# SURVEY OF BRUCELLOSIS IN SHEEP AND GOATS IN KADUNA NORTH SENATORIAL DISTRICT OF KADUNA STATE, NIGERIA

**BY**

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**M.SC./VET-MED/11994/2010-2011**

**A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE AWARD OF MASTERS OF SCIENCE IN VETERINARY MEDICINE**

**DEPARTMENT OF VETERINARY MEDICINE, FACULTY OF VETERINARY MEDICINE, ABU, ZARIA**

**FEBRUARY, 2013**

## DECLARATION

I hereby declare the originality of this work carried out by me in the Department of Veterinary Medicine, Ahmadu Bello University, Zaria under the supervision of Dr. S.N.A. Saidu, Professors A.K.B. Sackey and H.M Kazeem. The works of other investigators referred to in this study were duly acknowledged. No part of this thesis has been submitted previously for a degree or diploma at any university.

…….. ..... ……

Bilkisu Yunusa KALTUNGO Signature Date

## CERTIFICATION

This thesis titled "**SURVEY OF BRUCELLOSIS IN SHEEP AND GOATS IN KADUNA KADUNA NORTH SENATORIAL DISTRICT OF KADUNA STATE, NIGERIA" by** Bilkisu Yunusa KALTUNGO meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

| | | |
|---|---|---|
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## DEDICATION

This thesis is dedicated to my husband and children Halima, Aisha, Zainab, Khalil and Sulaiman for their understanding and support.

## ACKNOWLEDGEMENTS

All praise is due to Almighty Allah, the Beneficent and the Merciful for sparing my life up to this moment and for giving me the strength to carry out this work. May His peace and blessings be upon our noble prophet Muhammad (SAW), amin.

I am highly grateful to my Supervisors, Dr. S.N.A. Saidu, Prof. A.K.B. Sackey and Prof. H.M. Kazeem for their inspiration, continuous encouragement and persistent push that made it possible for me to bring this study to a successful completion.

I am highly indebted to Prof. J.O. Ayo for his encouragement and professional advice, and to Prof. C.A. Kudi for his support and effort in helping me to source the test kits. I also appreciate Prof. J.O.O. Bale and Prof. I. Ajogi for their professional advice and also providing relevant materials that aided my review of the literature.

I must sincerely acknowledge the moral and encouraging support of my husband, Dr. M.I. Waziri and my children for their patience, particularly at the time I was away from home in the process of completing this work, which was tremendous and worthy of note.

My special gratitude goes to my sister Dr. Z.Y. Kaltungo and brothers Akilu and Hamza Y. Kaltungo for their immense support which went a long way in helping to see this work to an end.

I wish to express my top appreciation to the Staff of the Bacterial Zoonosis Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Mallam Suleiman Yahuza, Mrs J.C. Atawodi, Mr. M.A. Mgbegha, Mr M.B. Odoba and Mr. K.C. Iwuanyanwu for their excellent technical support during the conduct of the laboratory aspect of this work.

I wish to appreciate the effort and support of Dr. F. Fasuyi, Nasiru Gidado and all the Veterinary staff of the LGAs where I obtained my samples for aiding the success of the sampling exercise.

I am grateful to Dr. B. Usman and Dr. U.B. Abubakar for their concern and brotherly advice. I must specially thank Dr. E. Ngbede for his contribution to the success of this work and most especially in helping in the statistical analysis of the results.

I also wish to acknowledge all those who have contributed in various ways to the success of this work whose names have not been specially mentioned.

## ABSTRACT

Brucellosis is a major economic and zoonotic disease of domesticated and wild animals. A survey of brucellosis in sheep and goats was carried out in four randomly selected Local Government Areas (LGAs) of Kaduna State, Nigeria. A total of 1021 (579 sheep, 442 goats) serum samples were used for the study. Rose Bengal Plate Test (RBPT), Serum Agglutination Test with ethylene diaminotetraacetic acid (SAT-EDTA) and Lateral Flow Assay (LFA) were the serological tests used in the study. Brucella seroprevalence rates of 31% and 22%, 17% and 7% and 6% and 1% for sheep and goats were recorded using the RBPT, SAT-EDTA and LFA, respectively. The result further revealed a seroprevalence rate of 2% and 1% for sheep and goats respectively with an overall prevalence of 3% with respect to *B. melitensis*. There was no statistically significant difference in the prevalence rates in sheep and goats ($P<0.05$). Seventy two and 122 lactating sheep and goats had 7% and 17% seroprevalence respectively using Milk Ring Test (MRT). Specie prevalence was statistically insignificant ($P<0.05$) in this study. Knowledge, attitude, and practices of herdsmen as it relates to brucellosis were evaluated using 70 structured questionnaires. Forty one (59%) of flock owners had good general knowledge of brucellosis only in the bovine species, their knowledge of the disease in sheep and goats was almost none existent. Their major sources of awareness were experienced farmers (68%), the media (32%), and livestock professionals (7%). Only 7 (10%) knew brucellosis as a zoonosis, but knew little of its signs in human. Flock management systems were intensive (3%), semi-intensive (23%) and extensive (74%). The study also revealed high level of animal exchange that involved borrowing (56%) or and lending out (71%) for the purpose of breeding. Also, (81%) of respondents experienced cases of abortion in their flocks. Sixty eight percent of

abortion occurred in third, 33% in second and 7% in first trimesters, respectively. Seroprevalence and poor farmer's perception of brucellosis in sheep and goats in the study area necessitates Government's strategic eradication and stakeholder awareness policies.

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## LIST OF ABREVIATIONS

| | | |
|---|---|---|
| BPAT | □ | Buffered Antigen Test |
| CARD | □ | Card Test |
| CDC | □ | Center for Disease Control |
| CFT | □ | Complement Fixation Test |
| CSF | □ | Cerebro Spinal Fluid |
| FAO | □ | Food and Agriculture Organization |
| FPA | □ | Flourescent Polarization Assay |
| FPSR | □ | False Positive Serological Reaction |
| HIT. | □ | Heat Inactivation Test |
| IFAT | □ | Indirect Immunoflorescent Test |
| KAP | □ | Knowledge Attitude and Practice |
| KDSG | □ | Kaduna State Government |
| LFA | □ | Lateral Flow Assay |
| LGA | □ | Local Government Area |
| LPS | □ | Lipopolysaccharide |
| MRT | □ | Milk Ring Test |
| OPS | □ | O-polysaccharide |
| PCR | □ | Polymerase Chain Reaction |
| PMLs | □ | Polymorphoneuclear leucocytes |
| RBPT | □ | Rose Bengal Plate Test |
| RLPS | □ | Rough Smooth lipopolysaccharide |
| SAT | □ | Serum Agglutination Test |
| SAT-EDTA | □ | Serum Agglutination Test With Ethylene Diaminotetraacetic Acid |
| SLPS | □ | Smooth lipopolysaccharide |
| SPAT | □ | Standard Plate Test |
| TNF | □ | Tumour Necrosis Factor |
| USA | □ | United States of America |
| USDA | □ | United States Department of Agriculture |
| WHO | □ | World Health Organization |

# CHAPTER 1

# INTRODUCTION

## 1.1 SCOPE OF THE STUDY

Small ruminants form an integral and important component of the pattern of animal production in most rural communities in Nigeria. Sheep and goats are widely distributed in Nigeria from rural to urban and peri-urban areas, representing about 63.7% of 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 important because they require low initial capital and maintenance costs and are able to utilize marginal land and crop residues (Winrock International, 1983). They also produce meat and milk and are easily cared for by most family members. They form a source of income that could be easily mobilized for paying some of the household expenditures, particularly in lean times. Nigeria derives substantial foreign exchange from exporting goat skin for the international leather industry (Junaidu*, et al*., 2010). 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). They also provide the bulk of meat consumed in rural areas in Nigeria (Saidu *et al*., 1991).

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).

The disease is of economic importance as well as of public health significance (OIE, 2009). There is the need to effectively control the disease in farms in order to ensure optimum production, economic return to production and protection of public health.

## 1.2 STATEMENT OF THE PROBLEM

Small ruminants in Kaduna State and Nigeria provide employment for both rural and urban dwellers who engage in the production and marketing of livestock and their by-products. They serve as a major source of animal protein. Brucellosis in sheep and goats has been reported in various parts of Nigeria (Falade, 1974, 1980; Bale, 1980; Ogundipe *et al.*, 1994). Apart from the grave zoonotic consequences, the economic losses associated with the disease are enormous and they include loss of offsprings, reduced milk production, and subsequent animal infertility (Esuruoso, 1974, 1977; Okoh, 1980; Chukwu, 1987). The endemicity of the disease in Nigeria prompted Esuruoso in 1976 to make use of available information on bovine brucellosis up to 1973 to draw up a brucellosis map in which he divided the country into areas of low to moderate as well as heavy infections (Ajogi, 1997). Yearly unconfirmed cases of abortion, stillbirth, and retained placenta have been reported in livestock in Nigeria and these might have been attributed to infection by *Brucella* species. (Falade, 1974). Brucellosis is a disease which can potentially affect livestock production, especially small ruminants, thereby affecting the supply of the much desired animal protein.

The prevalence of brucellosis in small and even large ruminants might constitute a significant hurdle for the development of the livestock industry in Nigeria. Therefore, early

and accurate diagnosis of the disease is important for initiating any effective control measure against the disease.

In most cases, humans contract brucellosis from animals unknowingly by direct contact with infected animals or their products, such as milk, milk products and meat or through handling animal deliveries. In humans the disease has both health and economic consequences, because infected humans often suffer from long term illness and incapacity which frequently prevents them from working efficiently (Nuru and Dennis, 1975).

This study is therefore aimed at determining the seroprevalence of *B. melitensis* in sheep and goats in four Local Government Areas in Kaduna North Senatorial District of Kaduna State, Nigeria.

## 1.3 IMPORTANCE OF THE PROBLEM

Small ruminants make up the bulk of the population of food animals in Nigeria totalling 51 million. This is made up of 28 million goats and 23 million sheep against the cattle population of 15.2 million (FAO, 2006). Kaduna State has an estimated population of 1,144,000 cattle, 832,000 sheep and 988,000 goats (KDSG, 2008)**.** The high small ruminant population in the State coupled with the socio-economical and cultural relationship between these animals and man (especially women and children) may predispose humans to infection from affected sheep and goats. Thus, this study is designed to determine the status of the disease in small ruminants in four LGAs from Kaduna Kaduna North Senatorial District of Kaduna State.

## 1.4 JUSTIFICATION FOR THE STUDY

The major occupation of the rural population of Kaduna State is agriculture, mainly involving producing food crops and rearing of livestock (KDSG, 2008).

The fact that there is an uncontrolled movement of small ruminants kept by pastoral and rural populations and that there is an increase in the prevalence of brucellosis in cattle (Ocholi *et al*., 2004), the occurrence of the disease among sheep and goats could be expected. Unfortunately, there appears to be no available literature/research establishing the status of small ruminant brucellosis in the study area. Thus, there is the need to bridge this gap.

Sheep and goats serve as easy sources of animal based protein especially to the rural communities in Nigeria. Furthermore, goat milk is fast gaining acceptance worldwide because of some of its advantages over cow milk (Haenlein, 2011). Hence, it becomes necessary to evaluate the status of brucellosis in these species of animals, with a view to advicing all stakeholders of small ruminant production on the possible risks.

Unconfirmed cases of abortion and stillbirth, among others, in small ruminants are continuously being handled by farmers and animal health workers. These and many others pose a risk of human infection that calls for attention. It is therefore imperative to appreciate the status of brucellosis in these species of animals.

Diagnosis of brucellosis has been based mainly on serological tests such as Rose Bengal Plate Test (RBPT), Milk Ring Test (MRT), and Serum Agglutination Test (SAT), among others, which are not without limitations such as having false positive or false negative

results. However, a recently introduced serological test, the Lateral Flow Assay (LFA) developed by The Royal Tropical Institute in the Netherlands that uses more specific monoclonal antibodies, is gaining acceptance worldwide for its simplicity, rapidity, and sensitivity (Abdoel *et al*., 2008). This study was set to determine the prevalence of *B. melitensis* in sheep and goats in Kaduna Kaduna North Senatorial District of Kaduna State. Furthermore, it was also designed to provide information on the farmers' knowledge, attitude and practices with regards to brucellosis and the risk associated with the disease in these animals.

## 1.5 AIM OF THE RESEARCH

To determine the current status of brucellosis in sheep and goats in four LGAs in Kaduna Kaduna North Senatorial District of Kaduna State, Nigeria.

## 1.8 OBJECTIVES OF RESEARCH

1) To determine the sero-prevalence of brucellosis in sheep and goats in the Kaduna North Senatorial District of Kaduna State, Nigeria
2) To determine the farmers' knowledge, attitude and practices (KAP) towards brucellosis in sheep and goats using structured questionnaire.

## 1.9 RESEARCH QUESTION

Does brucellosis exist in small ruminants in the Kaduna North Senatorial District of Kaduna State and what are the farmers' knowledge, attitude and practices (KAP) with respect to the disease

# CHAPTER 2

# 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). Abdulkadir (1989) reported that the *Brucella* organism has a wide host range among domestic and wild animals, and that the disease is also known as infectious abortion, "slinking of calf", swine brucellosis and Bang's disease. In humans, it is called Constantinople fever as well as fevers of Malta, Naples, Cyprus, Crete, Crimea, Levant, Syria, Mediterranean, Gilbraltar, Gastric, Undulant, milk, Remittent, Relapsing and Rock (Wikipedia, 2012).

A British Army surgeon, stationed on the Mediterranean Island of Minorca in Malta, described cases of chronic relapsing febrile illness in humans and cited Hippocrate's description of a similar disease more than 2,000 years earlier (Cleghorn, 1950). Vassallo (1992) reported that in 1863, an assistant Surgeon, Jeffrey Alien Marston, gave a very accurate description of the illness in troops invalided to Malta during the Crimean War, calling it "Mediterranean Remittent (or) Gastric Remittent Fever". He further reported that, in 1879, Surgeon Major H. Veale reported from the Royal Victoria Hospital at Nutley on Southampton Water on fever patients invalided from Gibraltar, Malta and Cyprus, pointing out the distinctions between this fever and malaria, enteric fever, and relapsing fever. 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). In 1893, Bruce named the organism *'Micrococcus melitensis*' from the Roman name for Malta. However, adding to the success of Sir David Bruce's work was Dr. Carruana-Secluna who played an important technical role in culturing the organism even though his name was not mentioned as a co-author (Rust, 2006). In the same year, 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). In 1897, a Danish veterinarian, Bernhard Bang isolated *Brucella abortus* as the cause of abortion in cattle and named it ''Bacillus of abortion".

The great breakthrough came in June 1905 when Dr (later Sir) Themistocles Zammit, successfully incriminated the Maltese Goat 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. 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.

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 Newzealand and Australia. *Brucella neotome* 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. More recently, another *Brucella* species has been isolated from marine mammals and was proposed to be named *Brucella maris* (Anon, 1997).

The popular name, undulant fever, originates from the characteristic undulance (or "wave-like" nature) of the fever, which rises and falls over weeks in untreated human patients. In

the 20th Century, this name, along with brucellosis, gradually replaced the 19th 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 AETIOLOGY

*Brucella* is a generic name for a group of organisms which is part of the α-2 subgroup of the Proteobacteriaceae. It 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 Terrestrial Manual, 2009).

The organisms are Gram-negative, facultative intracellular bacteria (Corbel, 1997b; Young, 2000). 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). Young (1995) reported that the organisms are strictly aerobic, non-encapsulated, and catalase and oxidase positive. They do not ferment carbohydrates and have variable urease activity. 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).

### 2.2.1 Taxonomic Tree

The genus *Brucella* (51BRUC) belongs to the family Brucellaceae (41BRUC), order Rhizobiales (31RHIZ), class Rhodospirilli (21RHOD) in the kingdom Proteobacteria (01PROT) (Wikipedia, 2013).

### 2.2.2 Morphology

*Brucella* organisms are small (0.5-0.7 by 0.6-1.5μm), non-motile, non-spore forming, non-encapsulated, coccobacilli which occur singly or more rarely in group of short chains or clusters (Ryan and Ray, 2004). Similarly, they do not have flagella or pilli and do not show bipolar staining. They are not truly acid fast but resist discoloration by weak acids, thus staining red with bluish background when Stamp's modification of Ziehl-Nelsen stain is used (Anon, 2001). The cellular and colonial morphology of the species of *Brucella* are similar in most respect (Deyoe, 1981). There are six well known species of the genus which include *B. abortus, B. melitensis, B. ovis, B. canis, B. neotome* and *B. suis* which are further divided into biotypes (Alton *et al*., 1975). More recently, 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 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 mandibular lymph nodes of wild red foxes (*Vulpes vulpes*) in Austria. An unnamed strain (*Brucella spp*. NVSL 07-0026) was recently isolated from a baboon (Wikipedia,

2012a). These reports therefore indicate that there is still a wide range of information to be explored regarding the genus *Brucella* and its host range.

*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).

## 2.3 HOST RANGE

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

A report from OIE (2009) stated that *Brucella melitensis,* has three biovars that mainly infects 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. Infections in sheep and goats can spill over into wild ruminants. Also from the same report it was found that, 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 host for *B. abortus* which is classified into seven biovars, namely biovars 1-6 and 9 (Mariana *et al*., 2010). *Brucella abortus* can also infect buffaloes, camels, deer, dogs, horses, goats, sheep, and man (Kudi *et al*., 1997). Other species of animals reported to be susceptible to *B. abortus* are turkeys, pigeons, pheasant, duck, goose, and chicken (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. However, 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. However, this pathogen 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). Nontheless, swine can also be infected by other *Brucella* species other than *B. sui* 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 seal (*Phoca vitulina*), dolphins (*Tursiops truncates*, *Delphinus delphis*, *Lagenorhynchus acutus* and *Stenella coeruleoalba*), whale (*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 recently identified *Brucella* species isolated from marine mammals, *B. ceti* and *B. pinnipedialis*, can also cause human brucellosis (Foster *et al*., 2007). It was also reported by Godfroid *et al*. (2005) that importantly, *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.

## 2.4 PATHOPHYSIOLOGY

According to Olsen *et al.* (2010), *Brucellae* are intracellular pathogens that localize in lymphoreticular cells throughout the body and have a predilection for reproductive tissues. Upon entry of the bacteria through the plasma membrane, they modify the endosomal compartment of phagocytic cells to allow replication and long-term survival, using various mechanisms to modify the host environment, resist oxidative killing, and modify their metabolism to survive in their preferred intracellular environment and that an unknown molecular and/or physiologic mechanisms may contribute to animal or species preference, recrudescence, and targeting of reproductive tissues in mammalian hosts.

