

**PREVALENCE OF BRUCELLA ANTIBODIES IN HORSES AND KNOWLEDGE,
ATTITUDE AND PRACTICES OF GROOMERS IN THREE LOCAL
GOVERNMENT AREAS OF KADUNA STATE, NIGERIA**

BY

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AUGUST, 2016

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GOVERNMENT AREAS OF KADUNA STATE, NIGERIA**

BY

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**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE
STUDIES AHMADU BELLO UNIVERSITY, ZARIA
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**DEPARTMENT OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA**

AUGUST, 2016

DECLARATION

I, declare that the work reported in this dissertationentitled “**Prevalence of *Brucella* Antibodies in Horses and Knowledge, Attitude and Practices of Groomers in three Local Government Areas of Kaduna State, Nigeria**” has been carried out by me in the Department of Veterinary Medicine. The information derived from the literature has been duly acknowledged in the text in the list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

AlhassanYunusa BABA-----

Signature

Date

CERTIFICATION

This dissertation entitled “PREVALENCE OF BRUCELLA ANTIBODIES IN HORSES AND KNOWLEDGE, ATTITUDE AND PRACTICES OF GROOMERS IN THREE LOCAL GOVERNMENT AREAS OF KADUNA STATE, NIGERIA,” by Alhassan Yunusa BABA meets the regulations governing the award of the degree of Master of Science (EQUINE MEDICINE) of Ahmadu Bello University, Zaria and is approved for its scholarly contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to my parents late Mallam Baba Yunusa Haruna and Fatima Abdullahi, my wife and daughter Ramlah for their understanding and great support.

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

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ABSTRACT

In a study to determine the prevalence of *Brucella* antibodies in horses and knowledge, attitude and practices of groomers, blood samples for serum were collected from 304 horses of various breeds, sexes, age - groups and purposes (uses) in three Local Government Areas of Kaduna State, Nigeria. The samples were analysed using Rose Bengal Plate Test (RBPT) and Serum Agglutination Test – EDTA (SAT - EDTA). The knowledge, attitudes and practices of the groomers in the various stables under study were determined using a structured questionnaire. From the study, the overall seroprevalences were 5.59% and 20.07% using RBPT and SAT-EDTA respectively. The seroprevalences by breed were 11.9% and 12.70% by RBPT and SAT-EDTA respectively for Arewa breed, 1.69% and 28.81% by RBPT and SAT-EDTA respectively for Argentine breed, 0.00% and 21.74% by RBPT and SAT-EDTA respectively for Sudanese breed and 0.00% and 16.21% by RBPT and SAT-EDTA respectively for Talon breed of horses. The corresponding seroprevalences by sex were 0.84% and 29.41% for females and 8.65% and 14.05% for males. The seroprevalences by age-group were 8.33%, 8.97%, 0.99% and 2.94% for 1 to 5 years old, 6 to 10 years old, 11 to 15 years old and above 15 years old respectively using the RBPT. Respective seroprevalences by purpose were 11.82%, 1.34%, and 2.22% for ceremonial, polo and racing horses using the RBPT. From the structured questionnaire, 37.50% of the respondents were aware of brucellosis and 22.50% ascribed their sources of information on the disease to be the media, 10.00% of the experienced groomers among the respondents and 5.00% professionals who attended to the veterinary care of their horses.

Of the respondents, 12.50% knew brucellosis to be zoonotic disease contracted through ingestion and 2.50% through contact. Considering clinical signs, 12.50% and 7.50% respectively reported night sweats and fever as clinical signs of brucellosis in man. Considering attitude of respondents towards brucellosis, 15.00% of the respondents reported lending out stallions for breeding, 2.50% did not borrow stallions for breeding because they considered brucellosis and trichomoniasis being reproductive diseases that could result through the use infected stallions. The study also reported 52.50% and 40.00% of the respondents were in the habit of lending and borrowing grooming tools respectively, even though they regarded such acts as capable of causing diseases like ulcerative lymphangitis (95.00%), ringworm (72.50%), dermatophilosis (5.00%) and thrush (7.50%). Similarly, 67.50% of the respondents participated in durbar and other tournaments and reported such participation to result in diseases and conditions like ulcerative lymphangitis (25.00%) and wounds (27.50%). The study further showed that, 50.00% of the respondents were grazing their horses where other animals grazed and even where there were reports of abortions by such animals without their horses coming down with brucellosis. All the respondents reported giving their horses' routine veterinary medical care, especially on babesiosis. From the study, it was concluded that *Brucella* antibodies were circulating in the blood of the sampled horses and that there were breed predisposition to the infection. Males were more seroprevalent than females while seropositivity increased with the age of the horses. There is the need to conduct further studies to determine the *Brucella* spp circulating among horses in the study area particularly that horses have been reported to graze on pastures where other animals had previously grazed and had history of abortions.

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LIST OF ABBREVIATIONS

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

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Brucellosis is a highly contagious, zoonotic, and economically important bacterial disease of animals and humans worldwide (OIE, 2000). It is also one of the most important infectious causes of reproductive disorders in domestic animals (OIE, 2000). The disease is also called contagious abortion, infectious abortion, and epizootic abortion. In horses it is called “fistulous withers” and “poll evil” (Megid *et al.*, 2010; Rust, 2012). In cattle, it is called ‘Bang’s Disease’ in tribute to the Danish veterinarian who was the pioneer in the study of the disease in this species (Megid *et al.*, 2010; Rust, 2012). The disease in humans is called “Malta fever”, “Mediterranean fever” and “Gibraltar fever” according to the region in which the illness was first described (Megid *et al.*, 2010; Rust, 2012). It is also known as undulating fever due to the oscillating temperature presented by infected persons. Clinical signs vary according to the animal species that is being infected and the infecting *Brucella* species (Rust, 2012).

Brucellosis is caused by members of the genus *Brucella* which is a Gram negative, facultative intracellular bacterium and can infect many animal species and man (Corbel, 1997; Young, 2000). Members of the genus are small (0.5-0.7 by 0.6-1.5µm), non-motile, encapsulated, and coccobacilli (Ryan and Ray, 2004). Genetically, ten species of the genus *Brucella* have been documented and they include *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotome*, *B. pinipedialis*, *B. ceti*, *B. muris* and *B. inapinata*. However, cross-species infections by these *Brucella* species have been reported (Foster *et al.*, 2007). For example, cattle can be infected by both *B. abortus* and *B. melitensis* at the same time

(Abdulssalam and Fein, 1976). *Brucella abortus* is the species of *Brucella* documented to cause brucellosis in horses (Kaltungo *et al.*, 2013).

Brucella neotomae, which affects desert rat, is not associated with human infections (Rust, 2012). *Brucella pinnipedialis* and *Brucella ceti* that were recently isolated from marine animals can also cause disease in humans (Xavier *et al.*, 2010). *Brucella melitensis* is the most virulent and most widely encountered of all the species (Bingol *et al.*, 1999). The organisms are capable of invading and surviving in phagocytic and non-phagocytic cells (Celli, 2006). Recently, another species *B. mariswas* was discovered in marine animals (OIE, 2000).

Brucella species can remain viable for several months in contaminated water, aborted materials, manure, wool, hay, equipment and clothing in conditions of high humidity, low temperature and if there is no exposure to sunlight (Alton and Forsyth, 1996). It has been reported by the same authors that they can, however, be destroyed by several hours of exposure to direct sunlight, surfactants such as 1% sodium hypochlorite, 70% ethanol, iodine/alcohol solutions, glutaraldehyde and formaldehyde.

