since this bull may have the opportunity of mating with female animals in other herds. Generally, most of the animal species kept by pastoralists have multiple economic advantages, except for donkeys which were only used for transport. This report is in agreement with Khan *et al.* (2015) who reported that donkeys are mostly used for transportation of a variety of goods.

Pastoralists in Katsina State identified cattle rustling, effects of transhumance, watering points, lack of/inadequate shelter, diseases, cost of medication and feeding as husbandry problems that affected livestock production. Of these problems, cattle rustling was ranked number one. This agrees with Suleiman *et al.* (2015) in a study in neighbouring Kaduna State who also identified

cattle rustling as a serious problem that could have a grave impact on the sustainability of pastoralism if left unchecked. This form of livelihood crime in Nigeria have been attributed to ‘subsistence’ and ‘commercial’ imperatives (Gueye, 2013). However, from studies in Uganda and Kenya, cattle rustling was said to have a bearing with loss of population resilience and was therefore devised as a means of wealth redistribution amongst pastoralists (Bond, 2014).

Pastoralists in their own way seem to have some understanding of epidemiology. They could observe that whenever transhumance pastoralists from neighbouring villages in Niger Republic had a stopover in their villages most of their livestock species became sick, though they were not able to identify specific diseases brought about by these transhumance pastoralists. Some other factors identified by these pastoralists like problems of livestock rearing were similar to those identified by Majekodunmi *et al.* (2013) in Jos Plateau, Nigeria.

As for pastoralists in Sokoto State, similar problems were identified like those of their counterparts in Katsina State. However, in Sokoto State, pastoralists could detect that each time transhumance pastoralists had a stopover with their camels from neighbouring villages in Niger Republic, most of their livestock species come down with ‘Chabo’ (FMD). Though there is paucity of information on the presence of FMDV in the dromedaries, Ularamu *et al.* (2015) reported the first evidence of this virus in camels sampled in different geopolitical zones of Nigeria. It is, however, contrary to the findings of Warney and Kaaden (2004) who reported that camels tested in Africa and the UAE were found to be serologically negative for FMD. In another study by Wernery and Kinne (2012), it was reported that dromedaries were not susceptible to FMD and did not transmit infection, even when in close contact with susceptible animals. These controversies may be subjected to future investigation in Nigeria to truly ascertain the role of the dromedaries in the epidemiology of FMD.

Brucellosis was ranked 6<sup>th</sup> in importance amongst diseases affecting small ruminants in Katsina and Sokoto States. This low ranking may be because losses due to brucellosis in small ruminants are subtler than those due to other diseases like Peste des Petites Ruminants (PPR) which are more glaring. Besides, it has been reported that most pastoralists have little or no knowledge of the disease in small ruminants (Kaltungo *et al.*, 2013). Despite the low ranking of brucellosis by pastoralists in both states, they could identify abortion as a major attribute of the disease. This perhaps may be an extrapolation of their knowledge from bovine brucellosis which they are more conversant with. This finding is very important and requires a keen attention because lochia being discharged after parturition is very rich in *Brucella* organisms and a potential source of contamination of pasture/feed and water thereby exposing susceptible animals to the infection. The matrix scoring was also able to reveal local names for other small ruminant diseases most of which depict the clinical presentation of such diseases and corresponds to the western case definition of such diseases.

Though pastoralists in the study were very knowledgeable about livestock diseases, their ability to relate particular clinical signs for some diseases could be said to be low as some of them ascribed high mortality and fever to brucellosis. Aldomy *et al.* (1992) reported that the main clinical features of *B. melitensis* infection in sheep and goats are abortion and birth of weak offspring that may die after parturition. The observation of Saidu *et al.* (1991) that pastoralists' knowledge of specific clinical signs of most livestock diseases was low could further be confirmed by what was obtained in this study. Also, they referred to anaplasmosis in small ruminants to be 'saifa' which literally means spleen in 'Hausa'. This coincidentally is the age-known nomenclature for anthrax in the same 'Hausa' language. This similarity in nomenclature could be due to the fact that both diseases

cause swelling of the spleen which might only be the lesion they use in identifying both diseases at port mortem.