Similarly, Anon. (2008) reported that shortly after gaining entry into the body, *Brucellae* are ingested by polymorphonuclear leukocytes (PMLs), which are attracted to the site of introduction. Lapaque *et al*. (2005) reported that it has been shown that the principal virulence factor for the organism is cell wall lipopolysaccharide (LPS). Normal serum factors, including complement, are involved in opsonization of the organisms to allow phagocytosis, but PMLs have limited ability to kill bacteria within phagocytes. Anon. (2008) reported further that a copper-zinc superoxide dismutase, O-polysaccharide, and nucleotide-like substances are among the factors that protect brucellae from being killed by PMLs.

*Brucellae* that are not killed by PMLs are ingested by macrophages, where they become localized within organs of the reticuloendothelial system (liver, spleen, bone marrow) and multiply in macrophages and monocytes. However, any organ or system can be involved in

brucellosis and these include the heart, joints, skin, the central nervous system, genitourinary system and the pulmonary system.

Shortly after infection, humoral antibodies directed against LPS and other cell wall antigens are produced. However, development of cell-mediated immunity is the principle mechanism of recovery. The host response to infection with *Brucella abortus* is characterized by the development of tissue granulomas indistinguishable from those of sarcoidosis. In contrast, infection with the more virulent species (*Brucella melitensis, Brucella suis*) more commonly results in visceral microabscesses (Anon., 2008). 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. In addition, *Brucella* species have relatively low virulence, toxicity, and pyrogenicity, making them poor inducer of some inflammatory cytokines such as tumor necrosis factor (TNF) and interferons. Also, they do not activate the alternative complement system. It is also thought that they inhibit programmed cell death.

After replication in the endoplasmic reticulum, the *brucellae* are released with the help of hemolysins and induced cell necrosis. Susceptibility to intracellular killing differs among species, with *B. abortus* readily killed while *B. melitensis* is rarely affected. This might explain the differences in pathogenicity and clinical manifestations in human cases of brucellosis (Lecaroz *et al*., 2006). Poole *et al*. (1972) reported that when the organism is introduced into a susceptible host, the bacterium penetrates the mucosal epithelium of the gastrointestinal tract and is transported, either free or within phagocytic cells, to regional

lymph nodes. If these bacteria do not remain localised or are not killed, they can spread to other organs, joints and bursae. This bacteraemic phase is subclinical and may take several weeks to some months (Animal Health Australia, 2005). The bacteria then localize in the pregnant uterus and udder or the testicles and accessory sex glands of the female and male host respectively. In pregnant cows, the chorioallantoic membrane becomes inflamed and ulcerated.

The bacteria can also spread via the blood stream to the foetus and the placenta. 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, cotyledons and foetal. It was also reported by Cutler *et al.* (2005) that the interaction of the bacteria with placental trophoblasts suggests its ability to acquire iron is vital, as the brucellae enter their acute replicative stage if there is a placental disruption which results in foetal loss or birth of weak and infected offspring. They further reported that iron acquisition is altered in the attenuated vaccine strain of *B. melitensis*, (Rev1) when compared with the virulent strain (16M).

Erythritol is believed to be important for determining tissue tropism for *Brucella*. Mutants of *Brucellae*, unable to utilize erythritol, are severely attenuated in ruminant hosts. Also, in vitro data suggests that *Brucella* metabolizing erythritol have a heightened requirement for iron, 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). Congenital infection can occur in newborn calves as a result of *in utero* infection and the infection may persist in a small proportion of calves which may also be serologically negative until after the first parturition or abortion (Parkinson, 2009). In mature, nonpregnant cattle, the bacterium localizes in the udder (Animal Health Australia, 2005). Infection of the udder is often clinically inapparent, with no gross lesions.

*Brucellae* localize and replicate primarily in macrophages in mammary secretions or in phagocytes where they form an important source of organisms for periodic reinfection (and potentially for infection of calves and humans via the milk). In sheep and goats, following introduction of the organism, the bacteria moves via the lymphatic vessels to reach the regional lymph nodes and if local defences fail in controlling the infection, the uterus will become infected. In a more advanced phase, *B. melitensis* can colonize the udder of lactating goats, resulting in acute mastitis with the production of clotted and watery milk and reduced milk yield (Enright, 1990).

## 2.5 CLINICAL SIGNS OF BRUCELLOSIS

### 2.5.1Clinical Signs in cattle

Clinical signs vary according to the animal hosts and the infecting *Brucella* species (Megid *et al*., 2010). *Brucella abortus* is the main species that affect cattle (Anon., 2006). However, *B. suis* and *B. melitensis* infections can also occur in cattle that are in contact with infected pigs or goats but result in less severe disease (Anon., 2011). The period of

incubation varies considerably and it is mainly influenced by gestation, exposure dose, age, and vaccination status (Nicoletti, 1980). However, the length of the incubation period is inversely proportional to the stage of gestation at the time of exposure and could range between 53-251 days (Thomsen, 1950).

The major clinical sign in pregnant cows is abortion which usually occurs from the 5$^{th}$ to the 8$^{th}$ month of gestation, though Bang in 1906 observed an abortion in a three-month pregnant heifer 56 days after feeding with the cotyledons from an aborting cow (Megid *et al*., 2010).

According to Acha and Szyfres (2003) infected pregnant females usually abort only once after which they acquire immunity and that as an alternative to abortion, premature, stillborn or weak calves may occur. Abortion is often followed by placental retention and metritis, which may cause permanent or transient infertility. Importantly, some infected cows will not exhibit any symptoms of the disease and give birth to normal calves (Nicoletti, 1980).

The incubation period of brucellosis in bulls has not been clearly determined and that infection of the reproductive tract may lead to orchitis, epididymitis, ampullitis, and seminal vesiculitis. Orchitis is occasionally manifested, and when it occurs it is usually unilateral, but both testicles may be affected. Testicular atrophy may also occur (Plant *et al.,* 2008).

### 2.5.2 Clinical Signs in pigs

According to OIE (2009a), the most common manifestation of brucellosis in female pigs is abortion, occurring very early or at any time during gestation. Also vaginal discharge is not often evident, and, in chronically infected pigs, infertility rather than abortion is the most relevant clinical sign of the disease.

In males, brucellosis is more likely to be persistent, with lesions in the genital tract often leading to interference with sexual activity, which can be temporary or permanent (OIE, 2009a). Furthermore, the boar may excrete brucellae in the semen without any apparent abnormality in the sex organs or interference with sexual activity. In both sexes, there may be swollen joints and tendon sheaths which may develop into lameness and, occasionally, posterior paralysis (Holyoake, 2010). Abscesses of different sizes frequently occur in organs and tissues (Acha and Szyfres, 2003).

Deyoe (1986) reported that the rate of abortion is higher in sows or gilts exposed to *B. suis* via the genital tract at the time of breeding and that abortions are influenced more by the time of exposure to the *B. suis* rather than by the stage of gestation. Furthermore, embryonic resorptions have been observed as early as 17 days following natural mating by boars disseminating *B. suis* through their semen. Little or no vaginal discharge is observed in early abortions. Affected sows rarely have a second abortion, and those infected before sexual maturity hardly ever abort (Acha and Szyfres, 2003).

It has been reported by Dayoe (1986) that some infected boars may not develop any localized genital infection. However, boars that do develop genital infection seldom recover

from it. Infertility and lack of sexual activity may occur in infected boars and is frequently associated with testicular involvement. More commonly, boars have infections in their accessory genital glands. However, this does not necessarily reduce fertility (Deyoe, 1986). Infection of the genital organs may last for a shorter period of time in the female than in the male (Acha and Szyfres, 2003).

### 2.5.3 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, and giving birth to weak offsprings who usually die during the peripartum (Aldomy *et al*., 1992). They also reported that animals generally abort only once. Alton (1990) observed that abortion in female goats occurred between 3 and 4 weeks after being experimentally infected with high doses of *B. melitensis*. However, in sheep, abortion may occur between 4 to 12 weeks after being experimentally infected and it seems that ewes are more resistant to abortion than does.

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. Also, intermittent shedding of the agent in milk occurs in animals with persistent infection of the udder. This may result in inflammation of the udder leading to reduced milk production in infected animals (The European Commission, 2001).

In rams and bucks, the infection can produce inflammation of the genital organs, 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. In the chronic stage, hygromas and joint inflammation can be observed in bucks (Enright, 1990).

Radostits *et al*. (2003) reported that the initial clinical signs in rams are bacteraemia which is accompanied by a mild systemic response. However, this soon resolves as the organism localises in the epididymis. Furthermore, 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 finding. 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 the *B. ovis* infected rams are going to develop lesions in their external genital organs.

### 2.5.4 Clinical Signs In horses

Brucellosis in horses could be asymptomatic for as long as two years post exposure or be associated with clinical disease (Cvetnic *et al.,* 2005). Infection in horses most frequently involves *Brucella abortus*. Furthermore, the disease is mainly recognized as an inflammation of the supraspinous and supra-atlantal bursae. These syndromes are known as

"fistulous withers" and "poll evil", respectively. The bursal sac becomes distended by a clear, viscous, straw-coloured exudate and develops a thickened wall. It may rupture, leading to a secondary point of infection. Unlike in infected cows, excretion of the organism in vaginal discharges appears to be short-lived. Other clinical signs reported in horses due to *B.abortus* infection are arthritis, intermittent lameness, lethargy and swelling of the carpal joint (Ocholi *et al.,* 2004).

### 2.5.5 Clinical Signs in dogs

Shin and Carmichael (1999) reported that infection with *B. canis* results in variable clinical signs associated with the reproductive system which varies from asymptomatic to mild, despite an ongoing systemic infection, with high morbidity and low mortality. The classic sign of canine brucellosis is late abortion, which can occur between 30-57 days of gestation. It is more common from 45 to 55 days of gestation in about 75% of the cases and abortions are followed by mucoid, serosanguineous, brownish or gray vaginal discharge that persists for up to six weeks.

Canine brucellosis does not change the presentation of oestrus and breeding (Hollet, 2006). The infection in the bitch can produce consecutive abortions and present litters of sick-born puppies that die a few hours to more than one month after delivery. Birth of apparently normal offsprings, which develop the disease later, can also occur (Shin and Carmichael, 1999; Hollett, 2006). Resorption or early embryonic death within 2 to 3 weeks after breeding can also occur (Shin and Carmichael, 1999).

In the male dog, history of failure to achieve intromission due to pain, unwillingness to ejaculate or successful internal ties without pregnancy is reported by the owners (Robles *et al*., 1998; Hollett, 2006). The main clinical manifestations are severe epididymitis, orchitis and prostatitis. Epididymitis usually begins 5 weeks after infection. An acute onset of inflammation with pain and swelling enables the detection of orchitis and epididymitis by physical examination. Scrotal dermatitis develops from a constant licking by the male leading to oedema and dermatitis which usually present secondary contamination by non-haemolytic staphylococci (Hollett, 2006).

Neiland and Miller (1981) reported that the clinical signs of infection due to *B. abortus* in dogs vary from mild fever to orchitis and testicular atrophy with shedding of the organisms in the urine. Dogs experimentally infected with *B. suis* were reported to be afebrile and asymptomatic without gross lesions (Mantur *et al*., 2007). They further reported that under natural conditions hindlimb weakness along with large and firm epididymitis could be observed to be associated with oligospermia and increased number of neutrophils in semen similar to what was observed in *B. canis* infection.

### 2.5.6 Clinical Signs in camels

The Office International des Epizooties (2009) reported that camels are not known to be primary host for any of *Brucella* organisms but they are susceptible to both *B. abortus* and *B. melitensis.* Camels infected with *B. melitensis* shed the organisms in milk and this poses a serious public health problem. Clinical signs of brucellosis in camels appear to be very rare.

### 2.5.7 Clinical Signs in humans

Human brucellosis is a life-threatening disease that may have variable clinical presentations (Colmenero *et al*., 2002). The incubation period varies between few weeks and several months. *Brucella melitensis* is the most important cause of human brucellosis worldwide (Lucero *et al*., 2005). The most common clinical signs of brucellosis are undulant fever in which the temperature can vary from 37ºC in the morning to 40ºC in the afternoon, night sweats with peculiar odour, chills and weakness (Megid *et al*., 2010). They also reported that malaise, insomnia, anorexia, headache, arthralgia, constipation, sexual impotence, nervousness, and depression are also common. Complications and involvement of internal organs can be diverse, depending on the site of infection, and can include encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis as well as spontaneous abortions in pregnant women infected with *Brucella* species in early stages of infection (Acha and Szyfres, 2003). 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 and Mantur, 2008). Though the illness can be protracted and debilitating, brucellosis is rarely fatal in humans (Madkour, 2001). However, if left untreated, up to 2% of patients will develop endocarditis which can be fatal.

It has been reported that among the four *Brucella* species known to cause disease in humans (*B. abortus, B. melitensis, B. canis, and B. suis*), *B. melitensis* is thought to be the most virulent and causes the most severe and acute form of brucellosis (Madkour, 2001). *Brucella melitensis* is also the most prevalent worldwide. A prolonged course of illness,

often associated with suppurative destructive lesions, is associated with *B. suis* infections. *Brucella abortus* is associated with mild-to-moderate sporadic disease that rarely causes complications and that *B. canis* infection has a disease course that is indistinguishable from *B. abortus* infection. *Brucella canis* infection has an insidious onset, causes frequent relapses, and does not commonly cause chronic disease in humans. *Brucella pinnipediae* and *B. cetaceae* are distinctive species that typically affect marine animals. However, these strains were recently described to cause disease in humans, mainly as neurobrucellosis (Wafa, 2011).

The timely and accurate diagnosis of human brucellosis continues to challenge clinicians because of its non-specific clinical features, slow growth rate in blood culture, and the complexity of its serodiagnosis (Colmenero *et al*., 1990; Memish *et al*., 2000).

## 2.6 PATHOLOGY OF BRUCELLOSIS

### 2.6.1 General pathology

Lesions due to brucellosis can be generalized, though they are mostly reported in the reproductive system (Meador *et al.,* 1989). Furthermore, the pathology may vary with the various *Brucella* species and the organs or tissues involved. Some aborted foetuses may appear normal while others are autolyzed or have variable amounts of subcutaneous oedema and bloodstained fluid in their body cavities (Animal Health Australia, 2005). Jackson and Pankay (1967) reported that histological findings in brucellosis usually include mixed inflammatory infiltrates with lymphocytic predominance and granulomas in up to 55% of cases and with necrosis.

In ruminant foetuses, the spleen and/or liver may be enlarged, and the lungs may exhibit pneumonia and fibrous pleuritis (Animal Health Australia, 2005). Abortions caused by *Brucella* spp. are typically accompanied by placentitis. The cotyledons may be red, yellow, normal or necrotic. In cattle and small ruminants, the intercotyledonary region is typically leathery, with a wet appearance and focal thickening. There may be exudates on the surface (The Office International des Epizooties, 2009).

In adults, granulomatous to purulent lesions may be found in the male and female reproductive tracts, the mammary gland, supramammary lymph nodes, other lymphoid tissues, bones, joints, and other tissues and organs. Also, mild to severe endometritis may be seen after an abortion (Animal Health Australia, 2005). They also reported that in bulls, *B. abortus* causes infection and swelling of the testicles that may not be obvious, but increasing pressure results in necrotic foci that coalesce and may lead to total testicular necrosis with sequestration by inflammatory thickening of the tunica. Nicoletti (1986) reported that *B. abortus* may also infect the accessory sex glands. Further, Brucellae in cattle may localize in the carpal and other bursae, where hygromas containing large number of bacteria may be found.

The The Office International des Epizooties (2009a) reported that in sows, *B. suis* biovar 2 infections are associated with nodules of varying sizes in internal organs, particularly seen more in the reproductive organs. Also, the lesions can also be seen in the spleen, liver, lung, and most other organs. Skin and subcutaneous tissues can also be affected. These nodules often become purulent but despite the presence of the nodules, the sow's body condition may remain good. In addition, Hall (1977) reported that in the sow, cattarhal metritis is

common and that abscess can frequently be found in the testes or seminal vesicles of the boar. Histologic findings in brucellosis usually include mixed inflammatory infiltration with lymphocytic predominance, granulomas (in up to 55% of cases), and with necrosis (Jackson and Pankay, 1967).

### 2.6.2 Pathology due to *Brucella ovis* infection

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. They also reported that there is 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 (The Office International des Epizooties, 2009).

### 2.6.3 Pathology due to *Brucella melitensis* infection

Infection due to *B. melitensis* in animals 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, palpable testicular alterations, necrotising orchitis and epididymitis with

subsequent granuloma, necrotising seminal vesiculitis and prostatitis could be observed. Acute mastitis with palpable nodules and the production of clotted and watery milk may occur (European Commission, 2001).

### 2.6.4 Pathology due to *Brucella canis* infection

Aborted puppies are often partially autolyzed and have evidence of generalized bacterial infection. Foetal lesions can include subcutaneous oedema, subcutaneous congestion and haemorrhages in the abdominal region, serosanguinous peritoneal fluid, and degenerative lesions in the liver, spleen, kidneys, and intestines are evident (Anon, 2012a).

The lymph nodes are often enlarged in affected species. The retropharyngeal and inguinal lymph nodes are often involved, but generalized lymphadenitis also occurs. The spleen is frequently enlarged, and may be firm and nodular. Hepatomegaly may also be seen. Scrotal oedema and scrotal dermatitis, epididymitis, orchitis, prostatitis, testicular atrophy and fibrosis may occur in some infected males, while metritis and vaginal discharge may be seen in infected females. Less commonly reported lesions include discospondylitis, meningitis, focal non-suppurative encephalitis, osteomyelitis, uveitis, and abscesses in various internal organs (Anon, 2012a).

Carmichael and Joubert (1988) reported that semen from infected dogs usually contains large numbers of abnormal sperm and inflammatory cells, especially during the first three

months following infection and chronically infected males may have azospermia, or reduced numbers of immature sperm cells.

### 2.6.5 Pathology due to *Brucella neotome* infection

In marine mammals, brucellosis has been linked to lesions in a few animals. These lesions include meningoencephalitis, subcutaneous abscesses, placentitis, abortion, epididymitis, chronic purulent or granulomatous orchitis, lymphadenitis, mastitis, spinal discospondylitis, peritonitis, and a mineralized lung granuloma (Foster *et al*., 1996). Others include hepatic abscesses, hepatic and splenic necrosis. There is also the presence of macrophage and or histiocytic cell infiltrations in the liver, spleen and lymph nodes. In dolphins with meningoencephalitis due to *B. neotome*, the lesions were described as severe, chronic, and widespread along with nonsuppurative meningitis which was most severe in the brainstem. The meningitis was observed to be accompanied by periventricular encephalitis (Gonzalez *et al*., 2002; Hernandez-Mora *et al*., 2008).