Brucellosis has been reported in many animal species in Nigeria (Falade *et al.*, 1975; Okoh *et al.*, 1978; Falade and Shonekan, 1981; Adamu and Ajogi, 1995; Bertuet *et al.*, 2010; Ehizibolo *et al.*, 2011). These reports seem to indicate that brucellosis is endemic and problematic in Nigeria. There are many factors that can affect the prevalence of the disease in various species of animals. These factors include climatic conditions, vegetation type, type of animal husbandry, animal species, sex, age, and diagnostic tests applied (Bercovich and Taaijke, 1990).

Horses are one of the most valuable animals in Nigeria (Musa, 2013). They are being used for ceremonial processions, polo and racing among others (Mshelia, 2013; Musa, 2013). They are also kept by the police and army for defense and security operations (RIM, 1992). They have also been used under the traditional setting for drought power in the production of local sugar commonly referred to as “Mazar Kwaila” in Kaduna State in particular (RIM, 1992).

Different breeds of horses have been reported to be kept in Kaduna State for various purposes which include polo, racing and traditional ceremonies (Garba, 2006).

1.2 Statement of Research Problems

Horses have been used for long as beast of burden for the production of local sugar as well as the sole means of long distance journeys before the advent of modern transport facilities (Mshelia, 2013). The coming of the European expeditions saw horses being used for haulage of European goods and even merchandise (Mshelia, 2013). Diseases such as bacterial, viral, protozoan and parasitic diseases have been shown to influence the role of horses in contributing to the national economy and private horse owners. To what extent brucellosis causes such effect in horses seems not to be fully investigated in Kaduna State. Thus, there is therefore the need to investigate the prevalence of equine brucellosis, especially that the primary *Brucella* species in horses is *B. abortus* which has been reported to be endemic among cattle in Nigeria. It is also known that pastoralists commonly use horses as a means of transporting the young ones during migration (Saidu *et al.*, 1991). The fact that the groomers are closely associated with horses, especially polo and racing ones through their grooming activities, can result in serious public health hazards, particularly if these groomers are not aware of the disease in horses.

1.3 Justification for the Study

The domestic equine population in Nigeria is made up of 340,000 horses and 940,000 donkeys (RIM, 1992; Anon, 1994). In Kaduna State, the horse population is estimated to be 2,500 (Aliyu, 2014). More than 90% of the estimated equine population is located within the semi and sub-humid zones of the country where they are used either as beasts of burden (for transport, threshing and caramel production) or in the case of horses for sports and ceremonial purposes such as durbar as well as the production of sugar (RIM, 1992; Mshelia, 2013; Musa, 2013). There is increasing use of horses for ceremonies, especially in Kaduna State during durbars where many horses are gathered. The groomers are known to be very close to these horses due to their activities in grooming them. They interchangeably borrow grooming tools from one horse to another. Therefore, horses with lesions around the polls and withers may be groomed, and without the grooming tools being properly washed and disinfected could be used on another horse (Mshelia, 2013). These therefore, could lead to spread of the disease. Polo and horse racing are both national and international programmes that involve either the movement of racing horses or horse owners in and out of the country which could be a potential means for the spread of equine brucellosis. Rust (2012) reported that brucellosis caused by *Brucella abortus* is a zoonotic and infectious illness to which humans became exposed as a result of domestication of animals and the establishment of animal husbandry as an important element following civilization.

In a situation whereby the horse owners and the horse boys are ignorant of brucellosis affecting horses, the disease could easily be spread among horses and even horse boys and their owners that actually develop the habit of close interaction with horses as reported by Mshelia (2013).

Breeding programmes for horses to reduce importation is capable of introducing equine brucellosis and other diseases like African Horse Sickness and equine babesiosis (Mshelia, 2013). With the seeming paucity of information on equine brucellosis among horses and horse owners as well as horse handlers and the horse boys, there is the need to conduct sero-prevalence and KAP studies on equine brucellosis in Kaduna State, Nigeria.

1.4 Aim of the Study

The aim of the study is to determine the prevalence of *brucella* antibodies in horses as well as to determine the horse owners' knowledge, attitude and practices with regards to brucellosis in horses in three LGAs of Kaduna State, Nigeria.

1.5 Objectives of the Study

The objectives of the study are:

- 1) To determine the prevalence of *brucella* antibodies in horses in three LGAs of Kaduna State using RBPT and SAT-EDTA.
- 2) To determine the knowledge, attitude, and practices (KAP) of groomers with regards to brucellosis in horses in three LGAs of Kaduna State using questionnaire.

1.6 Research Questions

1. Do horses in Kaduna State have *Brucella* antibodies?
2. Are there any relationships between the prevalence of *Brucella* antibodies in horses and epidemiological factors like age, sex, breed, purpose, management of horses and horse husbandry practices in Kaduna State?
3. Are groomers in the Kaduna State aware of the public health risks due to brucellosis?

CHAPTER TWO

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, sinking 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, Gibraltar, Gastric, Undulant, milk, Remittent, Relapsing and Rock (Wikipedia, 2012). Brucellosis is a major disease of domesticated and wild animals worldwide and is also an important zoonosis. Of the domesticated species, cattle, sheep, pigs and goats are most commonly affected, and reproductive failure is the most common clinical manifestation.

A British Army surgeon, stationed on the Mediterranean Island of Minorca in Malta, described cases of chronic relapsing febrile illness in humans and cited Hippocrates'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 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 Netley 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 (late 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 to Sir David Bruce’s work was Dr. Carruana – Sacluna 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 period, no one knew the sources 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. (late Sir) Themistocles Zammit, successfully incriminated the Maltese Goat as the animal host of *Micrococcus melitensis* in pure culture from infected blood. Synder (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 the 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 Keeferin (1924) as the cause of undulant fever in man in the USA. Evans (1981) was the one who showed that *Brucella* organisms were related morphologically, biochemically, and serologically (Vassallo, 1992).

Jahans *et al.* (1997) reported other species of *Brucella* of veterinary and public health importance that have since been isolated. For instance, *Brucella ovis*, the cause of epididymitis and abortion in sheep was isolated by Buddles and Boyes (1953) in New Zealand and Australia. *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*(OIE, 2000).

2.2 Epidemiology of Brucellosis

2.2.1 Distribution

Brucella organisms have a worldwide distribution, though 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 culture engages in animal husbandry. The epidemiology of brucellosis is therefore not static as changes in the 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 (Wikipedia, 2012).

Brucellosis had not been suspected in Nigeria until contagious 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 had 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 of animals 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 and this 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 socio – political problems.

Studies in various parts of Nigeria showed 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; Buhari, 2014). There was also a report of prevalence ranging from 0.4% to 8.6% based on abattoir surveys (Chukwu, 1987).

Brucella 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 zoonoses 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 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).

2.2.2 Aetiology

Brucella is a generic name for a group of organisms which is part of the $\alpha - 2$ 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 (*Brucella*) and opportunistic or soil bacteria (*Ochrobactrum*) (OIE, 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 –capsulated, 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).