The knowledge of the interviewees was quite appreciable in both states in terms of identification of a variety of remedies for different ailments of livestock. This finding agrees with that of Adekunle *et al.* (2012) who reported that livestock owners have a holistic understanding and approach in handling diseases and other problems in relation to their livestock than just the production aspect. They believe that, this forms the basis for, and complements the success of, all sustainable animal health-care programs in developing countries. For brucellosis in particular, firing of hygroma with subsequent application of 'Rai dore' (*Senna occidentalis*) powder was believed by the interviewees to have a curative effect as revealed by pastoralists in Zamfarawa Ward in Katsina State. This agrees with the report of Disa, (2016) that firing was remedy for brucellosis as revealed by farmers in Yobe State. Firing of tissues induces an active inflammatory condition which subsequently results in increase in blood supply to the area with subsequent increase in nutrient supply thereby enhancing the healing process. The heat due to firing may also kill the bacteria and restrict their dissemination. It may, however, not actually eliminate the *Brucella* organisms from the host tissues or organs. Besides, in the process of firing, the *Brucella*-rich hygroma fluid may accidentally sip out thereby contaminating the environment and exposing other animals and man to danger of infection. Chanting of verses of the holy Quran by pastoralists in Baure and Daura LGAs of Katsina State for cure of brucellosis is a long existing practice which is in agreement with the reports of Padmakumar (1998) and Adekunle *et al.* (2002) who stated that it is a magic or religious means of treating diseases. It was also recited before animals leave the enclosure and may sometimes be worn as amulets on the animals.

From the study, the interviewees reported using many plants for the treatment of livestock diseases. This could be due to transfer of traditional knowledge from the old to the young folk as reported by Kaltungo (2012), Buhari (2014) and Abdullahi *et al.* (2017). Their knowledge on treatment against brucellosis was, however, low as they reported very few plants to be used against the disease. This could be due to the insidious nature of the disease and as such affected animals may not be often presented for medical attention even though, at the long run, it has a high economic impact.

The findings from the PE in this study agrees with those of other researchers who reported that despite limited level of formal education of pastoralists, they are key in helping researchers in the investigations relating to knowledge of local diseases, their symptoms and local remedies to such diseases. They also help in designing plans and initiatives in surmounting their local problems (Catley *et al.*, 2012; Disa, 2016).

## CHAPTER SIX

### 6.0 CONCLUSIONS AND RECOMMENDATIONS

#### Conclusions

The study came out with the following conclusions:-

1. The animals sampled in Katsina and Sokoto States had antibodies to *Brucella melitensis* based on m-RBPT with sero-prevalence of 13.5% in both States.
2. There were mild (4.11% and 3.3%) and severe (11.3% and 6.3%) infections with *Brucella* species in small ruminants sampled based on SAT-EDTA in the two States respectively.
3. Small ruminants sampled recorded 76.5% and 77.0%; 23.5% and 23.0% for acute and chronic infections respectively in Katsina and Sokoto States based on 2-Mercaptoethanol Test (2-MET).
4. Sero-prevalence by c-ELISA in Katsina and Sokoto States respectively were higher in females (17.5 %) than in males (14.3 %) and also highest in the old (20.7%), followed by the mature (16.0%) and then the young (13.3 %).
5. Co-infections of *B. abortus* and *B. melitensis* at 4.1% and 5.0% in Katsina and Sokoto States respectively indicated that small ruminants were exposed to both *B. abortus* and *B. melitensis* in the two States.
6. Lack of watering points, effects of transhumance, , lack/inadequate shelter, diseases, cost of medication and feeding were factors militating against livestock production in Katsina and Sokoto States. Cattle rustling and conversion of cattle routes to cropland were peculiar to Katsina and Sokoto States respectively.