## 2.7 EPIDEMIOLOGY OF BRUCELLOSIS

### 2.7.1 Distribution of Brucellosis

*Brucella* organisms have a worldwide distribution, but brucellosis is more common in countries having poorly standardized or ineffective animal and public health programmes (Anon., 2007). Rust (2006) reported that brucellosis is a zoonotic infectious illness to which humans became vulnerable at some point after the domestication of animals and the establishment of animal husbandry as an important element following civilization. He also stated that human disease prevalence in any given area of the world closely parallels the

extent to which the indigenous cultures engage in animal husbandry. The epidemiology of brucellosis is therefore not static as changes in agent, host or environment may affect the complex interrelationship that exists between them thereby influencing the epidemiology of the disease. The complexity of the epidemiology and the serious difficulties for effective control measures against the disease arise from the involvement of the main producing domestic animals and humans in the spread of the infection (WHO/MZCP, 1998).

The Mediterranean countries of Europe, Northern and Eastern Africa, Near East countries, India, Central Asia, Mexico, and Central and South America are especially affected by this disease (Health Protection Agency, 2013).

Brucellosis had not been suspected in Nigeria until contageous abortion was reported in newly established stock farms (Earnshaw and O'Brien, 1928). Since then the existence of the disease as a zoonosis acquired through sheep and goats have been reported by various researchers in Nigeria (Halle and Ajogi, 1997).

The herding of livestock together greatly enhances the possibility of transmission of the disease from one group to another (Ocholi *et al*., 1993). It was reported by Esuruoso (1974) that generally brucellosis was more prevalent in government owned farms than in nomadic herds. Bale (1981) also reported that epidemiological investigations revealed that results obtained varied which may 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.

Though the nature of the disease in Nigeria, where people still consume unpasteurized milk and milk products is not clear (Cadmus *et al*., 2006). 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 ranges between 3.7% and 48.8% (Esuruoso and Hill, 1971; Esuruoso and Van Black, 1972; Esuruoso, 1974). Infection rates in nomadic herds have been shown to be between 0.4% and 26.0% (Nuru and Dennis, 1975; Ocholi *et al.*, 1996). There was also a report of prevalence ranging from 0.4% to 8.6% based on abattoir surveys (Chukwu, 1987).

B*rucella melitensis* is the least species-specific which can be isolated from a wide range of domestic and wild animals and is the cause of human brucellosis as well as being one of the most important zoonosis in the world, accounting for annual occurrence of more than half a million cases yearly (Pappas *et al.,* 2006). Risk of the disease is also proportional to the degree of contact with *Brucella*-infected animals, their excreta, or their edible by-products, particularly milk or cheese. Pasteurization of milk and care in the slaughtering of animals for meat and assurance of adequate cooking of meat are of great importance in preventing human brucellosis. Worldwide, *B. abortus* accounts for the largest number of human and veterinary cases of brucellosis (Rust, 2006). Corbel (1997a) reported that, in humans, brucellosis caused by *B. melitensis* in sheep and goats is by far the most important clinically apparent disease. The disease occurs worldwide, except in those 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 (Corbel, 1997a).

### 2.7.2 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. Furthermore, there is the possibility for cross infection among animal species, especially when they are kept in close contact. Adult animals are much more susceptible to infection by *Brucella spp.* than younger ones and such adults may be infected but may not show any clinical sign but generally show only a weak and transient serological response. Susceptibility is said to increases 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).

In cattle, sheep, goats and swine, susceptibility to brucellosis is greatest in sexually mature animals (Corbel, 2006). Furthermore, young animals are often resistant, though it should be noted that latent infections can occur and such animals may be hazardous when mature.

Corbel and Brinley-Morgan (1984) reported that dairy breeds of sheep appear more susceptible than those kept for meat production. Manthei *et al*. (2010) further 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 dairy cows and many beef bulls are maintained separately from the herd and their exposure to infection is therefore lessened.

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

Anon. (2010) reported that the survival of the organism in the environment may play a role in the epidemiology of the disease and that the ability of *Brucella species* to persist outside mammalian hosts is relatively high compared to most other non-sporing pathogenic bacteria and can survive for a long time in both hot and cold environments, particularly in moist conditions. The organism can survive in tap water for several months at 4 to 8 $^0$C, 2.5 years at 0 $^0$C, and several years in frozen tissues or medium (FAO, 2005). *Brucellae* can also survive for up to 60 days in damp soil, and for up to 144 days at 20 $^0$C, 40% relative humidity and at pH greater than 4. *Brucella* organisms can also survive freezing and thawing and also for several weeks in non-fermented milk (Merchant and Packer, 1975). Brucellae can survive for 30 days in urine, 75 days in aborted foetuses and more than 200 days in uterine exudates. In beddings contaminated with infected faecal material, *Brucella* will be destroyed at 56$^0$C- 61$^0$C within 4-5 hours (King, 1957).

Susceptibility of *Brucella* species to dyes such as thionin, basic fuschin methylviolet, 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 sulfate, 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 between species and biovars, a fact that has been used for routine typing tests for members of the genus (Anon., 2001).

Anon., (2012) reported that *Brucella* species are readily killed by most commonly available disinfectants including hypochlorite solutions, 70% ethanol, isopropanol, iodophores, phenolic disinfectants, formaldehyde, glutaraldehyde and xylene. However, organic matter and low temperatures decrease the efficacy of these disinfectants. The same author also reported that disinfectants that are effective in destroying *Brucella* on contaminated surfaces following one hour of exposure include 2.5% sodium hypochlorite, 2-3% caustic soda, 20% freshly slaked lime suspension, and 2% formaldehyde solution.

### 2.7.4 Effects of management practices

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

### 2.7.5 Transmission of brucellosis

Most infections result from ingestion of the bacteria either from diseased animals or contaminated feedstuffs. 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. Infection may also be acquired by respiratory exposure and by contamination of abraded skin and mucosal surfaces. Natural breeding results in infection in swine and dogs and, to a lesser extent, sheep and goats (Corbel, 2006). USDA (2000) reported also 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.

The use of pooled colostra for feeding newborn 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. Bulls that remain fertile and functionally active will shed *Brucella* organisms in the semen during the acute phase of the disease (FAO, 2005). Shedding, however, may cease or become intermittent (McCaughey and Purcell, 1973). 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).

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). Furthermore, goats usually shed *B. melitensis* in vaginal discharges for at least 2 to 3 months, but shedding usually ends within three weeks in sheep. It was also reported by OIE (2009) that *B. mellitensis* can be found in milk and semen and that infected ewes and does may shed the organism in milk and semen for prolonged periods leading to lambs and kids to become infected. Also, infection in sheep occurs mainly through the nasopharyngeal route and can also be transmitted from dam to kid/lamb in uterus or via the colostrum or milk.

Transmission of brucellosis in pigs occurs by both venereal and through the 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). In ewes, *B. ovis* can uncommonly cause abortion associated with placentitis beginning at 30 days of gestation. Infected ewes may give birth to weak lambs and there may be high neonatal mortality rate (Meinershagen *et al*., 1974).

In dogs, transmission of brucellosis usually occurs by breeding or ingestion of contaminated placental tissues, aborted foetuses or vaginal secretions from infected bitches. 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 (*Parafi laroides, 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 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 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 infected meat, unpasteurized milk and milk products (Williams, 1971). In addition to the food-borne and occupational infection, it is linked to travel and bioterrorism (Young 1995; Greenfield *et al*., 2002). *Brucella* is considered a biological weapon in the category B pathogen. Recently, other routes of transmission have been identified to include infection through breast milk (Tikare and Mantur, 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).

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.

### 2.7.6 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, the timing depends 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 production of IgG1 isotype of antibody and subsequently by IgG2 and IgA in small quantities (Corbel, 1972; Beh, 1974). Because of the early onset of IgM antibody production, theoretically, it would be best to measure this isotype as an indicator of exposure.

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 Stuart, 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.

*Brucella abortus* S19 is a widely used vaccine (Nielsen and Yu, 2010). They also reported that this organism is antigenically indistinguishable from pathogenic strains of *B. abortus*. However, 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, 1990a). However, some animals were found to have residual antibody leading to higher antibody levels in vaccinated animals.

## 2.8 DIAGNOSIS OF BRUCELLOSIS

All abortions in late gestation in domestic animals should be treated as suspected brucellosis and should be investigated. The clinical picture of brucellosis is not pathognomonic, although the herd history may be helpful (OIE, 2009a).

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. The primary binding assays directly measure the interaction of antibody and 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* O: 157, some strains of *Escherichia hermannii* and *Stenotrophomonas maltophila*, *Salmonella* group N (O: 30), and *Yersinia enterocolitica* O: 9. However, only *Yersinia enterocolitica* O: 9 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 to *Brucella spp*. S-LPS and the responses are usually transient in cattle (Mittal *et al*., 1985; Garin-Bastuji *et al*., 1999), but titers in serum and milk may be high and persistent (Mittal *et al*.,1985). However, the diagnosis of brucellosis is usually performed by a combination of methods. A definitive serological diagnostic technique is not available yet, in spite of being pursued for more than one century (Poiester *et al*., 2010). Among the diagnostic tests for the detection of brucellosis include direct smear examination, culture and isolation of the organism, animal inoculation, and serology.

### 2.8.1 Useful specimen for the diagnosis of brucellosis

For the diagnosis of brucellosis, the organism may be recovered from a variety of materials. In animals, the placenta is the most infective and with the greatest concentration of the

bacteria, followed by lymph nodes and milk and from blood in humans (Poiester *et al*., 2010). Furthermore, other materials include stomach contents, spleen and lung from aborted foetuses, vaginal swabs, semen, and arthritis or hygroma fluids from adult animals. From animal carcasses, the preferred tissues for culture are the mammary, medial and internal iliac, retropharyngeal, parotid and prescapular lymph nodes and spleen. All specimens must be packed separately and transported immediately to the laboratory cooled or preferably frozen in leak proof containers. For humans, blood for culture is the material of choice but specimens need to be obtained early in the disease.

There is no ideal tissue for the isolation of *Brucella* from marine mammals, unless gross lesions are found in tissues. The recommended tissues for the recovery of *Brucella* in marine mammals are the spleen, the mammary gland, the mandibular, gastric, external and internal iliac and colorectal lymph nodes, the testes and blood (Foster *et al*., 2002).

### 2.8.2 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, placentas or aborted foetuses stained with the Stamp modification of the Ziehl-Neelsen staining method. However, morphologically related micro-organisms such as*, Clamydophila abortus, Clamydia psittaci* and *Coxiella burnetti* can mislead in the diagnosis because they superficially resemble *Brucella spp* (Marin *et al*., 1996; Poiester *et al*., 2010). Accordingly, the isolation of *B. melitensis* 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).

### 2.8.3 Cultural and isolation of *Brucella* organism

This procedure may be performed by culturing body tissues or secretions such as blood, milk and virginal discharge (Poiester *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 Terrestrial Manual, 2009a; Poiester *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 CO2, phage typing and metabolic tests, which among other problems involve time, biosafety, trained personnel and somewhat ambiguous results. 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 (Poiester *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 (European Commission, 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. Therefore, selective media should be used to avoid overgrowth of fast growing agents. The most widely selective media used are the Kuzdas and Morse (Kuzdas and Morse, 1953) and the Farrell´s medium (Farrel, 1974). The Kuzdas and Morse use 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 (25mg), polymyxin B sulphate (5mg), nalidixic acid (5mg), nystatin (100,000 units), vancomycin (20mg), natamycin (50mg). As Farrell´s medium is rather inhibitory for some strains of *B. abortus*, *B. melitensis*, and *B. ovis*, a modified Thayer-Martin medium may be used together with Farrell's. This medium can be prepared with GC medium as basal medium supplemented with 1% haemoglobin and the following antibiotics per litre of medium: colistin methanesulphonate (7.5mg), vancomycin (3mg), nitrofurantoin (10mg), nystatin (100,000 units) and amphotericin B (2.5mg) (Marin *et al*., 1996).

### 2.8.4 Laboratory animal inoculation

Guinea pigs are more susceptible to *Brucella spp.* than any other laboratory animal. However, mice are also susceptible and can be substituted for guinea pigs (Avong, 2000; Ocholi, 2005; OIE Terrestrial Manual, 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 Terrestrial Manual, 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 Terrestrial Manual, 2009a).

### 2.8.5 Use of serology in the diagnosis of Brucellosis

Body fluids such as serum, uterine discharge, vaginal mucus, milk, or semen plasma from a suspected animal may contain different quantities of antibodies of the M, $G_1$, $G_2$, 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, 2005).

It was reported by Poiester *et al*. (2010) 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, disease. Traditionally, screening tests are inexpensive, fast and highly sensitive, but not necessarily highly specific. They further reported that 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-polysacharride (OPS). Virtually all serological tests for antibody to these bacteria use *B. abortus* antigen in the form of whole cells, smooth lipopolysacharride (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 *et al*., 1990). Other *Brucella spp*. such as *B. neotome* and *Brucella* of marine mammals can be detected serologically using *B. abortus* antigens (Poiester *et al*., 2010).

The commonly used serological tests include; MRT, SAT, SPT, CFT, 2-Mercaptoethanol Test (2-MET), Buffered Antigen Test (BPAT), and RBPT (Wikipaedia, 2011). Others include the Card Test (CARD), Rivanol Test, Coombs test, Indirect Immunoflourescent Test (IFAT), Heat Inactivation Test (HIT), Skin Test, immune-assay and molecular biology technique.

*2.8.5.1 MRT*

The MRT is essentially a rapid agglutination test carried out on whole milk or cream. Hematoxylin stained *Brucella* cells are added to whole milk and reaction is allowed to take place (Hubber and Nicoletti, 1986; McCaughey, 1972). Immunoglobulins present in the milk will, in part, be attached to fat globules *via* the Fc portion of the fat molecule (Poiester *et al*., 2010). The 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, 1990a). The milk ring test is prone to false reactions caused by abnormal milk derived from mastitis, colostrums and late milk from the lactation cycle (Kerr *et al*., 1959; Cunnigham, 1970; 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).

*2.8.5.2 SAT.*

It was reported by Hajdu and Baseda (1974) that the SAT measures IgM, IgG1 and IgG2 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 anibody 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). RLPS is commonly used as the main antigen for detection of antibody to *B. ovis* and *B. canis* (Poiester *et al*., 2010).

*2.8.5.3 SPAT.*

The SPAT was standardized to give similar results with that of the SAT titre. Later a dye stained antigen was used for ease of reading and could be used under field conditions. This test does not use a series of dilutions like the SAT does but 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 was also resistant to the prozone effect (Wikipedia, 2011).

*2.8.5.4 SAT -EDTA.*

Because of the lack of specificity of the SAT, an adaptation of the test, which involves the addition of EDTA, has 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). However, the test has been improved by use of a stable buffered antigen.

*2.8.5.5 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).

*2.8.5.6 RBPT.*

The 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. At neutral pH, this test can measure the presence of IgM, IgG1 and IgG2. However, IgM appears the more active. At the buffered pH of 3.65, RBPT apparently measures only IgG1. 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. 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. Low pH of the antigen enhances the specificity of the test while the temperature of the antigen and the ambient temperature at which the reaction takes place may influence the sensitivity and specificity of the test (Alton, 1981; Macmillan, 1990). Corbel (1972) observed that the sensitivity of the test was associated with fractions containing immunoglobulin IgG especially the $IgG_1$.

*2.8.5.7 CARD.*

It is a version of the RBPT test developed for use in Canada and used in the USA. It works similar to BPAT and RBPT (Stemshorn *et al*., 1985).

*2.8.5.8 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 (Poiester *et al*., 2010). Acrydine 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 (Poiester *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).

*2.8.5.9 CFT*

The CFT is a prescribed test for international trade (OIE Terresrial Manual, 2009). It is considered as the most specific and valuable serological test for brucellosis (WHO/MZCP, 1998; Poiester *et al*., 2010). It detects mainly $IgG_I$ antibodies than it does for antibodies of the IgM type which are partially destroyed during inactivation. Since antibodies of the $IgG_I$ 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. 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 (FAO, 2005). It is used mainly as a verification tool in the diagnosis of human brucellosis (WHO/MZCP, 1998). It may also test false negative when antibodies of the $IgG_2$ type hinder complement fixation (Nielsen *et al*., 1988; Macmillan, 1990).

*2.8.5.10 The 2-Mercaptoethanol Test.*

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 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).

*2.8.5.11 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 $IgG_2$ 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.

*2.8.5.12 The 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.

*2.8.5.13 The 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$^{o}$C for 15 minutes, cooled to 18$^{o}$C. The test is just like the tube agglutination test, the only difference being the heating.

*2.8.5.14 The 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 crossreacting 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 Terrestrial Manual, 2009). Similarly, it was reported by Cutler *et al*. (2005) 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 result interpretation, make this type of test impractical for high throughput diagnosis.

*2.8.5.15 Primary Binding Assays*

Conventional methods have limitations which led to the development of primary binding assay techniques. These tests are capable of rapidly and accurately detecting humoral antibodies to *Brucella* (Poiester *et al.,* 2010). Vaccination induces antibody thought to be of lower affinity due to a short exposure time to the antigen because it is eliminated by the immune system. Alternatively, antibody produced in response to natural infection is of higher affinity because the antigen is not removed quickly by the immune system and, therefore, persists for a much longer period (Nielsen *et al*., 1989). Thus, the competitive enzyme linked immunosorbent assay (cELISA) and the fluorescent polarization assay (FPA) were developed to overcome this problem. These tests are capable of distinguishing vaccinated animals or animals infected with cross-reacting organisms such as *Salmonella urbana* O: 30, *Escherichia coli* O: 116 and O: 157, and *Yersinia enterocolitica* serotype 9 from naturally infected animals, thereby reducing the number of false positive reactions and subsequent trace backs or slaughter of animals in an otherwise negative or healthy population (Gall and Nielsen, 2004).

However, Ramirez-Pfeiffer *et al*. (2008) reported that FPA can be routinely applied as an adaptable screening test for diagnosis of brucellosis in goats. They further reported that the test is rapid and simple and is not affected by vaccination. The test is not suitable for use in large screening programmes. RadioImmunoassay, which is suitable for large screening tests, is associated with medical hazards and the conjugate shelf life is short.

2.8.5.15.1 The 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 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. 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 ingredients/components involved (Nielsen and Yu, 2010).

2.8.5.15.2 FPA

The FPA assay was developed in 1996 (Nielsen *et al*., 1996). The basis 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 fluoroescein 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. 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).

2.8.5.15.3 *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 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. This assay is an excellent confirmatory assay for the diagnosis of brucellosis in most mammalian species.

*2.8.5.16 Molecular Biology Techniques*

The first brucellosis PCR-based test was introduced in 1990 (Fekete *et al*., 1990). The first species-specific multiplex PCR was called AMOS-PCR assay which is 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* (Huber *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* sp. and vaccine strains (Lopez-Goni *et al*., 2008).

### 2.8.6 Other Tests for Brucellosis

Urinalysis may likely demonstrate a sterile pyuria similar to tuberculosis while arthrocentesis can be performed for septic arthritis. The joint aspirate can demonstrate an exudative fluid with low cell count and predominance of mononuclear cells. Radiographic evaluations in infected animals may reveal evidence of acute or chronic *Brucella* leptomeningitis, subarachnoid haemorrhage or cerebral abscess following cranial radiography. Similarly, echocardiography can also be used to evaluate possible endocarditis. Mycotic aneurysms of the aorta or carotids may be observed on duplex arteriography. Furthermore, Bone marrow biopsy and liver biopsy may also be performed to obtain specimen for diagnosis, especially during the acute phase of the disease (Maloney and Fraser, 2006).