2.2.3 Taxonomic Tree of *Brucella* organisms

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

2.2.4 Morphology of *Brucella* spp

Brucella organisms are small (0.5 – 0.7 by 0.6 – 1.5µm), non – motile, non – spore forming, non – capsulated coccobacilli which occur singly or more rarely in group of short chains or clusters (Ryan and Ray, 2004). Similarly, they do not have flagellae or pili 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 – Neelsen stain is used (Anon, 2001). The cellular and colonial morphology of the species of *Brucella* are similar in most respects (Deyoe, 1981). There are six well known species of the genus which include *B. abortus*, *B. melitensis*, *B. ovis*, *B. canis* and *B. neotome*. They 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* though it was later divided into two species, *B. celi* 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 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 linkage (Bundle *et al.*, 1987). *Brucella ovis* and *Brucella canis* lack the OPS component and, as a result, their outer surface contains only rough lipopolysaccharide (RLPS) and protein antigens (Blasco, 1990).

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 with most other non – spore forming pathogenic bacteria and can survive for a long time in both hot and cold environmental

situations particularly in moist conditions. The organism can survive in tap water for several months at 4 to 8°C, 2.5 years at 0°C, and several years in frozen tissues or media (FAO, 2005). *Brucella* spp can also survive for up to 60 days in damp soil, and for up to 144 days at 20°C, 40% relative humidity and at pH greater than 4. *Brucella* organisms can also survive freezing and thawing and for several weeks in non – fermented milk (Merchant and Parker, 1975). Brucellae can survive for 40 days in urine, 75 days in aborted fetuses and more than 200 days in uterine exudates. In beddings contaminated with infected faecal material, *Brucella* will be destroyed at 56°C – 61°C within 4 – 5 hours (King, 1957).

Susceptibility of *Brucella* species to dyes such as thionin, basic fuchsin 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, pantothenic acid and biotin (Plammet, 1991). Its growth can be improved by the addition of serum or blood while susceptibility to dyes varies between and within biovars, a fact that has been used for routine typing tests for members of the genus (Anon, 2001).

Anon (2012) report that *Brucella* species are readily killed by most commonly available disinfectants including hypochlorite solutions, 79% 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.

2.2.5 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 the OIE (2009) stated that *Brucella melitensis* has three biovars that mainly infect sheep and goats. Most breeds of goats are readily infected, but sheep breeds vary greatly in susceptibility. *Brucellamelitensis* infections have also been reported occasionally in cattle, camels and dogs, and 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 been reported in alpine ibex in Italy and chamois in the French Alps. However, there is no evidence that these animals serve as reservoir hosts for domesticated sheep and goats.

Cattle are considered to be the preferential hosts for *B. abortus* which is classified into seven biovars, namely biovars 1 – 6 and 9 (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, pheasants, ducks, geese, and chickens (Emmel, 1930; Adesiyun and Abdu, 1984).

Horses can become infected with *B. abortus*, but in this case the bacterium has a preference for bursae, tendons, muscles, and joints. Thus, it is commonly found in cases of fistulous withers and poll evil, probably as a secondary invader (Hall, 1977). Fretin *et al.* (2008) reported that porcine brucellosis caused by *B. suis* biovars 1, 2 and 3, is considered an important re-emerging disease of domestic and wild pigs. However, this pathogen may also affect other animal species such as cattle, horses, rabbits, dogs, and humans. *Brucella suis* biovars 4 and 5 have been specifically associated with reindeer and rodents, respectively (Corbel, 2006). Swine can be infected by other *Brucella* species other than *B. suis* but the infection is invariably self-limiting (OIE, 2009a).

Canine brucellosis caused by *B. canis*, usually affects domestic dogs, wild carnivores, and rarely other domestic animals and man (Carmichael, 1990).

Brucella neotome is known to infect only the desert wood rat under natural conditions (Stoenner and Leckman, 1957).

Since the 1990s, marine strains of *Brucella* have been isolated from a variety of marine mammal species including seals (*Phoca vitulina*), dolphins (*Tursiops truncatus*, *Delphinus delphis*, *Lagenorhynchus acutus* and *Stenella coeruleoalba*), whales (*Balaenoptera acutorostrata*), and other species (Ewalt *et al.*, 1994; Ross *et al.*, 1994; Foster *et al.*, 1996).

Human brucellosis is mainly caused by *B. melitensis*, *B. suis* and *B. abortus* that 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 and with a few organisms (10 to 100) being sufficient to cause a debilitating chronic infection (Fugier *et al.*, 2007). It was also reported that most importantly, *B. canis*, a pathogen of dogs, has a comparatively low zoonotic potential, while *B. neotome* and *B. ovis*, which infect desert rats and sheep, respectively, are not associated with human disease (Godfroid *et al.*, 2005).

2.2.6 Transmission of brucellosis

Most infections result from ingestion of the bacteria either from diseased animals or contaminated feedstuffs. Aborted fetuses, 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). The United States Department of Agriculture 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 infected or exposed animals or purchase of animals from unscreened sources along with sharing of male breeding stock (USDA, 2000). The use of pooled colostrum 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 (OIE, 2009). 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. melitensis* 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 Kenney, 1970). Kaltungo (2013) reported that dogs are capable of spreading brucellosis by carrying aborted foetuses or placenta from one place to another.

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 (*Paraфи 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 sources of exposure predispose farmers, shepherds, to greater risk of contracting the disease. The non-occupational sources of exposure include ingestion of contaminated meat, unpasteurized contaminated milk and milk products (William 1971). In addition to the food – borne and occupational infection, transmission of *Brucella spp* has also been 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 indentified to include infection though 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).

AHA, (2005) report that despite the ability of flies and ticks to experimental transfer infection, their role in spreading *B. abortus* from infected to uninfected herds has not been established.

2.2.7 Pathophysiology

According to Olsen *et al.*, (2010), *Brucellae* are intracellular pathogens that localize in lymphoreticular cells throughout the body and have a predilection for the reproductive tissue. Upon entry of the bacteria into circulation 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 (Olsen *et al.*, 2010). Similarly, Anon (2008) reported that shortly after gaining entry into the body, *Brucellae* are ingested by polymorphonuclear leukocytes (PMLs). Normal serum factors, including complement, are involved in opsonization of the organisms to allow phagocytosis, but PMLs have limited ability to kill the bacteria within phagocytes. He further reported that a copper – zinc superoxide dismutase, O – polysaccharide, and nucleotide – like substances are among the factors that protect brucellae from being killed by PMLs. *Brucella* 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

principal mechanism of recovery. The host response to infection with *Brucella abortus* is characterized by the development of tissue granulomas indistinguishable from those of sarcoidosis/sarcocystosis. 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 the 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 inducers 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 being killed while *B. melitensis* is rarely affected. This might explain the differences in pathogenicity and clinical manifestation 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 localized 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 (AHA, 2005). The bacteria then localize in the pregnant uterus and udder or the testicles and accessory sex glands of the female and male hosts 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 fluids (AHA, 2005). 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, 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*, (Rev 1) 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, and scavenge through siderophores such as 2, 3 – dihydroxybenzoic acid or brucebactin. This may be linked with the requirement for affective 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 parturition or abortion (Parkinson, 2009). In mature, non pregnant cattle, the bacterium localizes in the udder (AHA, 2005). Infection of the udder is often clinically inapparent, with no gross lesions. *Brucellae* localize and replicate primarily in macrophages in the mammary secretions (with the potential for infection of calves and humans via the milk).