7. Small ruminant diseases commonly encountered by the respondents in Katsina state in order of importance were PPR (*gishu/gurda*), piroplasmosis (*saifa*), fasciolosis (*balku/hanta*), helminthosis (*matsattsaku*), CCPP (*huhu*), brucellosis (*bakkale/bari*), tick infestation (*kaska/koti*), footrot (*ciwon kofoto*) and arthritis (*kumburin guiwa*). While in Sokoto State they were: FMD (*chabo*), pox (*gaye/ado*), PPR (*iska/dan kap*), helminthosis (*matsattsaku*), fasciolosis (*balku*), CCPP (*huhu*), kata (*mura*), brucellosis (*bakkale/bari*) and footrot (*ciwon kofoto*).
8. Pastoralists were able to recognize diseases encountered in their locality by their clinical presentation and also had an appreciable understanding and approach to treatment of livestock diseases and other problems than just livestock production.
9. The PE has been found to be a handy method of obtaining reliable information directly from sources. It also has added advantages of fastness in gathering information, cheap and ensures confidence of the respondents from whom the information is sourced.
10. The PE stands to reveal rare source of traditional knowledge that could be used to develop orthodox medical approach in animal health care delivery.

## 6.1 Recommendations

1. Detailed epidemiology of *Brucella* infections, not only in small ruminants, but also in other livestock species in Katsina and Sokoto States should be conducted.
2. Isolation and characterization of circulating *Brucella* sp. in the study areas should be carried out and that serological surveys involving other livestock species and high risk groups like the pastoralists, veterinarians, butchers among others should be carried out, in order to assess the extent of the spread of *Brucella* infection in Katsina and Sokoto States.
3. Sero-spatial maps of *Brucella* infections in all states of Nigeria should be institutionalized as an element of active surveillance and control strategies as it provides visual pictures of the disease situation.
4. The colour map imageries deduced from the GIS could serve as visualized descriptive augmentations to the estimated sero-positivity of brucellosis in pastoralist settings in the areas studied. Thus, it can be used for decision-support systems for animal/human health policy makers by identifying areas in need of resources, and make decisions on resource allocation.
5. Small ruminant owners should be educated on the dangers of brucellosis in their animals and themselves, and the Governments in Katsina and Sokoto States and Federal should provide veterinary extension services with a view to educating livestock owners on animal diseases, especially zoonotic ones, in order to avert outbreaks of livestock diseases and improve on livestock development through technology transfer.
6. Researchers in livestock health should give a voice to members of rural communities to enhance understanding of animal health problems in their localities and the options for their prevention, control and surveillance.

7. The active ingredients in the plants identified by the pastoralists should be identified with a view to developing such plants into standard drugs as to enhance animal health care delivery in the study areas and in Nigeria as a whole.

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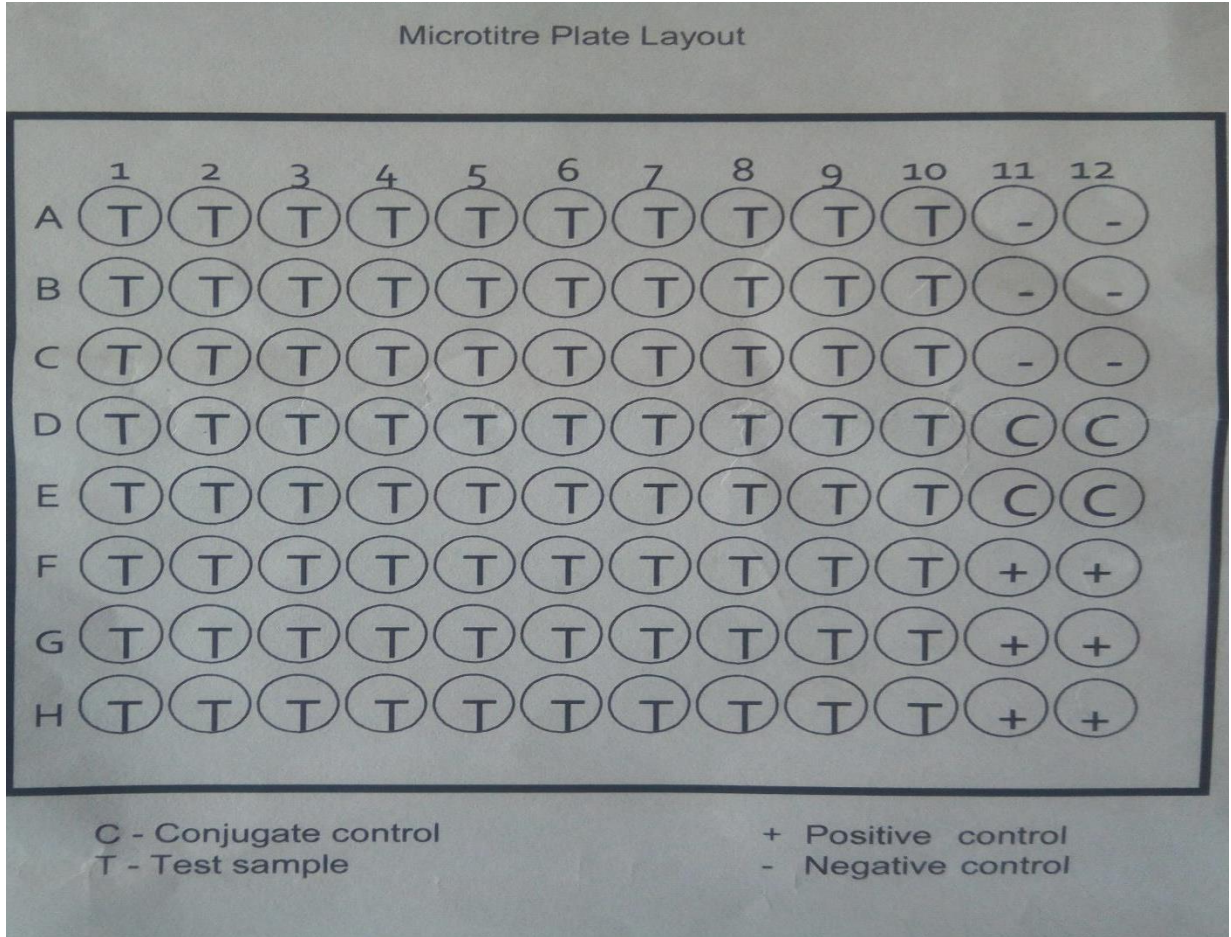
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# APPENDICES