## 2.9 DIFFERENTIAL DIAGNOSIS

Animal Health Australia (2005) reported that there are many potential causes of abortion in animals which can be confused with brucellosis. These could include infectious causes like

viral diseases such as infectious bovine rhinotracheitis and mucosal disease and infections with other organisms such as *Trichomonas foetus*, *Neospora caninum, Campylobacter foetus*, *Listeria monocytogenes*, *Sarcosporidia spp*, various *Leptospira* species and fungi. Likewise, viral diseases causing abortion which include Rift Valley fever and Wesselsbron disease, especially in sheep should be considered. There is also the need to consider chlamydiosis and coxiellosis. There are also a range of potential noninfectious causes resulting from nutritional and toxic factors.

Generally, brucellosis can be differentiated from these conditions due to its pathology, presentation and the excellent range of laboratory diagnostic methods that can be used.
In humans, the disease needs to be differentiated from diseases that are of acute fibrile nature which include typhoid fever, Q-fever, enteric fever, rheumatic fever, and cat scratch fever. Others include auto-immune disease, influenza, mononucleosis, cancer, malaria, disseminated fungal infection, cholecystitis, HIV, thrombophlebitis, gastroentritis, meningitis, sacroitiitis, and tuberculosis among others (Maloney and Fraser, 2006).

## 2.10 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). Furthermore, a combination of antibiotics should also be used to prevent relapse. 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).

### 2.10.1 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 streptomycine has effected some level of cure in sheep, though it is usually not economically feasible except in valueable rams (Cynthia, 2005). It was further reported that fertility may remain low even if the organism is eliminated. It was also reported by Radostits *et al.,* (2003) 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 dose of antibiotics, inadequate duration of treatment, high cost of medication, and failure to cure udder infection which could lead to a relapse (Radwan *et al*., 1992). Furthermore, due to the long term treatment regimen, it could lead to antibiotics residues in milk and meat of treated animals which could be passed onto the food chain of humans.

### 2.10.2 In Humans

FAO/WHO (1986) reported that, in humans, despite extensive studies over the past 15 years, the optimum antibiotic therapy for brucellosis is still disputed. The treatment recommended by the above organizations for acute brucellosis in adults is rifampicin at a dose of 600 to 900 mg and doxycycline given at a rate of 200 mg daily for a minimum of 6 weeks. Some still claim that the long-established combination of intramuscular

streptomycin with an oral tetracycline gives fewer relapses (Ariza *et al*., 1985). Alternative treatments were tried using the quinolones, aminoglycosides, streptomycin, gentamycin and rifampicin (Kaya *et al*., 2012).

## 2.11 PREVENTION AND CONTROL OF BRUCELLOSIS

Because brucellosis is a disease of major economic and zoonotic importance, a strategy for its control in animals, especially livestock, is essential in endemic areas. The initial aim of the strategy selected will be the reduction of infection in the animal population to such a level that the impact of the disease on human health as well as on animal health and production will be minimized (Kaplan, 1966).

Prevention and control of brucellosis include test and culling of positive reactors, quarantine, zoning, and tracing.

### 2.11.1 Test and culling of positive reactors

Serological testing of livestock should be routinely carried out using approved serological tests as was practiced in a test and slaughter strategy which was practiced in Australia before the total eradication of the disease in 1989. All animals reacting positively to such a test are destroyed or consigned for immediate slaughter at an approved abattoir, and tissues are submitted for *brucella* culture (Animal Health Australia, 2005). 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 (Animal Health Australia, 2005).

Cobel (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* free infections in ewes are generally prevented by controlling infections in rams.

### 2.11.2 Quarantine and movement controls

From the reports of Animal Health Australia (2005), any suspicion of brucellosis, quarantine must be immediately imposed on the affected herd or flock to ensure the containment of any infection after which such herds/flocks will require repeated serosurveillance to confirm their freedom from infection. 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.

### 2.11.3 Zoning

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 (Animal Health Australia, 2005).

### 2.11.4 Tracing

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 (Animal Health Australia, 2005).

### 2.11.5 Surveillance

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. On-farm activities also include examination of production records for evidence of abortions and/or infertility.

As cattle movements are the most likely way in which the disease is spread, special attention should be given to herds selling breeding animals and those with a history of recent introductions. Closed herds are unlikely to introduce *Brucellae* and breeding stock from such herds is unlikely to spread the disease. 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.

### 2.11.6 Depopulation of infected animals

Animals that are confirmed to be infected and are close to parturition or having a vaginal discharge pose a disease risk to personnel and are therefore preferably depopulated and disposed of immediately and should be burnt and/or buried (Animal Health Australia, 2005). Parkinson (2009) reported that the infection level is at its peak 4 days to before calving or abortion to 14 days afterwards in cattle. However, depopulation has serious economic impacts such as the availability 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.

### 2.11.7 Treatment of animal products and by-products

Precautions should be taken in the handling and disposal of the placenta, uterus and mammary gland from suspect animals on farms, abattoirs and animal by-product establishments. Furthermore, milk for human consumption must be pasteurized (Animal Health Australia, 2005).

### 2.11.8 Decontamination

Decontamination can be achieved with the aid of sunlight, high temperatures and a range of chemicals (Animal Health Australia, 2005). Furthermore, 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 desiccation of the organism. The spread of

infection can also be minimized by cleaning and disinfecting vehicles used to transport infected and suspect cattle.

### 2.11.9 Vaccination

Vaccination is an extremely important and effective facet of most control strategies of brucellosis infection but has the disadvantage that its use may confuse diagnosis by stimulating the production of hypersensitivity or antibodies notably IgG1 which is detectable by some serological tests. Thus antibody titres may persist for a prolonged period in a small proportion of vaccinated animals and this proportion increases with age at vaccination (Corbel, 1996). However, Nicoletti (1990a) and Animal Health Australia (2005) have reported that live vaccines have until now proved superior to inactivated products for the prevention of animal brucellosis.

*Brucella abortus* S19 and *B. melitensis* Rev.1 vaccines have proven effective against *B. abortus* in cattle and against *B. melitensis* and *B. ovis* in sheep and goats, respectively (Elberg, 1996; Nicoletti, 1990a). Both vaccines have the disadvantages of causing abortion in a proportion of pregnant animals, and of being pathogenic for humans (Nicoletti, 1990a). Similarly, Animal Health Australia (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, and 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, 1990a).

Xie Xin (1986) 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.

Although the S2 strain of *B. abortus* also gave a satisfactory protection rate in cattle (Nicoletti, 1990b), 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). They also reported that currently, the live attenuated *B. melitensis* Rev.1 vaccine appears to be the most generally effective vaccine strain available for prevention of animal brucellosis.

*Brucella abortus* strain 45/20 vaccine is normally administered as two doses, given 6 to 12 weeks apart, followed by an annual booster (CDC, 2005).

From the reports of Animal Health Australia (2005) it was stated that 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 US and that 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 a special assay and indicates that the animal has been previously vaccinated.

Corbel (2006) reported that vaccination against *B. ovis* is being practiced in New Zealand and some other countries, but not in the U.S. Also successful vaccines have been difficult to

develop for pigs. It seems that pigs are generally not vaccinated except in China. Furthermore, no vaccines have been developed for dogs. There does not also seem to be any successful vaccine developed preventing fistulous withers or poll evil in horses.

### 2.11.10 Wild animal control

Feral animals, including buffalo and deer, may become infected with brucellosis if they graze in the same area as domestic livestock. They should therefore be controlled by mustering or field destruction (Animal Health Australia, 2005; Corbel, 2006).

### 2.11.11 control of brucellosis in other species

Corbel (2006) reported control of brucellosis in other species of animals. For example canine brucellosis can be controlled as for livestock brucellosis usually by sanitation and the removal of infected dogs. Housing of dogs in individual cages also reduces the spread of the organism. Repeated testing and the removal of seropositive or culture-positive animals, combined with quarantine and testing of newly added dogs, have been used to eradicate brucellosis from some kennels. Long-term antibiotic therapy may be tried in some infected dogs followed by neutering as an additional control measure.

Specific control methods have not been established for brucellosis in marine mammals but the general principles of infection control in such animals include isolation, disinfection, and practice of good hygiene. Some authors suggest that centres involved in marine mammal rehabilitation should routinely screen animals for *Brucella spp* (Corbel, 2006).

Prevention of brucellosis in humans still depends on the eradication or control of the disease in animal hosts. The exercise of hygienic precautions to limit exposure to infection through occupational activities, and the effective heating of dairy products and other potentially contaminated foods like meat should be adequately considered (Corbel, 1997b).

### 2.11.12 Re-stocking of depopulated herd/flock

Restocking of a decontaminated herd/flock should be done after 30 days in hot season and slightly longer in the cold season as this assures minimal risk of reinfection because *Brucella* organisms are rapidly being inactivated by desiccation and sunlight (Animal Health Australia, 2005).

Corbel (2006) reported 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.

### 2.11.13 Human infection

Because brucellosis is a significant zoonosis, all people handling infective material, including live vaccines, should wear protective glasses, gloves and clothing and protect skin breaks from infection. There is no risk to the general public except from unpasteurised milk (Anon, 1998).

### 2.11.14 Public awareness

Media campaigns should highlight to farmers the importance of inspecting susceptible animals regularly and reporting abortions, the birth of weak or dead animals, or infertility. An abortion investigation programme that relieves producers of the costs of investigation is a useful strategy. Details of any imposed movement controls need to be readily available and clearly explained to the farmers. Given the important zoonotic implications, people at risk must be advised of appropriate occupational health and safety requirements and health authorities alerted to the potential for human infection (Maxwell and Bill, 2008).

## 2.12 ECONOMIC IMPLICATION OF BRUCELLOSIS

The economic implications associated with brucellosis are extensive and encompass both public health costs and loss of production in domestic livestock through loss of milk, meat, restrictions in international livestock trade and by diminished animal working power. The disease can have an effect on exports and constrain efforts to introduce exotic livestock breeds, which are more susceptible to the disease (Saleem *et al*., 2010).

## 2.13 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 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. Also, the expansion of animal industries and urbanization along with lack of hygienic measures in animal husbandry and in food handling can be said to partly account for brucellosis remaining as a public health hazard. Furthermore, it was also reported that expansion of international travels which stimulate the taste for exotic dairy goods such as fresh cheeses which may be contaminated, and the importation of such foods into *Brucella*-free regions, may contribute to the ever-increasing concern over human brucellosis (Corbel, 2006).

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 STUDY AREA

This study was conducted in the Kaduna Kaduna North Senatorial District of Kaduna State which comprises of seven Local Government Areas (LGAs). Kaduna State is located in the Northwest Geopolitical zone of Nigeria. It lies between latitudes $6^0$ and $11^0$ north and longitude $7^0$ and $44^0$ east, and is 1995 ft above sea level (Fig 3.1). It has distinct wet and dry seasons within the Guinea Savannah zone and part of the Sudan Savannah in Nigeria. The State shares boundaries with Katsina and Zamfara States to the North, Plateau and Bauchi States to the East, Nasarawa State and the Federal Capital Territory to the South, Niger State to the West and Kano State to the Northeast. Kaduna State is made up of 23 LGAs and occupies about 48,473.25 sq km, with a human population of over 6,066,562 people according to the 2006 census figures (KDSG, 2008).

Four out of the seven LGAs in Kaduna Kaduna North Senatorial District of Kaduna State were selected for the study using simple random sampling without replacement. These were Ikara, Makarfi, Sabon Gari and Soba LGAs. Three wards were further selected from each of the selected LGAs using the same method. The location of the flock, animal species, breed, age, and sex of each animal sampled were recorded. Ten animals were sampled per flock. A total of 250 animals from both sheep and goats of different age groups and sex were sampled in each LGA.

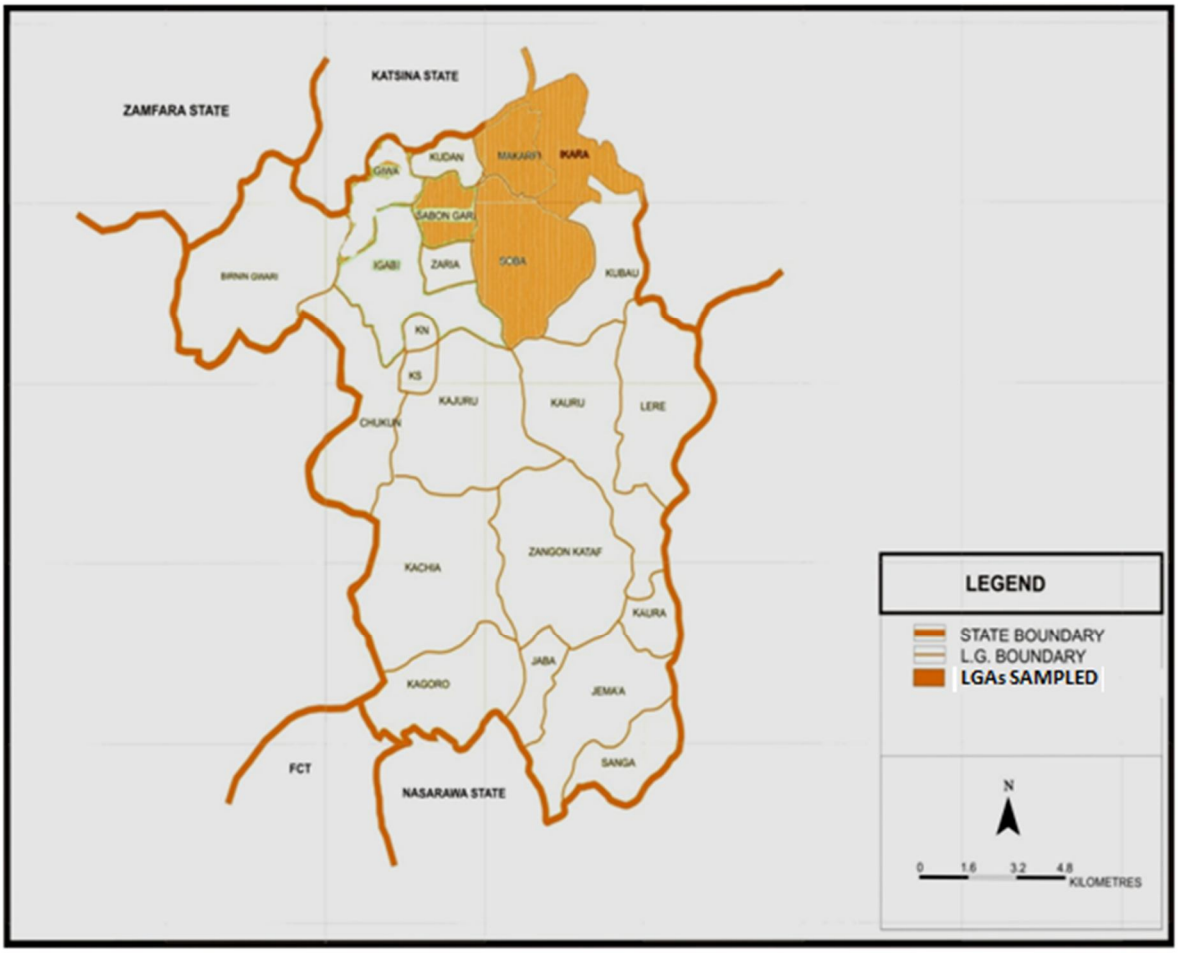



Fig 3.1 Illustrated map of Kaduna State showing the study area
Source: Urban and Regional Planning Department, Ahmadu Bello University, Zaria, Nigeria

## 3.2 METHODOLOGY

### 3.2.1 Animals used in the study

Pastoral and village level sheep and goats were used in the study. As much as possible, equal number of sheep and goats was sampled from the four selected LGAs that were selected. Flock selection was done based on random selection and farmers' consient.

### 3.2.2 Sample size determination

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

$$n = \frac{z^2pq}{d^2}$$

Where: n - Minimum sample size

z - Appropriate value for the standard normal deviation set at 95% confidence interval (1.96)

P- Prevalence (14.5% for sheep and 16.1% for goats; Bertu *et al*., 2010).

q – Complementary probability, 1 – p

d – Desired level of significance

$$\text{Sample size (Sheep)} = \frac{(1.96)^2 \times 0.145 \times (1-0.145)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.145 \times 0.855}{0.0025}$$

$$= 190.5 \approx 191$$

$$\text{Sample size (Goat)} = \frac{(1.96)^2 \times 0.161 \times (1-0.161)}{(0.05)^2}$$

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

$$= 207.6 \approx 208$$

To increase precision, the sample size was increased to 500 for each species making a minimum of total of 1000 samples.

### 3.2.3 Sample collection

*3.2.3.1 Blood sample collection*

Five mililitres (5ml) of blood sample was aseptically collected through the jugular vein from each goat and sheep using a 10ml syringe and 21G needle after proper restraint by an assistant. The blood was then immediately transferred into a 10ml clean plain EDTA free sampling bottle. The samples were then appropriately labelled based on the LGA, ward, and animal species. For example sample 1 collected from a goat in Sabon Gari LGA was labelled as SGG1 and that from sheep SGS1, and so on. The labelled bottles were then kept in a cooler and allowed to clot by allowing them in to remain in a slanting position in a receptacle to allow for separation of serum. The samples were then taken to the Bacterial Zoonosis Laboratory in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria in ice-packed containers where they were centrifuged at 1000g for 5 minutes to allow for proper separation of serum from the clotted blood. The serum was then siphoned, using a sterile

pasture pipette, into a 5 ml plastic serum tube which was appropriately labelled. All the extracted serum samples were then stored in the freezer at $-20^0$ C untill used.

*3.2.3.2 Milk Sample Collection*

Lactating animals earmarked for sampling were properly restrained by an assistant. The teats of the udder were cleaned and disinfected using 70% alcohol and dried using clean gauze bandage. The fore milk was stripped off to reduce contamination. About 5ml of fresh milk was then expressed from the udder into a clean sterile bottle which was labelled appropriately as in the case of blood sampling. The milk samples were then transported in ice box along with the blood samples to the laboratory where they were stored for at least 12 hours in the refrigerator at $4^oC$ after which they were analyzed.

**3.2.4 Questionnaire design and administration**

A structured questionnaire for collecting information on the knowledge, attitude and practice (KAP) for brucellosis was designed and administered to the herdsmen and household members who owned and reared the animals used in the study. The questionnaire was completed for all the selected flocks on a single visit during the sampling programme. The questionnaire was designed to comprise mostly closed ended (categorical) questions to ease data processing, minimize variation, and improve precision of responses (Appendix 1). The questions were translated to the herdsmen in Hausa language. The important flock and animal level data recorded included location of the flock, animal management practices, animal sourcing management, breeding and reproduction practices,

management of aborted foetuses, and information or awareness on brucellosis in animals and man.