In sheep and goats, following introduction of the organism, the bacteria move 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 in the udder of lactating goats, resulting in acute mastitis with the production of clotted and watery milk and reducing milk yield (Enright, 1990).

2.3 Clinical Signs of Brucellosis

2.3.1 Clinical 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 affects 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 incubation period 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 and 251 days (Thomsen, 1950).

The major clinical sign in pregnant cows is abortion which usually occurs from the 5th to the 8th month of gestation, though Bang in 1906 observed an abortion in a three – month pregnant heifer 56 days after feeding with 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, birth of premature, stillborn or weak calves may occur. In subsequent gestation, 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, thereby aiding the spread of the disease during mating (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, though both testicles may be affected. Testicular atrophy may also occur (Plant *et al.*, 2008).

2.3.2 Clinical signs in small ruminants

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 two months of gestation, placental retention, and giving birth to weak offsprings that usually die during the peripartum period (Aldomy *et al.*, 2004). 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 and 12 weeks after being experimentally infected and it seems that ewes are more resistant to abortion than does.

The E C, (2001) reported that approximately two thirds of acute natural infections of goats with *B. melitensis* 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.

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, with the scrotal sac being distended by either hemorrhagic or fibrino – purulent exudates. In the chronic stage, hygromas and joint inflammation can be observed in bucks. Radostits *et al.*, (2003) reported that the initial clinical sign in rams is bacteraemia which is accompanied by a

mild systemic response. However, this soon resolves as the organism localizes in the epididymis. Furthermore, the epididymitis can be profound, with sperm stasis and secondary spermatocele formation resulting in infertility. This acute phase is characterized by poor semen quality in the presence of scrotal oedema and inflammation. Palpable lesions in the epididymis and tunicae are often the primary clinical findings. However, lesions may not develop until after the acute syndrome has resolved and the latent period has elapsed (Radostits, 2003).

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

2.3.3 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 common 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 the 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 leading to 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 *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 Deyoe (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 glands may last for a shorter period of time in the female than in the male (Acha and Szyfres, 2003).

2.3.4 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).

2.3.5 Clinical signs in small animals (pets)

Shin and Carmichael (1999) reported that infection of small animals 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 (Shin and Carmichael, 1999). 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 that develop the disease later, can also occur (Shin and Carmichael,

1999; Hollet, 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; Hollet, 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 dermatitis develops from constant licking by the male leading to oedema and dermatitis which usually present secondary contamination by non – haemolytic staphylococci (Hollet, 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, asymptomatic and 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.

2.3.6 Clinical signs in camels

The OIE (2009) reported that camels are not known to be primary hosts 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.

2.3.7 Clinical Signs in Humans

Human brucellosis is known to be 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 37°C in the morning to 40°C 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, prostatitis in males as well as spontaneous abortions in pregnant women infected with *Brucella* species in early states 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 human (Madkour,

2001). However, if left untreated, upto 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 the most prevalent worldwide. A prolonged course of illness, often associated with suppurative destructive lesions, is associated with *B. suis* infection. *Brucella abortus* is associated with mild to moderate sporadic disease that rarely causes complications and that *B. 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 of the bacteria in blood culture, and the complexity of its serodiagnosis (Colmenero *et al.*, 1990; Memish *et al.*, 2000).

2.4 Pathology of Brucellosis

2.4.1 General Pathology of Brucellosis

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 fetuses may appear normal while others are autolyzed or have variable amounts of subcutaneous oedema and bloodstained fluid in their body cavities (AHA, 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 fetuses, the spleen and liver may be enlarged, and the lungs may exhibit pneumonia and fibrous pleuritis (AHA, 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 (OIE, 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 (AHA, 2005). It 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 OIE (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. 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, catarrhal metritis is common and that abscesses 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).

2.4.2 Pathology in Equidae

Fistulous withers is a chronic inflammatory disease of the supraspinatus bursa and associated tissues (Gaughan *et al.*, 1988; Rashmir-Raven *et al.*, 1990; Cohen *et al.* 1992).

Although infection by *B. abortus* has been associated with the condition (Duff 1937; O'Sullivan 1981), other infectious organisms and trauma can also cause the disease.

Indeed in geographical areas with a low prevalence of brucellosis in cattle, *B. abortus* is rarely isolated from cases of fistulous withers (Gaughan *et al.*, 1988; Cohen *et al.*, 1992). The bursa is approximately 5 cm wide and ranges in length from 5–11 cm, and has a capacity in

the normal horse of 30–90 ml. There may be lethargy and general stiffness. In most cases the bursa ruptures and purulent exudate is discharged from one or more fistulae. These fistulae may heal but may subsequently reform.

Extensive fibrosis may occur. Horses with fistulous withers that are seropositive to *B. abortus* are significantly more likely to have radiographic evidence of osteomyelitis of the underlying dorsal spinous processes than seronegative horses with fistulous withers (Cohen *et al.* 1992).

Poll evil (septic supra-atlantal bursitis) causes similar clinical signs to fistulous withers in the poll region. There is frequently pain and neck stiffness. Swelling of the region occurs which may be followed by discharge of purulent materials.

Mid- to late-term abortion in mares has been reported, but this appears to be rare (McNutt and

Murray, 1924; McCaughey and Kerr, 1967; Shortridge, 1967; Robertson *et al.*, 1973). *Brucella abortus* may be isolated from the foetus and foetal membranes, and the affected mares show a serological response to infection. Unlike infected cows, excretion of the organism in vaginal discharges appears to be short-lived. In most instances, mares have become infected by co-grazing pastures with infected cattle.

2.4.3 Pathology due to *Brucella canis* infection in Dogs and Cats

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.

2.4.4 Pathology due to other infections

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 (Foster *et al.*, 1996). In dolphins with meningoencephalitis due to *Brucella 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 (Hernandez – Mora *et al.*, 2008; Gonzalez *et al.*, 2002).

2.4.5 Susceptibility of *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 increase during pregnancy, and animals get more susceptible with the advancement of pregnancy. Bulls are said to be relatively more resistant than sexually mature heifers and less resistant than sexually immature heifers (Megid *et al.*, 2010). In contrast to bulls, boars are more likely to be sources for introducing *Brucella* organisms into a swine herd (Acha and Szyfres, 2001).

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 infection 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.

2.4.6 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 with most other non – spore forming pathogenic bacteria and can survive for a long time in both hot and cold environmental situations particularly in moist conditions. The organism can survive in tap water for several months at 4 to 8°C, 2.5 years at 0°C, and several years in frozen tissues or media (FAO, 2005). *Brucella* spp can also survive for up to 60 days in damp soil, and for up to 144 days at 20°C, 40% relative humidity and at pH greater than 4. *Brucella* organisms can also survive freezing and thawing and for several weeks in non – fermented milk (Merchant and Parker, 1975). Brucellae can survive for 40 days in urine, 75 days in aborted fetuses and more than 200 days in uterine exudates. In beddings contaminated with infected faecal material, *Brucella* will be destroyed at 56°C – 61°C within 4 – 5 hours (King, 1957).

Susceptibility of *Brucella* species to dyes such as thionin, basic fuchsin 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, pantothenic acid and biotin (Plammet, 1991). Its growth can be improved by the addition of serum or blood while susceptibility to dyes varies between and within biovars, a fact that has been used for routine typing tests for members of the genus (Anon, 2001).

Anon (2012) report 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.