## Appendix 1: Microtitre plate layout



## **Appendix 2: Checklist**

1. Introduction and purpose of visit (SSI)
2. Livelihood activities (SR, PP)
3. Livestock species kept (SR, PP)
4. Small ruminant husbandry practices and challenges (SR, PP)
5. Small ruminant diseases (local names) (SR, PP)
6. Clinical case definition of diseases (DIMS)
7. Access to veterinary services and traditional management of livestock diseases (EVK)
8. Questions and comments from the community
9. Transect walk and/or visit to households with a history of abortions.

**Appendix 3: Ethno veterinary remedies for livestock ailments in Katsina and Sokoto States, Nigeria.**

Ailment	Ethno veterinary approach	Route of administration
Retained Placenta	1. Crushed ‘Tsamia leaves’ ( <i>Tamarindus indica</i> ) in water.	Oral
	2. Crushed ‘yadiya’ ( <i>Lapadenia histata</i> )	Oral
	3. Soaked ‘tsaban gatari’ (metal part of an axe) with ‘ban adu’a’ ( <i>Balanites aegyptiaca</i> ’ bark)	Oral
Diarrhoea	1. crushed leaves of ‘kuka’ ( <i>Adansonia digitata</i> ) in water.	Oral
Bloat	Mixture of ‘gogai’ in groundnut oil	Oral
PPR	1.Mixture of ‘kanwa’ (pottash) with ‘tafarnuwa’ ( <i>Allium sativa</i> )	Oral
FMD	1. Solution of pounded ‘Bagaruwa’ ( <i>Acacia nilotica</i> ).	Oral
	2.Pounded onion	Topical
	3. Paste of Lime and ‘Bagaruwa’ ( <i>Acacia nilotica</i> ).	Topical
	4. Fermented ‘tuwo’ (Cooked grain)	Topical
Fasciolosis	1. Pound dried leaves of ‘garahoni’ ( <i>Mormodica charanlia</i> ) with ‘madaci’ ( <i>Khaya senegalensis</i> ) and add water.	Oral
Sheep/Goat pox	1.Solution of raw cow milk and ‘kuka’ ( <i>Adansonia digitata</i> )	Oral

**Appendix 4: Inadequate housing (arrow) facility for animals in Illela Ward in Illela Local Government Area of Sokoto State making small ruminants to mix with dogs**