## 3.3 EVALUATION OF SERUM AND MILK SAMPLES

### 3.3.1 The RBPT

The stored sera samples were thawed and then subjected to RBPT as described by Alton *et al.* (1975) using RBPT antigen sourced from Onderstepoort Biological Products Ltd, South Africa. About 0.03ml of RBPT antigen and equal amount of the test serum were placed alongside on a white ceramic tile and then mixed thoroughly. The tile was rocked gently for 4 minutes (rotation period). The sample was classified positive if any agglutination was observed and negative if no agglutination was observed.

### 3.3.2 The 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.

#### *3.3.2.1 Preparation of stock solution for SAT-EDTA*

One litre of phosphate buffer saline solution containing 5mM of Disodium EDTA was prepared for the modified SAT technique. The final solution contained 0.5% Phenol crystals, 0.85% (W/V) Sodium chloride, and 5mM Disodium ethylene diaminotetraacetic

acid (EDTA). The buffer also contained 31.2g sodium hydrogen orthophosphate ($NaH_2PO_4$) and 28.39g disodium hydrogen orthophosphate ($Na_2HPO_4$). Twenty eight millilitres of $NaH_2PO4$ and 72ml of $Na_2HPO_4$ were mixed to make the required PH of 7.2. All the constituents were weighed with an electric digital balance (Mettler P 165). They were then dissolved in pre-heated measured amounts of distilled water to make-up the buffer solution.

*3.3.2.2 Procedure for SAT-EDTA*

Phenol saline with EDTA buffer solution containing 5g phenol crystals, 8.5g sodium chloride, and 1.8612g Disodium EDTA, dissolved in 100ml of warm distilled water was prepared. A 1:10 dilution of the concentrated SAT antigen with the prepared buffer with a pinch of 0.02% Safranin O (to provide contrast to the agglutination reaction) was made for each day's work. A 96-well "U bottom" microtitre plate (Cooke Micro–Titer® System) was set up on the work table. Labeled serum vials were placed on the work table according to positions of the wells already labeled A-H and a corresponding vertical numbering of the wells were made (Appendix XXII). A representative entry of the sample details was made in the laboratory record book. Using automatic micropipette (Dragonmed®) 40µl of the buffer solution was measured out into the first well and 25µl into each of the remaining microtitre wells. This was followed by the addition of 10µl of test serum into the first microtitre well using a fresh disposable pipette plastic tip for each test sample which was later on discarded. A twofold serial dilution was done by transferring 25µl aliquots from the first well up to the fifth well. 25µl of the aliquot was discarded after the last well. The

content of the working dilution of the SAT antigen was mixed gently and 25µl 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 to prevent evaporation of the contents in the wells and incubated for 20 hours at $37^0$C in an incubator (Gallenkemp®, Germany) after which the results were read.

### 3.3.3 The LFA

The Antigen Rapid *B. melitensis* Antibody (Ab) Test Kit used for the LFA contained the *B. melitensis* test kit, Assay diluents (3ml), and a capillary tube (Plate I).

The thawed sera samples were analysed according to the manufacturer's instructions (Binote Inc., Republic of Korea). For each test serum, 20 µl of thawed serum was placed into the sample window of the test device followed by the addition of 4 drops of the provided diluents. The test results were read after 20 minutes by visual inspection for staining of the test and control lines (Plate II). Tests were scored negative when staining was observed only on the control line and scored positive when staining was observed on both the test and control lines.




**Plate I**: Contents of *B. melitensis* Antibody Test Kit obtained from Binote Inc. Republic of Korea.




**Plate II:** Test outcomes showing a negative (left) and a positive (right) following the application of the Antigen Rapid *B. melitensis* antibody test kit using sampled sera from sheep and goats.

### 3.3.4 The MRT

The MRT was performed as described by Alton *et al*. (1975) using MRT antigen sourced from Onderstepoort Biological Products Ltd, South Africa. The antigen and milk samples were placed on the work table for 1 hour to attain room temperature. A series of test tubes were labelled to correspond with those on the milk samples (Appendix XXIII). A drop (0.03ml) of the antigen was then placed in each test tube to which 1ml of the milk sample in the corresponding tube was added. The test tubes were then covered and the mixture was incubated in a water bath (Kottermann) at 37$^{o}$C for 1 hour after which the results were read (Appendix XXIV).

### 3.3.5 Statistical analysis

Data obtained from the serological tests were reduced and presented using descriptive statistics using Microsoft Excel Spreadsheet. Chi square statistical test was used to test for significant association between categorical variables using SPSS version 17.0 (2009), P-value< 0.05 was considered statistically significant.

# CHAPTER 4

# RESULTS

## 4.3 DISTRIBUTION OF SHEEP AND GOATS AND THEIR SEROPREVALENCE RATES FOR BRUCELLOSIS IN THE STUDY AREA.

From the total of 1021 sheep and goats sampled from the four LGAs of the State, two hundred and fifty three representing 25% of the total number of animals sampled were from Ikara, while 233 (23 %), 285 (28%), and 250 (24 %) were from Makarfi, Sabon Gari and Soba LGAs respectively (Table 4.1). Also, of the 1021 small ruminants sampled, 579 (56.9%) were sheep while 442 (43.3%) were goats (Table 4.3).

Based on the three serological tests used for *Brucella* antibodies detection the highest detection rate 68 (29) was obtained using RBPT, the lowest rate 2 (1%) was found using LFA from Makarfi LGA and Sabo Gari LGAs respectively (Table 2).

Among the 579 sheep tested, 148 (26%), 64 (11%), and 14 (2%) were positive for brucellosis based on the RBPT, SAT-EDTA, and LFA respectively (Table 4.3). Similarly, of the 442 goats, 114 (26 %), 49 (11%), and 11 (3 %) were positive based on the RBPT, SAT-EDTA, and LFA respectively. Among the sheep sampled from the LGAs, those from Makarfi LGA had the highest RBPT 13 (33 %) positive cases while thse from Soba LGA had the lowest RBPT seroprevalence of 31 (22 %). Furthermore, the sheep from Soba LGA had the highest SAT-EDTA seroprevalence 19 (14%) while those from Makarfi LGA had the lowest value of 3 (8%). Withrespect to the LFA, the sheep from Makarfi LGA had the lowest seroprevalence of 0 (0%) while the highest seroprevalence of 8 (6%) was recorded in sheep from Soba LGA. With regards to the goats, those from Makarfi and Sabon Gari LGAs had the highest RBPT seroprevalence of 55 (29 %) and 16 (29%) respectively while

those from Soba LGA had the lowest seroprevalence of 21 (19 %). Also from Table 4.3, the highest and lowest SAT-EDTA seroprevalence for goats were from Soba LGA with 22 (20%) and from Sabon Gari LGA with 2 (4%), respectively. The lowest LFA seroprevalence in goats was recorded from Sabon Gari LGA with 0 (0 %) while the highest LFA seroprevalence of 4 (5 %) was from Ikara LGA.

The results of the serological tests have shown that sheep and goats from Sabon Gari LGA had the highest prevalence to brucellosis with 82 positive cases representing 31% based on the RBPT while animals from Soba LGA had the highest seroprevalence rates based on the SAT-EDTA and the LFA with 43 (17%) and 15 (6%) positive cases, respectively. The results obtained according to the various districts within the 4 LGAs are as presented in Fig 4.1 and Appendices II to V.

**Table 4.1:** Distribution of Sheep and Goats sampled for brucellosis in the 4 LGAs in Kaduna North Senatorial District of Kaduna State.

| | Number of Animals Sampled | | |
|---|---|---|---|
| | Sheep | Goats | Total |
| LGA | No (%) | No (%) | No (%) |
| Ikara | 170 (16.7) | 83 (8.1) | 253 (25) |
| Makarfi | 40 (4.0) | 193 (18.9) | 233 (23) |
| Sabon Gari | 229 (22.4) | 56 (5.5) | 285 (28) |
| Soba | 140 (13.7) | 110 (10.8) | 250 (24) |
| Total | 579 (56.7) | 442 (43.3) | 1021 (100) |

**Table 4.2:** The rate of detection of *Brucella* anibodies in sheep and goats using three different serological tests from the four 4 LGAs in Kaduna North Senatorial District of Kaduna State.

| LGA | No. of Samples Tested | Number (%) Positive by test type | | |
|---|---|---|---|---|
| | | RBPT No (%) | SAT-EDTA No (%) | LFA No (%) |
| Ikara | 253 | 61 (24) | 34 (13) | 8 (3) |
| Makarfi | 233 | 68 (29) | 16 (7) | 3 (1) |
| Sabon Gari | 285 | 81 (24) | 22 (8) | 2 (1) |
| Soba | 250 | 52 (21) | 41 (16) | 12 (5) |
| **TOTAL** | 1021 | 265 (26) | 115 (11) | 28 (3) |

RBPT ⟶ Rose Bengal Plate Test
SAT-EDTA ⟶ Serum Agglutination Test with Ethylene Diaminotetra acetic Acid
LFA ⟶ Lateral Flow Assay

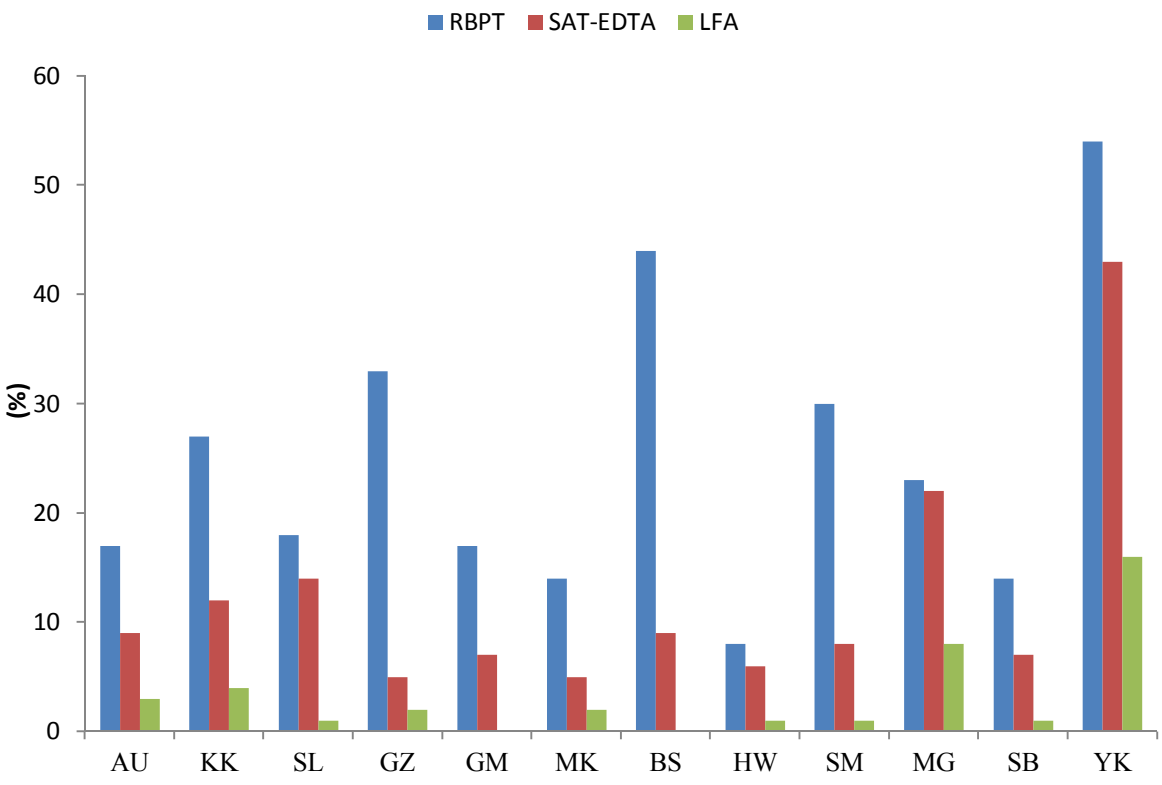



**Fig. 4.1:** Results of serological tests on brucellosis in sheep and goats from the twelve Districts of the four LGAs in Kaduna North Senatorial District of Kaduna State

**KEY**:

| **Ikara LGA** | **Makarfi LGA** | **Sabon Gari LGA** | **Soba LGA** |
|---|---|---|---|
| AU= Auchan | GZ= Gazara | BS= Basawa | MG= Maigana; |
| KK= Kurmin Kogi | GM= Gimi | HW= Hanwa | SB= Soba |
| SL= Saulawa | MK Makarfi | SM = Samaru | YK= Yakasai. |

The sampled sheep and goats in Basawa District of Sabon Gari had the highest seroprevalence with RBPT 44 (45%) while those in Maigana district of Soba LGA had the highest seroprevalence of 21 (26%) and 8 (9%) with the SAT-EDTA and the LFA respectively. All the districts recorded positive results to the RBPT and SAT-EDTA. Similarly, all the districts except Gimi tested positive to the LFA. The RBPT seroprevalence in all the districts tested ranged were 8 (9%) in Hanwa district to 44 (44%) in Basawa district, both of which in Sabon Gari LGA of the State. Similarly, the SAT-EDTA seroprevalence ranged between 5 (5 %) and 21 (26%) in Gazara Makarfi LGA and Maigana (Soba LGA), However, the LFA seroprevalence ranged between 0 (0%) in both Gimi (Makarfi LGA) and Basawa (Sabon Gari LGA) and 8(9%) in Maigana district of Soba LGA (Fig.4.1)

There was a statistically significant difference ($P = 0.0001$, $\chi^2 = 0.243.8$) in the rates obtained with the three different serological tests that detected *Brucella* antibodies in sheep and goats.

**Table 4.3:** The rate of detection of *Brucella* antibodies in sheep and goats using the three different serological tests from the four LGAs in Kaduna North Senatorial District of Kaduna State.

| LGA | Number of animals tested | | Number of animals seropositive using the three tests | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | RBPT | | SAT-EDTA | | LFA | |
| | Sheep | Goat | Sheep No. (%) | Goat No. (%) | Sheep No. (%) | Goat No. (%) | Sheep No. (%) | Goat No. (%) |
| Ikara | 170 | 83 | 39 (23) | 22 (27) | 22 (13) | 12 (15) | 4 (2) | 4 (5) |
| Makarfi | 40 | 193 | 13 (33) | 55 (29) | 3 (8) | 13 (7) | 0 (0) | 3 (2) |
| S/Gari | 229 | 56 | 65 (28) | 16 (29) | 20 (9) | 2 (4) | 2 (1) | 0 (0) |
| Soba | 140 | 110 | 31 (22) | 21 (19) | 19 (14) | 22 (20) | 8 (6) | 4 (4) |
| **TOTAL** | 579 | 442 | 148 (26) | 114 (26) | 64 (11) | 49 (11) | 14 (2) | 11 (3) |

RBPT ⟶ Rose Bengal Plate Test
SAT-EDTA ⟶ Serum Agglutination Test with Ethylene Diaminotetra acetic Acid
LFA ⟶ Lateral Flow Assay

The results of the serological tests according to the species of animal and the district in the various LGAs are presented in Appendices VI to IX. With regards to the RBPT, sheep from Basawa district in Sabon Gari LGA had the highest seroprevalence of 37 (44 %) while sheep from Hanwa district in Sabon Gari LGA had the lowest seroprevalence of 7 (11 %). Similarly, the highest SAT-EDTA seroprevalence in sheep was from Saulawa district in Soba LGA 8 (20%). However, the highest LFA seroprevalence of 5 (11%) occurred in sheep from Maigana district of Soba LGA. The lowest LFA seroprevalence was 0 (0%)

which was recorded in sheep from Saulawa district of Ikara LGA, Gazara, Gimi and Makarfi districts of Makarfi LGA, Basawa district of Sabon Gari LGA, and Soba district of Soba LGA. The highest RBPT seroprevalence in goats was 6 (50%) from Basawa district of Sabon Gari LGA, while lowest RBPT seroprevalence of 1 (4%) was recorded from Hanwa district of Sabon Gari LGA. Similarly, the highest SAT-EDTA seroprevalence in xcgoats was 11 (22%) from Yakasai district in Soba LGA while the lowest seroprevalence of 0 (0%) was recorded from Basawa district of Sabon Gari LGA. The highest LFA seroprevalence recorded in goats was 3 (6%) from Yakasai district in Soba LGA while the lowest was 0 (0%) from Gimi district in Makarfi LGA as well as all the three districts from Sabon Gari LGA.

There was no statistically significant difference ($P = 0.9422$, $\chi^2 = 0.005249$) in prevalence rates of brucellosis between sheep and goats.

## 4.4 PREVALENCE OF BRUCELLOSIS IN SHEEP AND GOATS BASED ON SEX IN 4 LGAs IN KADUNA NORTH SENATORIAL DISTRICT OF KADUNA STATE

The number of sheep and goats sampled according to their sex and associated seroprevalence rates for brucellosis using RBPT, SAT-EDTA and LFA tests are presented in Table 4.5. Altogether, 145 male and 876 female sheep and goats were sampled. Out of the 145 males animals sampled, 114 were rams and 31 were bucks, similarly for the female animals, 465 were ewes and 411 were does as presented in Table 4.5.

**Table 4.4**: Distribution of Sheep and Goats sampled for brucellosis according to sex in 4 LGAs in Kaduna North Senatorial District of Kaduna State.

| LGA | No. of Animals Sampled | Male | | Female | |
|---|---|---|---|---|---|
| | | Sheep (%) | Goat (%) | Sheep (%) | Goat (%) |
| Ikara | 253 | 22 (2.2) | 6 (0.6) | 148 (14.5) | 77 (7.5) |
| Makarfi | 233 | 10 (1.0) | 11 (1.1) | 30 (2.9) | 182 (17.8) |
| Sabon Gari | 285 | 51 (5.0) | 9 (0.9) | 178 (17.4) | 47 (4.6) |
| Soba | 250 | 31 (3.0) | 5 (0.5) | 109 (10.7) | 105 (10.3) |
| TOTAL | 1021 | 114 (11.2) | 31 (3.0) | 465 (45.5) | 411 (40.3) |

**Table 4.5:** Sex prevalence of brucellosis in sheep and goats from the 4 LGAs in North Senatorial District of Kaduna State.

| LGA | Sex | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Male No Positive (%) | | | | Female No Positive (%) | | | |
| | No Sampled | RBPT | SAT-EDTA | LFA | No Sampled | RBPT | SAT-EDTA | LFA |
| Ikara | 28 | 8 (29) | 2 (7) | 0 (0) | 225 | 53 (24) | 32 (14) | 8 (4) |
| Makarfi | 21 | 7 (33) | 1 (5) | 0 (0) | 212 | 61 (29) | 15 (7) | 3 (1) |
| S/Gari | 60 | 15 (25) | 1 (2) | 0 (0) | 225 | 67 (30) | 21 (9) | 2 (1) |
| Soba | 36 | 5 (14) | 3 (8) | 2 (6) | 214 | 49 (23) | 40 (19) | 13 (6) |
| **TOTAL** | 145 | 35 (3.4) | 7 (0.7) | 2 (0.2) | 876 | 230 (22.5) | 108 (10.6) | 26 (2.6) |

RBPT ⟶ Rose Bengal Plate Test
SAT-EDTA ⟶ Serum Agglutination Test with Ethylene Diaminotetra acetic Acid
LFA ⟶ Lateral Flow Assay

The results for the serological tests for brucellosis and the seroprevalence according to sex and district in the various LGAs are presented in Appendices X to XIII and fig 4.2 The highest RBPT seroprevalence rate of 5 (42%) was recorded in the males from Gazara district of Makarfi LGA while the lowest value was 0 (0%) from Gimi district of the same LGA. Similarly, the highest SAT-EDTA seroprevalence rates in males were 2 (33%) and 1 (33%) from Saulawa district in Ikara LGA and Gimi districts in Makarfi LGAs, respectively. The lowest SAT-EDTA prevalence of 0 (0%) was recorded in Auchan and

Kurmin Kogi (Ikara LGA), Gazara and Makarfi (Makarfi LGA) and Hanwa and Samaru (Sabon Gari LGA). With respect to the prevalence using LFA, the highest value of 1 (10%) was recorded in Maigana district of Soba LGA while 0 (0%) was recorded in the males from all the districts from Ikara, Makarfi, and Sabon Gari LGAs.