2.4.7 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

bethe custom to house animals in close space and this also facilitates transmission. However, contrary to this view and going by the data from studies 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 (AHA, 2005).

2.4.8 Immune response to *Brucella* infection

Antibody response to *B. abortus* will be used as an example because it has been most studied in detail (Neilsen and Yu, 2010). *Brucella* infection in cattle results in the production of early IgM isotype, appearing usually 5 – 15 days after exposure but may be delayed, with the timing depending on the route of exposure, the dose of the bacteria and the health status of the animals (Beh, 1973). The IgM antibody response is followed very shortly by the production of IgG1 isotype of antibody and subsequently by IgG2 and IgA in small quantities (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). Neilson 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 the 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.

2.5 Diagnosis of Brucellosis

All abortions in late gestation in domestic animals should be treated as suspected brucellosis and should therefore 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 Poester *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 cholera* O1, *Escherichia coli* O:157, some strains of *Escherichia hermannii* and *Stenotrophomonas maltophilia*, *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 titres 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 (Poester *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.

2.5.1 Specimen for the diagnosis of brucellosis

For the diagnosis of brucellosis, the organism may be recovered from a variety of materials. In animals, the greatest concentration of the bacteria is found in the placenta, followed by lymph nodes and milk and from blood in humans (Poester *et al.*, 2010). Furthermore, other materials include stomach contents, spleen and lung from aborted fetuses, 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).

2.5.2 Direct Smear microscopic examination of samples

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 fetuses stained with the stamp modification of the Ziehl – Neelsen staining method. However, morphologically related micro – organisms such as, *Chlamydophila abortus*, *Chlamydia psittaci* 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).

2.5.3 Culture and isolation of *Brucella* species

This procedure may be performed by culturing body tissues or secretions such as blood, milk and vaginal 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 ascetic fluids. Growth of the bacteria is unequivocal proof of infection (OIE, 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 ssp.* 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 (Poester *et al*, 2010). Optimum pH for growth of *Brucella* varies from 6.6 to 7.4; culture media should be adequately buffered near pH 6.8 for optimum growth. The optimum growth temperature is 36 – 38°C. However, most strains grow between 20°C and 40°C (EC, 2001).

Most *Brucella* strains, particularly *B. abortus* biovar 2 and *B. ovis*, grow better in media containing 5 – 10% of sterile (equine or bovine) serum free from *Brucella* antibodies. Frequently, field samples are contaminated with other bacteria and/ or fungi. Therefore, selective media should be used to avoid overgrowth of fast growing agents. The most widely selective media used are those of Kuzdas and Morse (Kuzdas and Morse, 1953) and Farrell (Farrell, 1974). The Kuzdas and Morse use the following antibiotics and quantities per liter of basal medium: 100mg 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 is 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), nitrofuratoin (10mg), nystatin (100,000 units) and amphotericin B (2.5mb) (Marin *et al.*, 1996).

2.5.4 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, 2009a). Animal inoculation may be either subcutaneously or through abraded skin in guinea – pigs or, preferably, intravenously or intraperitoneally in mice (Alton *et al.*, 1988; OIE, 2009). The spleen of mice is cultured 7 days after inoculation while serum samples of guinea pigs are subjected to specific tests 3 and 6 weeks after inoculation (OIE, 2009a).

2.5.5 Use of serology in the diagnosis of brucellosis

Body fluids such as serum, uterine discharge, vaginal mucus, milk, or semen from a suspected animal may contain different quantities of antibodies of the M, G₁, G₂, 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 Poieter *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 – polysaccharide (OPS). Virtually all serological tests for antibody to these bacteria use *B. abortus* antigen in the form of whole cells, smooth lipopolysaccharide (SLPS) or OPS (Nielsen and Yu, 2010; Poester *et al.*, 2010) while rough lipopolysaccharide (RLPS) or protein antigens are commonly used as the main antigen for detection of antibody to *B. avis* and *B. canis* (Classo, 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 (Poester *et al.*, 2010).

The commonly used serological tests include; MRT, SAT, SPT, CFT, 2 – Mercaptoethanol Test (2 – MET), Buffered Antigen Test (BPAT), and RBPT (Wikipedia, 2011). Others include the Card Test (CARD), Rivanol Test, Coombs test, Indirect Immunofluorescent Test (IFAT), Heat Inactivation Test (HIT), Skin Test, immunoassays and molecular biology techniques.

2.5.5.1 Rose Bengal Plate Test (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. It was considered that while the test gave few false negative results, it gave many false positives, possible 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 IgG1.

2.5.5.2 Serum Agglutination Test (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 (Poester *et al.*, 2010). For this reason, the test is only suitable for herd testing, rather than for individual animals. Furthermore, the presence of post- vaccinal antibody can complicate the results (Corbel and Brinley – Morgan, 1984). The SAT does not detect antibodies to *B. canis* and *B. ovis* because these rough strains of the organism do not have O – polysaccharide on their surfaces (Ndyabahinduka and Chu, 1984; Poester *et al.*, 2010). RLPS is commonly used as the main antigen for detection of antibody to *B. ovis* and *B. canis* (Poester *et al.*, 2010).

2.5.5.3 Standard Plate Agglutination Test (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. There is no use of a series of dilutions in this test like in the SAT, but it is standardized to give a result equivalent to a SAT titre of 1:100 (positive). Stemshorn *et al.*, (1985) considered that it could give positive results when SAT was negative by virtue of its use of high saline (8%) and higher serum concentrations. It was also resistant to the prozone effect (Poester *et al.*, 2010).

2.5.5.4 SAT – EDTA

Because of the lack of specificity of the SAT, and adaption of this test, which involves the addition of EDTA, has proven to significantly increase the test specifically (Poester *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.

2.5.5.5 Buffered Antigen Test (BPAT)

The main advantage of this test is the reduction of non specific test reactions. It is directed at testing for IgG (Angus and Barton, 1984).

2.5.5.6 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).

2.5.5.7 The Rivanol Test

Some non – specific reactivity may be removed by precipitation of high molecular weight serum glycoprotein which is the basis of this test (Poester *et al.*, 2010). Acridine dye such as rivanol (2- ethoxy – 6, 9 – diaminoacridine lactate) is used to precipitate glycoproteins from serum solutions which, in this case, is mainly IgM leaving mostly IgG in the serum (Nicoletti, 1969). The precipitate is then removed by centrifugation. The supernatant is

tested using rapid plate agglutination test with undiluted serum or a tube test using serum dilutions starting from 1:25 and because the protocol is fairly labour intensive, precipitation tests are generally used as confirmatory tests (Poester *et al.*, 2010). The test is capable of distinguishing between vaccinated and infected animals and chronic carriers, interpretation of the test is, however, difficult (Abdulkadir, 1989).

2.5.5.8 Complement Fixation Test (CFT)

The CFT is a prescribed test for international trade (OIE, 2009). It is considered as the most specific and valuable serological test for brucellosis (WHO/MZCP, 1998; Poester *et al.*, 2010). It detects mainly IgG1 antibodies than it does for antibodies of the IgM type which are partially destroyed during inactivation. Since antibodies of the IgG₁ 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 correlation 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 (Poester *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 IgG₂ type hinder complement fixation (Nielsen *et al.*, 1988; Macmillan, 1990).