As for the female sheep and goats, highest rate based on RBPT was 40 (49%) recorded in Basawa district of Sabon Gari LGA, while the lowest rate 7 (10%) was recorded in Hanwa district also of Sabon Gari LGA. Similarly, the highest SAT-EDTA rate was 19 (25%) from Maigana district of Soba LGA while the lowest rate of 5 (6%) was recorded in Gazara district of Makarfi LGA. The highest LFA seroprevalence was 7 (9%) in Maigana district of Soba LGA while the lowest LFA rate of 0 (0%) was recorded in Gimi district of Makarfi LGA and Basawa district of Sabon Gari LGA.

There was no statistically significant difference (P = 0.4111) in the prevalence rates of brucellosis between the male and female animals.

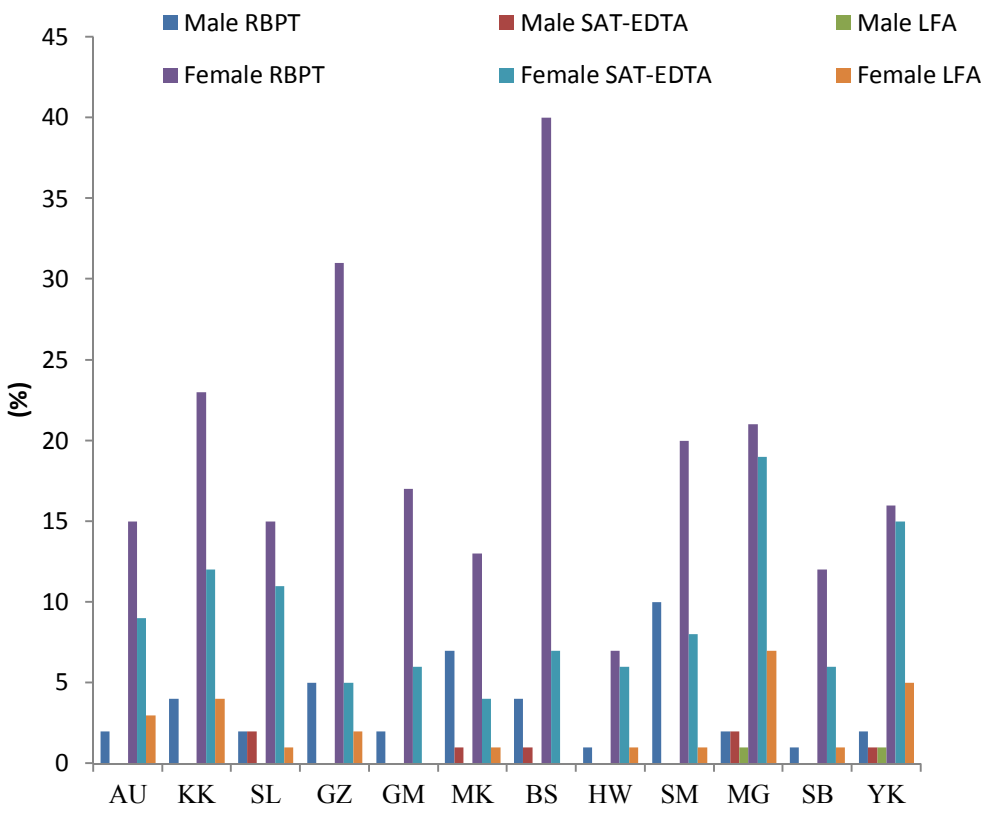



**Fig. 4.2:** Seroprevalence of brucellosis in sheep and goats based on the three serological tests according to sex from the 4 LGAs in Kaduna North Senatorial District of Kaduna State.

**KEY**:

| **Ikara LGA** | **Makarfi LGA** | **Sabon Gari LGA** | **Soba LGA** |
|---|---|---|---|
| AU= Auchan | GZ= Gazara | BS= Basawa | MG= Maigana; |
| KK= Kurmin Kogi | GM= Gimi | HW= Hanwa | SB= Soba |
| SL= Saulawa | MK Makarfi | SM = Samaru | YK= Yakasai. |

## 4.3 SEROPREVALENCE OF BRUCELLOSIS IN SHEEP AND GOATS ACCORDING TO AGE FROM THE 4 LGAs IN KADUNA NORTH SENATORIAL DISTRICT OF KADUA STATE.

The number of sheep and goats sampled and evaluated using the 3 serological tests based on age in the various LGAs is presented in Table 4.6 and Appendices XII to XVIII

**Table 4.6**: Seroprevalence of brucellosis in sheep and goats according to age from 4 LGAs Kaduna North Senatorial District of Kaduna State

| | | | LGAs | | | | |
|---|---|---|---|---|---|---|---|
| **Age group** | | | **Ikara** | **Makarfi** | **Sabon Gari** | **Soba** | **TOTAL** |
| < 1yr | No +ve (%) | No Sampled | 12 | 1 | 12 | 37 | 62 |
| | | RBPT | 2 (17) | 0 (0) | 4 (33) | 6 (16) | 12 (1.2) |
| | | SAT-EDTA | 1 (8) | 0 (0) | 1 (8) | 4(11) | 6(0.6) |
| | | LFA | 0 (0) | 0(0) | 0(0) | 4(11) | 4(0.4) |
| 1-3 yrs | No +ve (%) | No Sampled | 128 | 114 | 234 | 149 | 625 |
| | | RBPT | 26(20) | 29(25) | 69(30) | 27(18) | 151(14.8) |
| | | SAT-EDTA | 14(11) | 7(6) | 18(8) | 23(15) | 62(6.1) |
| | | LFA | 4(3) | 1(1) | 2(1) | 6(4) | 13(1.3) |
| > 3yrs | No +ve (%) | No Sampled | 113 | 118 | 39 | 64 | 334 |
| | | RBPT | 33(29) | 39(33) | 7(18) | 21(33) | 100(9.8) |
| | | SAT-EDTA | 19(17) | 9(8) | 3(8) | 16(25) | 47(4.6) |
| | | LFA | 3(3) | 2(2) | 0(0) | 5(8) | 10(0.3) |

RBPT ⟶ Rose Bengal Plate Test
SAT-EDTA ⟶ Serum Agglutination Test with Ethylene Diaminotetra acetic Acid
LFA ⟶ Lateral Flow Assay

Of the 62 sheep and goats of less than one year of age that were evaluated for brucellosis antibodies using the RBPT the highest rate of 1 (50%) was recorded in Basawa district of Sabon Gari LGA while the lowest rate of 0 (0%) was recorded in Auchan and Saulawa (Ikara LGA), all those from Makarfi LGA, and the animals Hanwa district of Sabon Gari LGA. For the SAT-EDTA test, the highest of 2 (16%) was recorded in Maigana district of Soba LGA while the lowest of 0 (0%) were recorded in Auchan and Saulawa (Ikara LGA), all the animals of this age group from all the districts from Makarfi LGA, those from Basawa and Hanwa districts in Sabon Gari LGA, from Soba distict in Soba LGA. Based on the LFA test, the highest rate of 2 (15%) for the age group was recorded in Maigana district of Soba LGA, while all the districts in Ikara, Makarfi, and Sabon Gari LGAs had a 0 (0%).

Of the total 625 sheep and goats within the age group of 1-3 years that were tested, the highest RBPT rate of 37 (45%) was recorded in Basawa district of Sabon Gari LGA. The lowest RBPT rate of 7 (10%) was recorded from Hanwa district in Sabon Gari LGA. Similarly, the SAT-EDTA showed the highest rate of 12 (21%) was recorded in Maigana district (Soba LGA) while the lowest of 2 (4%) was recorded in Auchan district in Ikara LGA. Within the same age group the highest rate based on the LFA test was 3(6%) recorded in Kurmin Kogi district in Ikara LGA while the lowest was 0 (0%) recorded in Gazara and Gimi districts (Makarfi LGA), Basawa (Sabon Gari LGA), and Soba district of Soba LGA.

Similarly, of the 334 sheep and goats that were aged three years and above, 21(48%) from Gazara in Makarfi LGA had the highest rate based on the RBPT while the least in the age group was 7 (10%) recorded in sheep and goats from Hanwa district of Sabon Gari LGA. Within the same age group, the rate based on the SAT-EDTA 7 (47%) recorded in Maigana district of Soba LGA. The lowest rate based on the same test was 0(0%) recorded in Samaru district in Sabon Gari LGA. The results of the LFA test for this age group showed the highest prevalence of 3(20%) recorded in Maigana district in Soba LGA while the lowest rate of 0 (0%) was recorded in Saulawa (Ikara LGA), Gimi and Makarfi (Makarfi LGA), and all the districts in Sabon Gari LGA.

There was no statistically significant difference ($P = 0.1096$; $\chi^2 = 4.432$ in prevalence rates of brucellosis between the three different age groups of the sheep and goats evaluated.

## 4.6 RESULTS OF THE MILK RING TEST

The results obtained on the prevalence of *Brucella* antigens present in the milk samples of sheep and goats used in the study indicated that of the 194 milk samples tested by MRT, collected, 45 (23%) were positive (Fig. 4.3). The highest rate for *Brucella* antigen in the milk of the sampled sheep was 8 (40%) out of 20 sheep from Ikara LGA, followed by those from Makarfi LGA with 1 (25%), 1 (15%) from Soba LGA and 3 (11%) from Sabon Gari LGA. Similarly, the highest prevalence rate for *Brucella* antigen in milk of goats determined by the MRT was in Sabon Gari LGA with 10 (40%) out of the 25 goats that

tested positive followed by those from Ikara, Makarfi, and Soba LGAs with 10 (40%),7 (24%), and 7 (14%) respectively.

There was no statistically significant difference (P = 0.1925, $\chi^2$ = 1.698) in the prevalence rates of *Brucella* antigens present in the milk sampled from sheep and goat.

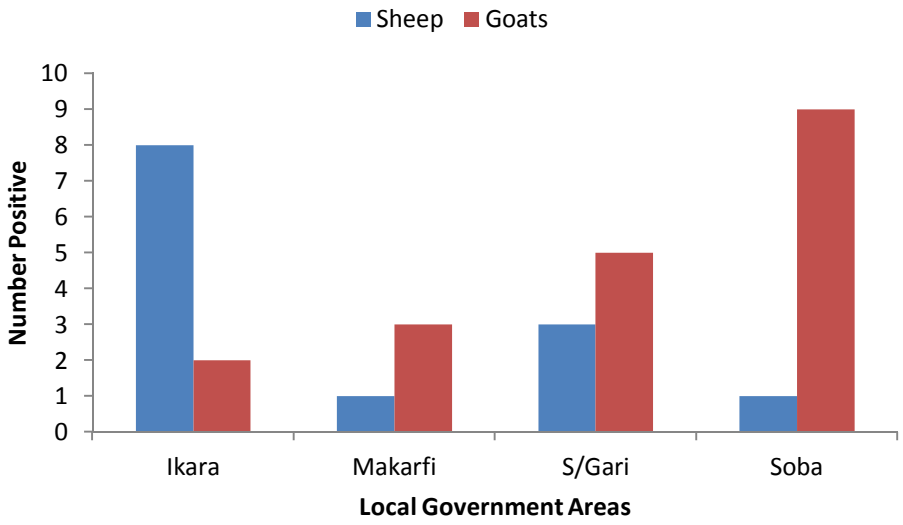



**Fig 4.3** Frequencies of *Brucella* antigen positive cases in the milk of sheep and goats in the 4 LGAs of the Kaduna North Senatorial District of Kaduna State.

## 4.7 RESULTS OF THE ANALYSIS OF RESPONSE TO KNOWLEDGE OF BRUCELLOSIS IN ANIMALS

Of the 70 people administered with the structured questionnaire, 41 (59%) were fully aware of brucellosis in cattle but not in sheep or goats while 29 (41%) were not aware of the disease at all. Sources of awareness were found to be the media 13 (32%), through contact with experienced farmers with 28 (68%), and from professionals 3 (7%). (Table 4.7 and Fig 4.4)

**Table 4.7**: Results of the response on awareness to brucellosis and sources of information in 4 LGAs in the Kaduna North Senatorial District of Kaduna State.

| LGA | No. of Respondents | Awareness of brucellosis | | Source of Awareness | | |
|---|---|---|---|---|---|---|
| | | Aware No. (%) | Not aware No. (%) | Media No. (%) | Experienced Farmer No. (%) | Professionals No. (%) |
| Ikara | 19 | 11 (15.7) | 8 (11.4) | 4 (5.7) | 7 (10.0) | 0 (0.0) |
| Makarfi | 16 | 13 (18.8) | 3 (4.3) | 1 (1.4) | 13 (18.8) | 2 (2.9) |
| S/Gari | 18 | 12 (17.1) | 6 (8.6) | 6 (8.6) | 6 (8.6) | 0 (0.0) |
| Soba | 17 | 5 (7.1) | 12 (17.1) | 2 (2.9) | 2 (2.9) | 1 (1.4) |
| TOTAL | 70 | 41 (58.6) | 29 (41.4) | 13 (18.8) | 28 (40.0) | 3 (4.3) |

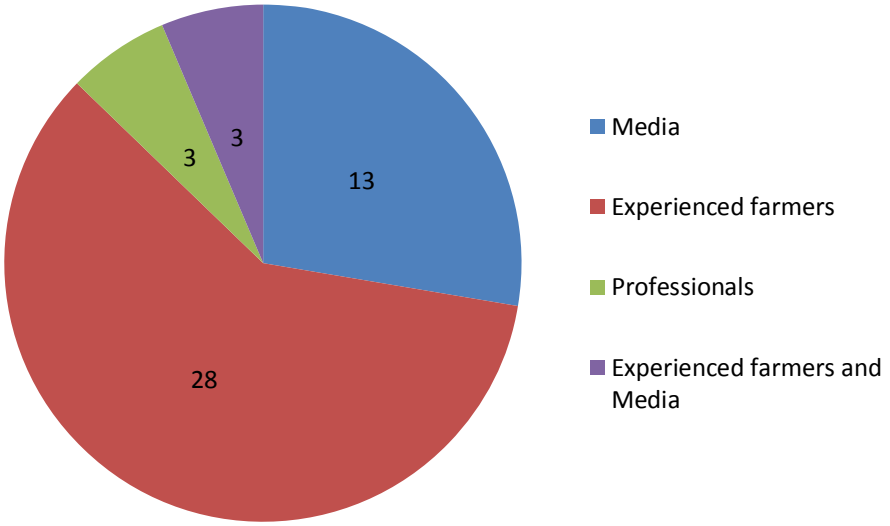



**Fig. 4.4:** Pie chart of responses on the questionnaire administered on sources of Awareness to brucellosis in sheep and goats 4 LGAs in the Kaduna North Senatorial District of Kaduna State.

### 4.7.1 Respondents' knowledge of brucellosis as a zoonosis

Seven out of the 70 respondents i.e 10 % indicating having knowledge on the zoonotic status of brucellosis (Table 4.8). Among the 7, 2 believed humans acquire brucellosis through ingestion while 3 others believed men acquire the infection mainly through contact. Respondents from Sabon Gari and Soba LGAs did not know how men can contract the disease. In particular, all the respondents from Soba were completely not aware of the disease in man while only one from Sabon Gari LGA indicated some knowledge of the disease in man. Two of the respondents gave signs and symptoms of the disease in man to include; night sweats, intermittent fever, headaches, joint pains, weakness, and vomiting. All the respondents from Sabon Gari and Soba LGAs did not associate any signs to the disease in man.

**Table 4.8:** Analysis of the response on the questionnaire on awareness of brucellosis as a zoonosis

| LGA | Number of Respondent | Awareness of Brucellosis | | Way of Infection | | Recognition of Brucellosis | | |
|---|---|---|---|---|---|---|---|---|
| | | Aware (%) | Not aware (%) | Ingestion | Contact | Night Sweats | Intermittent Fever | Others |
| Ikara | 19 | 1(1.4) | 19(27.1) | 1(1.4) | 0(0.0) | 1(1.4) | 1(1.4) | 0(0.0) |
| Makarfi | 16 | 5(7.1) | 11(15.7) | 1(1.4) | 3(4.3) | 2(2.9) | 2(2.9) | 2(2.9) |
| Sabon Gari | 18 | 1(1.4) | 17(24.3) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) |
| Soba | 17 | 0(0.0) | 17(24.3) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) |
| **TOTAL** | 70 | 7(10.0) | 64(91.4) | 2(2.9) | 3(4.3) | 3(4.3) | 3(4.3) | 2(2.9) |

### 4.7.2 Results of the responses on the questionnaire on management practices on sheep and goats

The management practices employed by the interviewed farmers in the study area showed that 2 (3%) of the farmers managed their animals based on an intensive system. However, 16 (23%) managed them on semi-intensive system while majority 52 (74%) managed them on an extensive system. Also, most of the respondents indicated keeping other species of animals like cattle, poultry, horses, cats, and dogs (Appendix XVIII). Majority of the respondents indicated that with respect to goats the Sokoto red goats, followed by the Sahel goat, Kano Brown and then Kano Brown/Sahel crosses were the main breeds kept in that order. For sheep, the Yankasa breed was the most predominant, followed by the Uda, Balami and to a lesser extent the Balami and Yankasa crosses (Appendix XIX).

On animal sourcing, the analysis of the responses in relationship to management shows that 49 (70%) of the respondents sourced their animals from the market, 4 (6%) from established farms, and 13 (20%) from other sources. Furthermore, majority of the respondents 45 (64%), did nothing in terms of quarantine to newly acquired animals prior to adding them to the existing flock. However, 6 (9%) indicated having practiced some level of quarantine, with 2 (3%) having the animals treated while 0 (0%) vaccinated before adding to the existing flock.