2.5.5.9 The 2 – Mercaptoethanol Test

The 2 – *Mercaptoethanol Test* (2 – MET) is an adoption 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 monomeric molecules and therefore unable to agglutinate. However, IgG can also be reduced giving false negative results, in general, reduction of IgM increases specification (Poiester *et al.*, 2010).

2.5.5.10 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 IgG₂types (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.

2.5.5.11 Indirect Immunofluorescent Antibody Test(IFAT)

The IFAT test is specific, rapid and sensitive and is used as confirmatory test. It is used for detecting antibodies in sera of humans.

2.5.5.12 Heat Inactivation Test(HIT)

With experimental cases, the HIT is very sensitive at early stages of infection. The test is based on the observation that two types of *Brucella* agglutinins IgM and IgG are found and can be differentiated on the basis of stability at 65°C for 15 minutes, cooled to 18°C. The test is just like the tube agglutination test, the only difference being the heating (Bale, 2008).

2.5.5.13 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 the test may aid in the interpretation of serological reactions thought to be false positive serological reactors due to infection with cross reacting bacteria, especially in brucellosis – free area (Pouillot *et al.*, 1997; Saergerman *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 animals. The test can be relied upon for clinical surveillance and epidemiological surveys (FAO/WHO, 1986). It is of great importance in areas with low prevalence and areas known to be free

from brucellosis (Bercovich, 1998). The test uses brucellin which involves injecting it into the flank or intrapalpebrally and measuring the thickness of the skin (Weildmann, 1991; Cheville *et al*, 1994). Not all infected animals react, therefore, this test alone cannot be recommended as the sole diagnostic test or for the purposes of international trade (OIE, 2009). Similarly, 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.

2.5.5.14 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 Nielson, 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 programme. RadioImmunoassay, which is suitable for large screening tests, is associated with medical hazards and the conjugate shelf life is short.

2.5.5.15 Lateral Flow Assay(LFA)

The LFA is a simplified ELISA for the qualitative detection of antigen specific antibodies in serum or whole blood samples (Christopher *et al.*, 2010). The assay is based on the binding of specific antibodies to antigen immobilized on a test strip (cellulose membrane matrix). It allows 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).

2.5.5.16 Fluorescence Polarization Assay(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 (Poester *et al.*, 2010). In brucellosis serology, small molecular weight submit of OPS is labeled with fluorescein isothiocyanate and used as the antigen. When testing serum, blood or milk, if antibody to the OPS is present, the rate of rotation of the labeled 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).

2.5.5.17 Milk Ring Test (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 (Poester *et al.*, 2010). The immunoglobulins detected by MRT are IgM and IgA. This test may be applied to individual animals or to pooled milk samples using a larger volume of milk relative to the pool size (MacMillan, 1990a). The milk ring test is prone to false reactions caused by abnormal milk derived from mastitis, colostrums and milk from late in 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).

2.5.5.18 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 antibodies and from cross reacting antibodies. 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.

2.5.5.19 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 was used to identify and differentiate *B. abortus* biovars 1, 2 and 4, *B. melitensis*, *B. ovis* and *B. suis* biovar 1, based on the polymorphism arising from species – specific localization of the

insertion sequence IS711 in the *Brucella* chromosome (Bricker and Halling, 1994). In addition to the commonly used PCR assays, a new multiplex – PCR assay was developed that specifically identified *B. neotomae*, *B. pinnipedialis*, *B. ceti*, and *B. microti* (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 *Buce – ladder* multiplex PCR assay was also developed for identification and differentiation of *Brucella* sp. and vaccine strains (Lopez – Goni *et al.*, 2008).

2.5.6 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 (Maloney and Frasser, 2006).

Similarly, echocardiography can also be used to evaluate possible endocarditis. Mycotic aneurysms of the aorta or carotids may be observed on duplex arteriography (Maloney and Fraser, 2006). Bone marrow biopsy and liver biopsy may also be performed to obtain specimens for diagnosis, especially during the acute phase of the disease (Maloney and Fraser, 2006).

2.6 Differential Diagnoses

AHA (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 *Leptopira* 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 (AHA, 2005).

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 febrile 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, gastroenteritis, meningitis, and tuberculosis among others (Maloney and Fraser, 2006).

2.7 Treatment of Brucellosis

Brucella strains are intracellular pathogens that infect host macrophages. Hence, the antibodies 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).

2.7.1 Treatment in Animals

Anon (2002) reported that there is no practical treatment for infected animals. Long – term antibiotic treatment is sometimes successful in infected dogs. However, it is difficult to cure (Ettinger and Feldman, 1995). Also, a concurrent treatment with chlortetracycline and streptomycin has effected some level of cure in sheep, though it is usually not economically feasible except in 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 these diseases in cattle is generally unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland and the reproductive organs. In general, failure in treatment of brucellosis has been attributed to reasons like the use of incorrect doses of antibiotic, 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.

2.7.2 Treatment 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 mentioned organizations for acute brucellosis in adults is rifampicin at a dose rate of 600 to 900 mg and doxycycline given at a rate of 200mg daily for a minimum of 6 weeks. Some researchers 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).

2.8 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 testing and culling of positive reactors, quarantine, zoning, and tracing.

2.8.1 Quarantine and animal movement control

From the reports of AHA (2005), at 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.

2.8.2 Testing and culling of positive reactors

Serological testing of livestock should be routinely carried out using approved serological tests as in a test and slaughter strategy which was practiced in Australia before the total eradication of the disease 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 (AHA, 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 (AHA, 2005).

Corbel (2006) reported that the prevalence of *B. ovis* can be decreased by examining rams before the breeding season and culling rams with palpable abnormalities. However, he further reported that palpable lesions are not always found in all infected rams and therefore

laboratory testing of rams should also be considered. Test and removal methods directed at rams can eradicate this organism from a flock. Also, *B. ovis* infections in ewes are generally prevented by controlling infections in rams.

2.8.3. 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 of the movement of animals between the zones will therefore have to be enforced (AHA, 2005).

2.8.4 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 (AHA, 2005).

2.8.5 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.

2.8.6 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 (AHA, 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.

2.8.7 Decontamination

Decontamination can be achieved with the aid of sunlight, high temperatures and a range of chemicals (AHA, 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.

2.8.8 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 AHA (2005) have reported that live vaccines have until now provided 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, AHA (2005) reported that *Brucella abortus* RB51 infection in humans is possible but has not been documented. *Brucella melitensis* Rev. 1 has also been evaluated for the vaccination of cattle in countries where *B. melitensis* infection in sheep and goats is widespread, and experimental studies have shown that *B. melitensis* Rev. 1 vaccine provided immunity to *B. melitensis* equal to or superior to the immunity induced by *B. abortus* 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. Furthermore, 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 AHA (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.

2.8.9 Wild animal control

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

2.8.10 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).

2.8.11 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 dessication and sunlight (AHA, 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.

2.8.12 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).

2.9 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. In horses, losses may be due to aborted fetuses and weak foals which may die after some hours or days. 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).

2.10 Public Health Significance 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, 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, he 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 THREE

MATERIALS AND METHODS

3.1 List of Materials

Syringes and needles, cotton wool, methylated spirit, hand gloves, face masks, plain sample bottles, serum bottles, ice packs, coolers, deep freezer, white ceramic tile, microtitre plates, microtitre pipette tips, automatic micropipette, foil paper, serum bottle racks, pasteur pipette, tissue paper, normal saline, applicator sticks, incubator, Rose Bengal Plate Test antigen, SAT-EDTA antigen (*B. abortus*), safranin, serum, hand sanitizer, lab-coat.

3.2 Study Area

This study was conducted in three Local Government Areas of Kaduna State, Nigeria. Kaduna state is located in the northwest geopolitical zone of Nigeria. It lies between latitudes 6° and 11° north and longitudes 7° and 44° 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 sqkm, with a human population of over 6,006,562 people according to the 2006 census figures (KDSG, 2008). The domestic equine population in Nigeria is made up of 340,000 horses and 940,000 donkeys (RIM, 1992; Anon, 1994). In Kaduna State, the horse population is estimated to be 2,500 (Aliyu,

2014). More than 90% of the estimated equine population are used as either beasts of burden (transport, threshing and caramel production) or in the case of horses for sports and ceremonial purposes such as durbar as well as the production of sugar. A wide range of crops are grown in the state, including: guinea corn, millet, maize, cow pea, and groundnut. In addition, the State possesses a large livestock population mainly made up of cattle, goats, and sheep. The horses are mainly managed on extensive, semi-intensive, and intensive systems by their owners. The horse owners provide a form of shelter (stables) for them, where they are kept. During the day the horses are usually taken out to graze. Some of these shelters, especially those for traditional rulers, are made of wood and zinc roofing, while those for polo and racing are usually kept in fenced stables. The horse owners usually provide some feed supplementation to the horses owned by traditional rulership, especially in the dry season. These horses are usually involved in polo and racing tournaments as well as durbar participation.

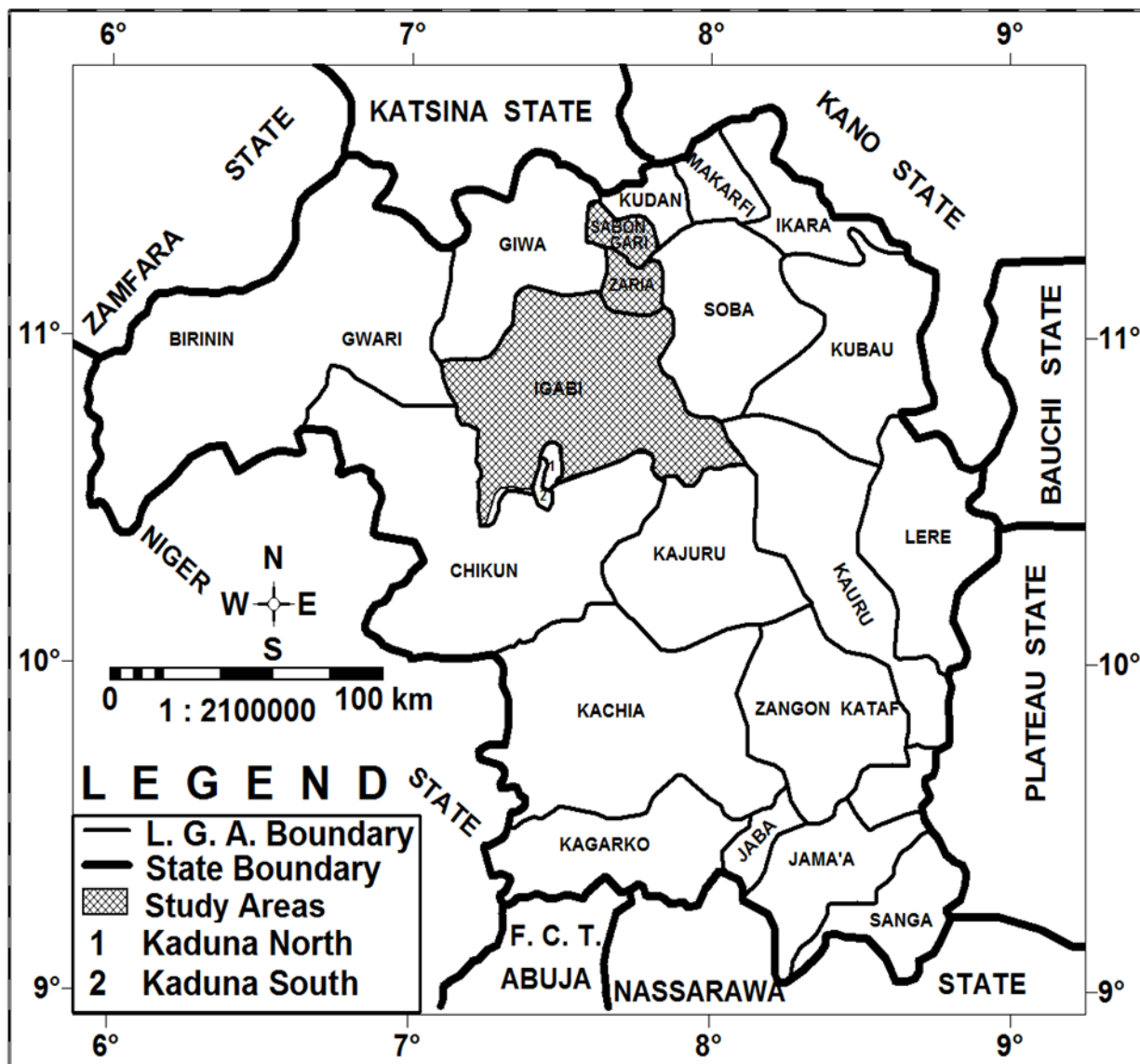


Fig 3.1: Map of Kaduna state showing study area
Source: Department of Geography, Federal College of Education, Zaria

Three out of the twenty three LGAs of Kaduna State were selected using purposive sampling. These were Igabi, Sabon Gari, and LGAs. The location of the stables, animal species, breed, age, purpose and sex of each animal sampled were recorded.

3.2 Methodology

3.2.1 Study design

A cross- sectional study was carried out with Purposive/Judgemental sampling method.

3.2.2 Horses used in the study

Ceremonial, polo, and racing horses kept by horse owners were used in the study. Adult horses above three years of age were sampled from the three selected LGAs that were selected. Stable selection was done based on purposive selection and owners' willingness.

3.2.3 Sample size determination

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

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

Where:

n =Minimum sample size

z = Appropriate value for the standard normal deviation set at 96% confidence interval (1.96).

p = Prevalence (14.7% as reported by Ehizibolo *et al.*(2011).

q = Complementary probability, 1 – p

d- Level of significance 5% (0.05), but figure decreased to 0.04 to increase the sample size.

$$\text{Sample size (horses)} = \frac{1.96^2 \times (0.147 \times 1 - 0.147)}{(0.04)^2}$$

$$n = \frac{0.147 \times 3.8416 \times 0.853}{0.0016}$$

$$0.0016$$

$$n = \frac{0.8162683}{0.0016}$$

$$n = 301.$$

3.2.4 Sample collection

Blood samples were collected from horses in stables for subsequent collection of serum. Judgemental sampling method was used. The stables encountered were of two extremes viz: those with very few horses, usually between one to five, especially the traditional rulerships who kept their horses for durbar and other ceremonies and those with large stables of usually twenty horses and above. All horses encountered with groomers that had a few horses were sampled while few horses were skipped in those from large stables.

Structured, pretested questionnaires were also administered horse owners, and groomers to access knowledge, attitude and practices of such respondents towards brucellosis in horses in the study area.