### 4.7.3 Results of responses on the questionnaire on breeding practices by the sheep and goats owners

The results have shown a very high level of exchange of animals in form of borrowing involving 39 (56%), and lending involving 50 (71%) of the respondents. Also, 57 (81%) of the respondents indicating having a history of abortion in their flock with 4 (7%), 19 (33%) and 39 (68%) of the respondents noting abortion occurring in the first, second, and third trimesters, respectively. However, 5 (9%) of the respondents indicated not to be sure of the stage of pregnancy when the abortion occurred (Appendix XX). The result also showed 12 (21%) of the respondents noticing the abortion affecting 1-10% of the flock, 22 (39%) of respondents had it affecting 11-20%, while 23 (40%) indicated abortion affecting 20% of the flock. Furthermore, on reproduction and breeding practices 24 (34%) of the respondents indicated that a separate delivery pen was used during delivery while 45 (66%) of the respondents indicated none (Appendix XX).

On the practices employed by the respondents on dealing with aborted foetus, the results showed that 6 (9%) of the respondents burry aborted foetuses, 1 (1%) burn them, while 51

(73%) indicated dumping such foetuses. Also of the respondents, 41 (59%) indicated having a history of retained placenta, 17 (24%) did not, while 10 (14%) were not sure about retained placenta (Appendix XXI).

# CHAPTER 5

# DISCUSSION

This study designed to investigate the seroprevalence of *B. melitensis* in sheep and goats in 4 LGAs located in the Kaduna North Senatorial District of Kaduna State revealed an overall sero-prevalence 3% based on the LFA and a range of between 1 and 31% based on the RBPT SAT-EDTA, and LFA tests. These differences observed across the 4 LGAs could be attributed to the types of management practices of herdsmen. Sheep and goats are usually allowed to roam about in the villages for the village level animals while the migratory habit of the pastoralists could account for infection in these small ruminants since they are allowed to mix freely with cattle, a practice which Ocholi *et al*. (2004) reported to be increasing the risk of infection in them. The same situation can be speculated for the differences at the district levels.

The results of the study showed that more animals were seropositive using the RBPT and SAT-EDTA when compared to the LFA test. The high seroprevalence rate obtained using the RBPT could be attributed to its relatively low specificity and very high sensitivity which could be as a result of possible reaction to other SLPS *Brucella* species especially *B. abortus* since sheep and goats are herded together with cattle (Poiester *et al.*, 2010). This observation is in line with that of Gerbier *et al*. (1997) in which they stated that some other Gram-negative bacteria (e.g *Vibrio cholerae* O1, *Escherichia coli* O: 157, some strains of *Escherichia hermannii* and *Stenotrophomonas maltophila*, *Salmonella* group N (O: 30), and *Yersinia enterocolitica* O: 9) which have LPS O-chains similar to those of Brucellae are capable of giving false positive reactions. These organisms have agglutinins, mainly IgM, which are capable of reacting with *Brucella* antigens which may lead to a false positive

reaction (Corbel, 1972). However, the RBPT can be used as a screening test to ascertain exposure of animals to infection due to *Brucella spp.*

The lower seroprevalence rates obtained using the SAT-EDTA could be attributed to increase in specificity of this test and agrees with the findings of Bertu *et al*. (2010) who also reported a prevalence rate of 9.3% and 5.2% using the RBPT and the SAT respectively.

The least seroprevalence rate recorded using the LFA is indicative of the high specificity with this assay since it detects antibodies due to *B. melitensis* only. Due to the high specificity and simplicity coupled with the fact that it does not involve any expertise or refrigeration, it is advocated that this assay (LFA) should be used for serological survey of the disease.

Furthermore, the ability of any serological test to reliably detect brucellosis also depends on the presence of detectable antibodies at the time of examination. However, some infected animals may inevitably elude detection if antibodies are not in circulation during the time of blood collection (FAO, 2004). This may explain the relatively lower seroprevalence rate in this study.

The prevalence of brucellosis based on the serological tests used in the sheep and goats were 2% and 1%, respectively, giving an overall prevalence of 3%. This is comparable with the findings of Brisibe *et al*. (1993) who reported prevalence rates of 2.5% in Maiduguri metropolis and that of Falade and Shonekan (1982) who reported a prevalence of 2.56% in Ibadan, in western Nigeria. However, Shehu *et al*. (1999) also reported a higher prevalence

rate of 6.6% in a seroprevalence study of brucellosis in ruminants in Bauchi and environs. Okoh (1980) reported a relatively higher prevalence of 14.5% in a sheep breeding center in Kano. In an earlier study conducted in Northern Nigeria Bale *et al*. (1980) reported a prevalence of 14.1%.

A low prevalence of 1% was reported in goats in this study which does not agree with the reports of Brisibe *et al*. (1993) who reported a prevalence of 2.8% in Northern Nigeria. Other researchers like Falade, (1981), Ogundipe *et al*. (1994) and Shehu *et al*. (1999) reported higher prevalence rates of 9.0%, 5.88%, and 4.75%, respectively. Furthermore, in a recent study in a goat flock in Abeokuta, Ojo *et al.* (2007) reported an alarming prevalence rate of 45.75%. The difference in the seroprevalence by different workers could be due to difference in the accuracy of the different diagnostic methods used among other reasons (Poiester *et al.*, 2010).

Although 56.9% of the animals sampled were sheep among both sheep and goats, sheep were found to be more seropositive than the goats using all the three tests. Nonetheless, 56.7% of the animals sampled were sheep, nonetheless, this finding does not agree with the findings of Bertu *et al*. (2010) who got seroprevalence of 14.1% and 16.1% in sheep and goats, respectively, eventhough more goats were sampled compared to sheep in their studies. The opposite is the case in this study as only 43.3% of the animals evaluated were goats. The absence of any significant differences in the prevalence rates between the sheep and the goats as observed in this study could be due to the fact that, in most cases, the animals are flocked together and as such they have equal exposure potential.

From the study, female animals were more seropositive than their male counterparts. The difference could be because of the fact that more females were sampled since they formed the bulk of the animals in the flocks as they were kept for breeding purposes. Generally, owners keep fewer males mainly for the purpose of breeding the females. Another reason for fewer males could be explained by the fact that rams are usually fattened and sold during Eid celebrations. In any case, the higher prevalence obtained could be due to the fact that females are more susceptible than males as reported by Keppie *et al*. (1965).

The results have revealed that sheep and goats within the age range of 1-3 years and those above 3 years of both sexes showed the highest seroprevalence rate for brucellosis. Although, more animals within this age bracket were sampledin this study, the finding is in agreement with the reports of Aulakh *et al.* (2008) and Abubakar *et al*. (2010) who reported the incidence to be high in sexually mature animals. The animals within this age range are normally actively involved in breeding. Therefore the occurrence of brucellosis in the group may have serious economic implications in terms of loss through reproductive wastages involving abortions, still births, infertility, sterility, and reduced milk production. It may also mean that this particular age group are capable of spreading the infection since they mix among themselves from different flocks. Furthermore, it is also important to note that animals in this age group are more likely to be sold out for slaughter or for other reasons thereby serving as sources for human infections.

The fact that sheep and goats below one year of age were seropositive to brucellosis could be due to the fact that they might have been exposed through suckling of their dams. Such

animals might also have contracted the infection through contaminated pasture or water during grazing or at watering points thereby serving as potential source of spread to other animals in the future.

The high prevalence rate observed using the MRT in this study is of great significance because goat milk is becoming increasingly important and that pastoralists use it for traditional medicine (Anon., 2012; Saidu, 2012). Also, the fact that goat milk has several advantages over cow milk it can be considered as a source of public health risk (Adamu *et al*., 2012 and Anon., 2012). However, there is the possibility that some of the positive MRT result could have been due to certain factors like end of lactation, mastitis, presence of colostrum, or a hormonal disorder could result in false positive. The effects of these factors were however not investigated during the course of this study. Despite these limitations, the MRT remains an important screening test for brucellosis considering the fact that some other tests are more problematic and cumbersome.

The occurence of brucellosis in sheep and goats could also be attributed to direct contact with infected animals, especially via pasture, due to the use of common grazing fields and watering points. When Brucella associated abortions occur, there is the likelihood of contaminating such feeding and watering resources thereby exposing the other species of animals to risk. The fact that most sheep and goats are kept together with cattle could further explain the occurrence of brucellosis in these animals.

The knowledge of the respondents on brucellosis as obtained in this study could be expected as a number such respondents, especially pastoralists, might have come across the disease in cattle. Furthermore, knowledge is usually transmitted from the elders to the

youths as revealed by this study. Similarly extension technology delivery through the use of radio and television, along with activities of the livestock Rearers' Workshop programmes over the years have contributed in making these livestock owners knowledgeable on many diseases including brucellosis (Saidu *et al*., 1980; Ardo, 1998; El-Nafaty, 1998).

The understanding by respondents that brucellosis can be zoonotic may similarly be due to their interactions with more experienced farmers within their communities and with professionals as deduced from this study. This finding has shown that sheep and goat owners in the study area were more than ordinary with respect to their level of awareness. The closeness of some of the districts investigated to A.B.U. Zaria makes it easier for many of such respondents to have contact and information on veterinary care for their animals. Also, the ambulatory services of the Veterinary Teaching Hospital have provided another avenue in creating awareness of the disease.

However, their seemingly little knowledge on the disease brucellosis in sheep and goats could be because as pastoralists, they attach more importance and interest to cattle than sheep and goats due to their greater economic benefits. The fact that the pastoralists were more conversant with the disease than their village level counterparts could be explained by the fact that pastoralists interact more with livestock since it is their main source of livelihood (Fulani and Saidu, 1988; Saidu, 1990). Their understanding of the disease being zoonotic as well as the clinical signs of the disease in man can similarly be explained by their proximity to relevant educational institutions for information and access to better

veterinary services. Furthermore, the ambulatory services of the Veterinary Teaching Hospital are extended to some of these Fulani pastoralist settlements.

The majority of the sheep and goats owners interviewed in this study were engaged in the extensive husbandry system. This agrees with the findings of Saidu and Isitor (1989) who reported similar findings. Their adoption of this system could be influenced by factors of level of education and lack of access to resources to invest in order for more efficient systems. Furthermore, their keeping of various breeds of livestock could explain their diversification, even in livestock development (Saidu and Isitor, 1989).

The fact that most of the respondents bought their new animals from open markets could be understood as these markets serve as the main, easy and reliable sources of acquiring new animals. This could be due to the fact that presently, most elite farms and even government livestock farms are slowing down in direct livestock production. The result of this study has shown that the respondents had little knowledge on the significance of quarantine after purchase of animals. This could be considered to be due to inability of such keepers to appreciate the role of quarantine, while for the pastoralists they seem to be always on the move which apparently may not favour this practice.

The fact that such animals were allowed to mix and even breed during grazing could account for the potential risk they are exposed to in contracting diseases, including those that impact on reproduction. This also leads to greater risk of them coming down with brucellosis. The high percentage of abortions in the animals can be associated with brucellosis especially that many of the animals aborted during the second and third

trimester. The management practices on aborted foetuses as reported by the respondents could mean increased chances of spread of the organisms in nature and contamination of the grazing and watering points for the unsuspecting pastoralist herds and flocks.

# CHAPTER 6

# SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

## 6.1 SUMMARY

The study revealed that small ruminants (sheep and goats) in the four LGAs (Ikara, Makarfi, Sabon Gari and Soba) of the Kaduna North Senatorial District of Kaduna State had antibodies to *Brucella spp* based on RBPT, SAT-EDTA, LFA, and MRT serological techniques. The sampled sheep were more seropositive than goats and this could be explained by the fact that most of the sheep are herded with cattle especially in pastoralist herd than the goats. However, this was not statistically significant. The study has further shown that the disease is more prevalent in females than in males. This could be attributed to the fact that more females are kept longer in the flock than males since they form the bulk of the breeding animals and that only one or two males may be required for breeding in a given flock. Another reason could be that males (rams and bucks) are usually sold or used for religious festivals, as well as for day to day slaughter for consumption in rural communities. Furthermore, sheep and goats within the age range of one to three years and above were more seropositive than those of less than one year old. This could be due to their longer stay in the flock which would have given them more exposure opportunities than the younger ones. The study also demonstrated that RBPT was more sensitive than SAT-EDTA and LFA in detecting infected animals and that *B. melitensis*, in addition to *B. abortus*, exists in the study area.

The presence of antibodies in the milk of some of sheep and goats connotes the potential public health significance of brucellosis in the study area. The lack of overt clinical signs of

brucellosis along with the seeming absence of investigation of cases of abortion, stillbirth and birth of weak lambs and kids in the study area by veterinary authorities may make the environment ripe for the spread and survival of the *Brucella* organisms in the area. Further the study has shown that risk of infections in humans engaged in the consumption of poorly cooked meat 'Balangu/Suya' along with consumption of unpasteurized milk from these animals since the bulk of the meat eaten in rural areas, like most of the study areas, is from sheep and goats, as well as the consumption of unpasturised milk, especially by small children of the pastoralist, from infected animals. This is particularly so as pastoralists as well as rural communities use goat milk as part of traditional medicine.

The study has similarly demonstrated the risk that could be exposed to butchers in the study area as livestock owners usually sell female animals that abort more than once to the butchers should such animals be infected with *Brucella* organisms. Small ruminant owners who are in the habit of manually relieving dystocia or retained placenta membranes could similarly run the risk of acquiring infection through break in their skin continuity should such animals be infected.

The KAP of the respondents has clearly demonstrated their awareness of the existence of brucellosis, in cattle. They knew the signs of the disease in man often take necessary measures to control the disease by the use of traditional methods in their animals. The study has further shown that many of the small ruminant owners interviewed especially the pastoralists were aware of how the disease is spread among animals.

Some of the respondents were in the habit of the quarantining new additions to their flocks or herds. However, once these animals mixed with the regular members of the flock, infections could still occur since such added animals were not routinely screened for brucellosis before adding them to the flock. Thus, there is still room for the spread of the disease in the absence of screening. Similarly, since animals were allowed to mix with other animals from other flocks with chances of field breeding, infected males and females could spread the disease to clean ones.

## 6.2 CONCLUSIONS

From this work it was concluded that:

1. *Brucella* antibodies were present in blood and milk of sampled sheep and goats in four LGAs (Ikara, Makarfi, Sabon Gari and Soba) of the Kaduna North Senatorial District of Kaduna State.
2. Sheep were more seropositive than goats.
3. Females were more seropositive than males.
4. Sheep and goats of one to three years and above were more seropositive than those of less than one year of age.
5. RBPT was more sensitive than SAT-EDTA and LFA.
6. The KAP of respondents in the study area indicated that many of them were aware of brucellosis mainly in cattle and that the disease has potentials for being zoonotic in the study area.

## 6.3 RECOMMENDATIONS

1. A national epidemiological survey is needed to determine *Brucella* biotypes, magnitude and distribution of brucellosis in both humans and ruminants in the study area and Nigeria as a whole to enable Government to develop a national control programme against the disease in view of its economic and public health significance.
2. High risk groups like the veterinarians, butchers and livestock handlers and the general public should be given health education on the nature of the disease and on how to minimize the risk of transmission of the disease through animal contact and milk products produced from unpasteurized milk.
3. Further study involving more animal species in the study area and the nation as a whole is advocated with a view to having a better understanding of the epidemiology of the disease in the study area and the country as a whole.

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## APPENDICES

APPENDIX I

FORM NO.....

DATE.....

STRUCTURED QUISTIONNAIRE ON BRUCELLOSIS IN SMALL RUMINANTS IN KADUNA NORTH SENATORIAL DISTRICT OF KADUNA STATE, NIGERIA

FLOCK HISTORY

1) Name of owner (optional).....
2) Location.....
3) Flock size.....
4) Breed.....
5) System of management:

   Intensive ( )

   Semi-intensive ( )

   Extensive ( )

6) Do you keep other animals YES ( ) NO ( )
7) Specify type.....
8) Do you introduce new animals into the flock YES ( ) NO ( )
9) How? Quarantine ( ) treat before ( ) vaccinate ( ) do nothing ( )
10) What is the source of new stock? Market ( ) Established farm ( ) other ( )
11) Do you borrow males for breeding YES ( ) NO ( )
12) Do you give out breeding males? yes ( ) no ( )

13) Any history of abortion in the flock in the last one year YES ( ) NO ( )

14) At what stage of pregnancy did it occur 1st ( ) 2nd ( ) 3rd ( ) trimester

15) What proportion of the flock was affected? 1-10% ( ) 11-20% ( ) above 20% ( )

16) Do you have a separate delivery pen YES ( ) NO ( )

17) How do you dispose of your aborted materials? Burying ( ) Burning ( ) Dumping ( ) other ( )

18) Do you have a history of retained placenta in the flock since last one year? YES ( ) NO ( )

19) Have you heard of brucellosis (Bakkale) before? YES ( ) NO ( )

20) If yes from what source? Media ( ) experienced farmers ( ) professionals( )

21) How do you recognise the disease? Retained placenta ( ) Abortion ( ) other ( )

22) How do you prevent your goats against brucellosis? vaccination ( ) other ( )

23) If vaccination, what is your source of vaccine? Market ( ) other ( )

24) Are you aware you can get infected with brucellosis? Yes ( ) no ( )

25) How do you think you can get infected? Contact ( ) ingestion ( )

26) Do you know the signs of brucellosis in humans? YES ( ) NO ( )

27) If above is yes, what signs do you observe? Intermittent fever ( ) night sweats ( ) other ( ).

THANK YOU

## APPENDIX II

### Serology for Ikara Local Government Area

| District | No. of Tested Samples | Positive for Serological Tests | | |
|---|---|---|---|---|
| | | RBPT (%) | SAT-EDTA (%) | LFA (%) |
| Auchan | 80 | 17 (21) | 9 (11) | 3 (4) |
| Kurmin Kogi | 105 | 27 (26) | 12 (11) | 4 (4) |
| Saulawa | 68 | 17 (26) | 13 (21) | 1 (2) |
| TOTAL | 253 | 61 (24) | 34 (13) | 8 (3) |

## APPENDIX III

### Serology for Makarfi Local Government Area

| District | No. of Tested Samples | Positive for Serological Tests | | |
|---|---|---|---|---|
| | | RBPT (%) | SAT-EDTA (%) | LFA (%) |
| Gazara | 98 | 36 (37) | 5 (5) | 2 (2) |
| Gimi | 84 | 17 (20) | 7 (8) | 0 (0) |
| Makarfi | 51 | 15(28) | 4(10) | 1 (2) |
| TOTAL | 233 | 68 (29) | 16 (7) | 3 (1) |

## APPENDIX IV

### Serology for Sabon Gari Local Government Area

| District | No. of Tested Samples | Positive for Serological Tests | | |
|---|---|---|---|---|
| | | RBPT (%) | SAT-EDTA (%) | LFA (%) |
| Basawa | 97 | 44 (45) | 8 (9) | 0 (0) |
| Hanwa | 89 | 8 (9) | 6 (7) | 1 (1) |
| Samaru | 99 | 30 (28) | 8 (8) | 1 (1) |
| TOTAL | 285 | 82 (28) | 22 (8) | 2 (1) |

APPENDIX V

Serology for Soba Local Government Area

| District | No. of Tested Samples | Positive for Serological Tests | | |
|---|---|---|---|---|
| | | RBPT (%) | SAT-EDTA (%) | LFA (%) |
| Maigana | 85 | 23 (27) | 21 (26) | 8 (9) |
| Soba | 57 | 13 (25) | 6 (12) | 1 (2) |
| Yakasai | 108 | 18 (16) | 16 (13) | 6 (7) |
| TOTAL | 250 | 54 (22) | 43 (17) | 15 (6) |

APPENDIX VI

Prevalence per species for Ikara Local Government Area

| District | No. of Tested Samples | | RBPT (%) | | SAT-EDTA (%) | | LFA (%) | |
|---|---|---|---|---|---|---|---|---|
| | Sheep | Goat | Sheep | Goat | Sheep | Goat | Sheep | Goat |
| Auchan | 52 | 28 | 11(21) | 6(21) | 5(10) | 4(14) | 2(4) | 1(4) |
| Kurmin Kogi | 77 | 28 | 16(21) | 11(39) | 9(12) | 3(11) | 2(3) | 2(7) |
| Saulawa | 41 | 27 | 12(29) | 5(19) | 8(20) | 5(19) | 0(0) | 1(4) |
| **TOTAL** | 170 | 83 | 39 (15) | 22 (7) | 22 (7) | 12 (5) | 4 (2) | 4 (2) |

APPENDIX VII

Prevalence per species for Makarfi Local Government Area

| District | No. of Tested Samples | | RBPT (%) | | SAT-EDTA (%) | | LFA (%) | |
|---|---|---|---|---|---|---|---|---|
| | Sheep | Goat | Sheep | Goat | Sheep | Goat | Sheep | Goat |
| Gazara | 3 | 95 | 1(33) | 35(37) | 0(0) | 5(5) | 0(0) | 2(2) |
| Gimi | 10 | 74 | 4(40) | 13(18) | 1(10) | 6(8) | 0(0) | 0(0) |
| Makarfi | 27 | 24 | 8(30) | 7(29) | 2(7) | 2(8) | 0(0) | 1(4) |
| **TOTAL** | 40 | 193 | 13(6) | 55 (24) | 3 (1) | 13 (6) | 0(0) | 3 (1) |

APPENDIX VIII

Prevalence per species for Sabon Gari Local Government Area

| District | No. of Tested Samples | | RBPT (%) | | SAT-EDTA (%) | | LFA (%) | |
|---|---|---|---|---|---|---|---|---|
| | Sheep | Goat | Sheep | Goat | Sheep | Goat | Sheep | Goat |
| Basawa | 85 | 12 | 37(44) | 6(50) | 8(9) | 0(0) | 0(0) | 0(0) |
| Hanwa | 64 | 25 | 7(11) | 1(4) | 5(9) | 1(4) | 1(2) | 0(0) |
| Samaru | 80 | 19 | 21(26) | 9(47) | 7(9) | 1(5) | 1(5) | 0(0) |
| **TOTAL** | 229 | 56 | 65 (23) | 16 (6) | 20 (7) | 2 (1) | 2 (1) | 0 (0) |

APPENDIX IX

Prevalence per species for Soba Local Government Area

| District | No. of Tested Samples | | RBPT (%) | | SAT-EDTA (%) | | LFA (%) | |
|---|---|---|---|---|---|---|---|---|
| | Sheep | Goat | Sheep | Goat | Sheep | Goat | Sheep | Goat |
| Maigana | 47 | 38 | 13(28) | 8(21) | 11(23) | 8(21) | 5(11) | 1(3) |
| Soba | 35 | 22 | 11(31) | 2(9) | 3(9) | 3(14) | 0(0) | 1(5) |
| Yakasai | 58 | 50 | 7(12) | 11(22) | 5(9) | 11(22) | 3(5) | 3(6) |
| **TOTAL** | 140 | 110 | 31(12) | 21(8) | 19 (8) | 22 (9) | 8(3) | 4(2) |

APPENDIX X

Prevalence per sex in Ikara Local Government Area

| District | Sex | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | | | | Female | | | |
| | No. of Tested Samples | Positive (%) | | | No. of Tested Samples | Positive (%) | | |
| | | RBPT | SAT-EDTA | LFA | | RBPT | SAT-EDTA | LFA |
| Auchan | 12 | 2(17) | 0(0) | 0(0) | 68 | 15(22) | 9(13) | 3(4) |
| Kurmin Kogi | 10 | 4(40) | 0(0) | 0(0) | 95 | 23(24) | 12(13) | 4(4) |
| Saulawa | 6 | 2(33) | 2(33) | 0(0) | 62 | 15(24) | 11(18) | 1(2) |
| **TOTAL** | 28 | 8(3.2) | 2(0.8) | 0(0) | 225 | 53(20.1) | 32(12.6) | 8(3.2) |

APPENDIX XI

Prevalence per sex in Makarfi Local Government Area

| District | Sex | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | | | | Female | | | |
| | No. of Tested Samples | Positive (%) | | | No of Tested Samples | Positive (%) | | |
| | | RBPT | SAT-EDTA | LFA | | RBPT | SAT-EDTA | LFA |
| Gazara | 12 | 5(42) | 0(0) | 0(0) | 86 | 31(36) | 5(6) | 2(2) |
| Gimi | 3 | 0(0) | 1(33) | 0(0) | 81 | 17(21) | 6(7) | 0(0) |
| Makarfi | 6 | 2(33) | 0(0) | 0(0) | 45 | 13(29) | 4(9) | 1(2) |
| **TOTAL** | 21 | 7(3.0) | 1(0.4) | 0(0.0) | 212 | 61(26.2) | 15(6.4) | 3(1.3) |

APPENDIX XII

Prevalence per sex in Sabon Gari Local Government Area

| District | Sex | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | | | | Female | | | |
| | No of Tested Samples | Positive (%) | | | No of Tested Samples | Positive (%) | | |
| | | RBPT | SAT-EDTA | LFA | | RBPT | SAT-EDTA | LFA |
| Basawa | 15 | 4(27) | 1(7) | 0(0) | 82 | 40(49) | 7(9) | 0(0) |
| Hanwa | 19 | 1(5) | 0(0) | 0(0) | 70 | 7(10) | 6(9) | 1(1) |
| Samaru | 26 | 10(39) | 0(0) | 0(0) | 73 | 20(27) | 8(11) | 1(1) |
| **TOTAL** | 60 | 15(5.3) | 1(0.4) | 0(0.0) | 225 | 67(23.5) | 21(7.4) | 2(0.8) |

APPENDIX XIII

Prevalence per sex in Soba Local Government Area

| District | Sex | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | | | | Female | | | |
| | No of Tested Samples | Positive (%) | | | No of Tested Samples | Positive (%) | | |
| | | RBPT | SAT-EDTA | LFA | | RBPT | SAT-EDTA | LFA |
| Maigana | 10 | 2(20) | 2(20) | 1(10) | 75 | 21(28) | 19(25) | 7(9) |
| Soba | 8 | 1(13) | 0(0) | 0(0) | 49 | 12(25) | 6(12) | 1(2) |
| Yakasai | 18 | 2(11) | 1(6) | 1(6) | 90 | 16(18) | 15(17) | 5(6) |
| **TOTAL** | 36 | 5(2.0) | 3(1.2) | 2(0.8) | 214 | 49(19.6) | 40(16.0) | 13(5.2) |

APPENDIX XIV

Prevalence per age in Ikara Local Government Area

| District | Age Range (yrs) <1 | | | | 1-3 | | | | Above 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Positive (%) | | | | Positive (%) | | | | Positive (%) | | |
| | No of Tested Samples | RBPT | SAT-EDTA | LFA | No of Tested Samples | RBPT | SAT-EDTA | LFA | No of Tested Samples | RBPT | SAT-EDTA | LFA |
| Auchan | 3 | 0(0) | 0(0) | 0(0) | 47 | 7(15) | 2(4) | 1(2) | 30 | 10(33) | 7(23) | 2(7) |
| Kurmin Kogi | 9 | 2(22) | 1(11) | 0(0) | 47 | 12(26) | 6(13) | 3(6) | 49 | 13(27) | 5(10) | 1(2) |
| Saulawa | 0 | 0(0) | 0(0) | 0(0) | 34 | 7(21) | 6(18) | 1(3) | 34 | 10(29) | 7(21) | 0(0) |
| **TOTAL** | 12 | 2(0.1) | 1(0.4) | 0(0.0) | 128 | 26(10.3) | 14(5.5) | 4(1.6) | 113 | 33(13.0) | 19(7.5) | 3(0.8) |

APPENDIX XV

Prevalence per age in Makarfi Local Government Area

| District | Age Range (yrs) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | <1 | | | | 1-3 | | | | Above 3 | | | |
| | | Positive (%) | | | | Positive (%) | | | | Positive (%) | | |
| | No of Tested Samples | RBPT | SAT-EDTA | LFA | No of Tested Samples | RBPT | SAT-EDTA | LFA | No of Tested Samples | RBPT | SAT-EDTA | LFA |
| Gazara | 0 | 0(0) | 0(0) | 0(0) | 54 | 15(28) | 2(4) | 0(0) | 44 | 21(48) | 3(7) | 2(5) |
| Gimi | 1 | 0(0) | 0(0) | 0(0) | 33 | 7(21) | 3(9) | 0(0) | 50 | 10(20) | 4(8) | 0(0) |
| Makarfi | 0 | 0(0) | 0(0) | 0(0) | 27 | 7(26) | 2(7) | 1(4) | 24 | 8(33) | 2(8) | 0(0) |
| **TOTAL** | 1 | 0(0) | 0(0) | 0(0) | 114 | 29(12.5) | 7(3.0) | 1(0.4) | 118 | 39(16.7) | 9(3.9) | 2(0.9) |

APPENDIX XVI

Prevalence per age in Sabon Gari Local Government Area

| | Age Range (yrs) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | <1 | | | | 1-3 | | | | Above 3 | | | |
| | | Positive (%) | | | | Positive (%) | | | | Positive (%) | | |
| District | No of Tested Samples | RBPT | SAT-EDTA | LFA | No of Tested Samples | RBPT | SAT-EDTA | LFA | No of Tested Samples | RBPT | SAT-EDTA | LFA |
| Basawa | 2 | 1(50) | 0(0) | 0(0) | 83 | 37(45) | 6(7) | 0(0) | 12 | 4(33) | 2(17) | 0(0) |
| Samaru | 8 | 3(38) | 1(13) | 0(0) | 83 | 25(30) | 7(8) | 1(1) | 8 | 2(25) | 0(0) | 0(0) |
| Hanwa | 2 | 0(0) | 0(0) | 0(0) | 68 | 7(10) | 5(7) | 1(2) | 19 | 1(5) | 1(5) | 0(0) |
| **TOTAL** | 12 | 4(1.4) | 1(8) | 0(0) | 234 | 69(24.2) | 18(6.3) | 2(0.7) | 39 | 7(2.5) | 3(1.1) | 0(0.0) |

APPENDIX XVII

Prevalence per age in Soba Local Government Area

| District | Age Range (yrs) <1 | | | | 1-3 | | | | Above 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Positive (%) | | | | Positive (%) | | | | Positive (%) | | |
| | No of Tested Samples | RBPT | SAT-EDTA | LFA | No of Tested Samples | RBPT | SAT-EDTA | LFA | No of Tested Samples | RBPT | SAT-EDTA | LFA |
| Maigana | 13 | 2(15) | 2(16) | 2(15) | 57 | 15(26) | 12(21) | 3(5) | 15 | 6(40) | 7(47) | 3(20) |
| Soba | 5 | 1(20) | 0(0) | 0(0) | 33 | 4(12) | 2(6) | 0(0) | 19 | 8(42) | 4(21) | 1(5) |
| Yakasai | 19 | 3(16) | 2(11) | 2(11) | 59 | 8(14) | 9(15) | 3(5) | 30 | 7(23) | 5(17) | 1(3) |
| **TOTAL** | 37 | 6(2.4) | 4(1.6) | 4(1.6) | 149 | 27(10.8) | 23(9.2) | 6(2.4) | 64 | 21(8.4) | 16(6.4) | 5(2.0) |

# APPENDIX XVIII

## Respondents' Attitude on Small Ruminants Keeping

| Local Government Area | Respondents | Flock size | Species | | | | | | | | Management System | | | Keeping of Other Animals | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Goat(Sampled) | | | | Sheep(Sampled) | | | | | | | | | | | |
| | | | Sokoto Red | Sahel | Kano Brown/Sahelcross | Kano Brown | Yankasa | Balami | Uda | Balami/Yankasa cross | Intensive | Extensive | Semi-intensive | Cattle | Poultry | Horse | Cat | Dog |
| Ikara | 19 | 1425 | 121 | 0 | 0 | 0 | 184 | 0 | 0 | 3 | 0 | 19 | 0 | 18 | 18 | 0 | 0 | 18 |
| Makarfi | 16 | 378 | 324 | 0 | 0 | 0 | 103 | 0 | 0 | 0 | 0 | 16 | 0 | 15 | 16 | 0 | 0 | 16 |
| S/ Gari | 18 | 149 | 46 | 4 | 1 | 8 | 227 | 2 | 31 | 1 | 1 | 7 | 10 | 9 | 14 | 1 | 4 | 6 |
| Soba | 17 | 156 | 121 | 0 | 0 | 0 | 147 | 4 | 5 | 0 | 1 | 10 | 6 | 5 | 13 | 0 | 1 | 1 |
| **TOTAL** | 87 | 2108 | 612 | 4 | 1 | 8 | 661 | 6 | 36 | 4 | 2 | 52 | 16 | 47 | 61 | 1 | 5 | 41 |

APPENDIX XIX

Respondents' Attitude on Small Ruminants Sourcing

| Local Government Area | Respondent | Source of Animal | | | Method of Addition of New Animals to Existing flock | | | |
|---|---|---|---|---|---|---|---|---|
| | | Market | Established Farm | Other | Quarantine | Treat Before | Vaccinate | Do Nothing |
| Ikara | 19 | 19 | 0 | 0 | 0 | 0 | 0 | 19 |
| Makarfi | 16 | 16 | 0 | 0 | 0 | 0 | 0 | 16 |
| S/Gari | 18 | 7 | 2 | 9 | 3 | 2 | 0 | 4 |
| Soba | 17 | 7 | 2 | 4 | 3 | 0 | 0 | 6 |
| **TOTAL** | 70 | 49 | 4 | 13 | 6 | 2 | 0 | 45 |

APPENDIX XX

Mode of Small Ruminants breeding by Respondent

| Local Government Area | No of Respondents | Breeding Males | | | | History of Abortion | | Period of Abortion(Trimester) | | | | Proportion of Flock Affected by Abortion (%) | | | Delivery Pen | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Borrow | | Lend Out | | | | | | | | | | | | |
| | | Yes | No | Yes | No | Yes | No | 1st | 2nd | 3rd | Not Sure | 1-10 | 11-20 | Above | Yes | No |
| Ikara | 19 | 19 | 0 | 19 | 0 | 19 | 0 | 0 | 10 | 15 | 0 | 0 | 4 | 15 | 0 | 19 |
| Makarfi | 16 | 9 | 6 | 12 | 4 | 16 | 0 | 4 | 2 | 9 | 3 | 1 | 9 | 6 | 8 | 8 |
| Sabon Gari | 18 | 6 | 12 | 7 | 13 | 11 | 7 | 0 | 3 | 9 | 0 | 7 | 3 | 1 | 3 | 14 |
| Soba | 17 | 5 | 12 | 12 | 5 | 11 | 6 | 0 | 4 | 6 | 2 | 4 | 6 | 1 | 13 | 4 |
| **TOTAL** | 70 | 39 | 30 | 50 | 22 | 57 | 19 | 4 | 19 | 39 | 5 | 12 | 22 | 23 | 24 | 45 |

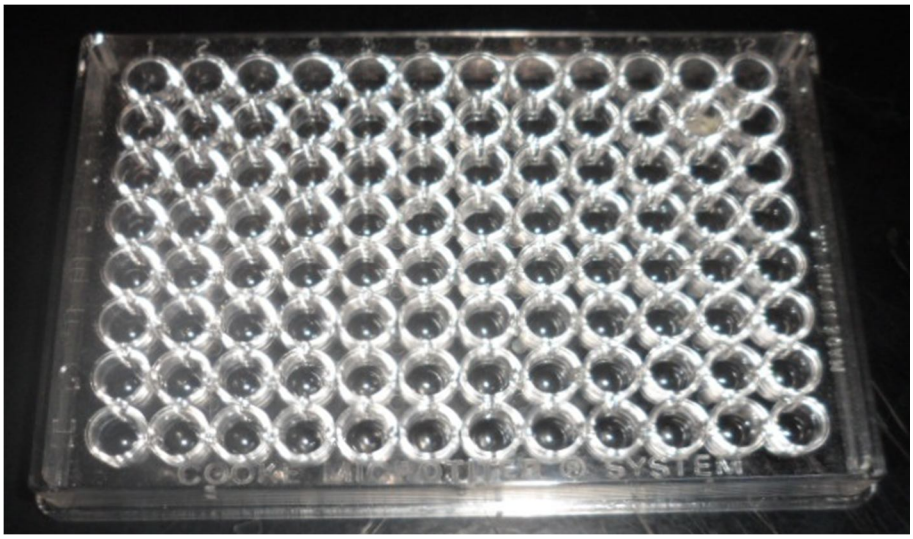
APPENDIX XXI

Respondents' management of aborted foetus

| Local Government Area | Respondent | Management of Aborted foetuses | | | Any History of Retained Placenta in the flock | | |
|---|---|---|---|---|---|---|---|
| | | Burry | Burn | Dump | Yes | No | Not Sure |
| Ikara | 19 | 1 | 0 | 18 | 16 | 3 | 0 |
| Makarfi | 16 | 0 | 0 | 16 | 13 | 0 | 3 |
| Sabon Gari | 18 | 1 | 1 | 10 | 5 | 9 | 2 |
| Soba | 17 | 4 | 0 | 7 | 7 | 5 | 5 |
| **TOTAL** | 70 | 6 | 1 | 51 | 41 | 17 | 10 |

APPENDIX XXII

Uncoated microtitre plate ("U bottom") used in the study



APPENDIX XXIII

Appropriately labelled empty test tubes ready for MRT



APPENDIX XXIV

Milk samples obtained from sheep and goats incubated in a water bath at $37^0$C and used for the MRT.



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