3.2.5 Blood sample collection

Five millilitres (5ml) of blood sample were aseptically collected through the jugular vein from each horse 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 I collected from a horse in Sabon Gari LGA was labelled as SG/DM/A1, and so on. The labelled bottles were then kept in a cooler and allowed to clot by allowing them 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°C until used.

3.2.6 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 grooms and horse owners who owned and groom the horses used in the study (Appendix 1). The questionnaires were completed for all the selected stables on a single visit during the sampling programme. The questionnaire was designed to comprise mostly closed ended (categorical) questions to ease data processing, and improve precision of responses. The questions were translated to the grooms in Hausa language. The important stable 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 Laboratory Investigation

3.3.1 Serology

The first serological tests were conducted by Wright and Semple in 1897 after which different researchers carried out modifications on the Wright and Semple tests or performed new sets of tests entirely.

3.3.1.1 The RBPT

The stored sera were thawed to room temperature (25⁰C) and then subjected to RBPT as described by Alton *et al* (1975) using RBPT antigen sourced from Onderstepoort Biological Products Ltd, South Africa (Plate I). 0.03ml of RBPT antigen and equal amount of the test serum were placed alongside on a white ceramic tile and then mixed thoroughly using a clean tooth pick which was discarded after use. The tile was rocked gently using both hands for 4 minutes (rotation period). The sample was classified positive if any agglutination was observed and negative if no agglutination was observed (Plate II).

3.3.1.2 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.

3.3.1.2.1 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 diaminetetraacetic 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 millilires of NaH_2PO_4 and 72nl 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.

3.3.1.2.2 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 rectangular 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 well already labeled A – H and a corresponding vertical numbering of the wells were made (Plate 1). A representative entry of the sample details was made in the laboratory record book. Using automatic micropipette (Dragonned[®]), 40 μl of the buffer solution was measured out into the first well and 25 μl 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 two-fold serial dilution was done by transferring 25µl aliquots in each well, and 25µl was discarded after the last well. The content of the working dilution of the SAT antigen was mixed gently by tapping the container and 25µl added to each well. Finally, the contents in the microtitre plate were gently mixed by tapping the edges of the plate for 20seconds. The microtitre plates were then covered to prevent evaporation of the contents in the wells and incubated for 20 hours at 37°C in an incubator (Gallenkemp[®], Germany) after which the results were read. The result was considered positive when there was a red dot surrounded by a circular zone at the bottom of the well of the microtitre plate and negative when there was no circular zone that was surrounded by a dot (Plate III).

3.4 Data Analysis

Data obtained from serological tests and questionnaires were presented in tables and analysed using SPSS version 20.0 statistical package. Descriptive statistics, Chi square and Fishers Exact tests were used to test for association between categorical variables. P values <0.05 were considered significant.

CHAPTER FOUR

RESULTS

4.1 Distribution of Horses and Prevalence rates of *Brucella* Antibodies in Kaduna State, Nigeria

A total of 304 horses were sampled from the study area (Table 4.1). Out of these, 161 were from Igabi while 38 and 105 were from Sabon Gari and Zaria LGAs respectively. Furthermore, 126 (41.45%) of these horses were Arewa breeds while 118 (38.81%), 23 (7.57 %) and 37 (12.17%) were Argentine, Sudan and Talon breeds of horses respectively. Among horses in Igabi LGA, 37 (22.98%) were Arewa breed while 118 (73.29%), 3 (1.86%) and 3 (1.86%) were Argentine, Sudan and Talon breeds respectively. The horses in Sabon Gari LGA included 11 (28.95%) Arewa, 5 (13.16%) Sudan and 22 (57.89%) Talon breeds. Similarly, of the 105 horses in Zaria LGA, 78 (74.29%) were of the Arewa breed while 15 (14.29%) and 12 (11.42%) were of the Sudan and Talon breeds. No Argentine horses were sampled in Sabon Gari and Zaria LGAs.

4.2 Prevalence of *Brucella* Antibodies in Horses

Seventeen (5.59%) out of the 304 horses sampled were seropositive for *Brucella* antibodies using the RBPT while 61 (20.07%) were positive using the SAT-EDTA (Table 4.2). Two (1.24%) and 44 (27.33%) of the 161 horses from Igabi LGA were positive using RBPT and SAT – EDTA respectively while of the 38 horses from Sabon Gari LGA 1 (2.63%) and 5 (13.16%) were respectively positive using RBPT and SAT – EDTA. Similarly, of the 105 horses from Zaria LGA 14 (13.33%) and 12 (11.43%) were respectively positive using the

RBPT and SAT – EDTA. Some of the samples that were positive are presented in Appendix 2.

4.3 Prevalance of *Brucella* Antibodies in Horses by Breed in Kaduna State, Nigeria

Fifteen (11.90%) and 16 (12.70%) of the Arewa horses sampled were seropositive for *Brucella* antibodies using RBPT and SAT – EEDTA respectively (Table 4.3). Similarly, 2 (1.69%) and 34 (28.81%) Argentine horses were positive using RBPT and SAT – EDTA while of the 23 Sudanese horses none was positive using RBPT while 5 (21.74%) were positive using SAT – EDTA. As for the Talon horses, 6 (16.22%) were positive for *Brucella* antibodies with none being positive by the RBPT.

Table 4.1: Distribution of horses sampled for *brucella* antibodies in three LGAs of Kaduna State, Nigeria.

LGA	No Sampled (%)	Breed of horses Sampled			
		Arewa	Argentine	Sudan	Talon
Igabi	161 (52.96)	37 (22.98)	118 (73.29)	3 (1.86)	3 (1.86)
Sabon Gari	38 (12.50)	11 (28.95)	0 (0)	5 (13.16)	22 (57.89)
Zaria	105 (34.54)	78 (74.29)	0 (0)	15 (14.29)	12 (11.42)
Total	304 (100)	126 (41.45)	118 (38.81)	23 (7.57)	37 (12.17)

Table 4.2: Prevalence of *Brucella* antibodies in horses sampled in three LGAs of Kaduna State, Nigeria

LGA	Number of Samples tested	Number (%) Positive by test type	
		RBPT (%)	SAT-EDTA (%)
Igabi	161	2 (1.24)	44 (27.33)
Sabon Gari	38	1 (2.63)	5 (13.16)
Zaria	105	14 (13.33)	12 (11.43)
Total	304	17 (5.59)	61 (20.07)

RBPT: Fisher's exact test=16.521; df = 2; p = 0.000

SAT-EDTA: $X^2 = 10.533$; df = 2; p = 0.005

Table 4.3:Prevalence of *Brucella* antibodies in horses by breed sampled in three LGAs of Kaduna State, Nigeria.

LGA	Horse Breeds											
	Arewa			Argentine			Sudan			Talon		
	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA
Igabi	37	0 (0.00)	6 (16.22)	118	2 (1.69)	34 (28.81)	3	0 (0.00)	2 (66.67)	3	0(0.00)	1 (33.33)
Sabon Gari	11	1 (9.09)	1 (9.09)	0	0 (0.00)	0 (0.00)	5	0 (0.00)	0 (0.00)	22	0(0.00)	4 (18.18)
Zaria	78	14(17.95)	9 (11.54)	0	0 (0.00)	0 (0.00)	15	0 (0.00)	3 (20.00)	12	0(0.00)	1 (8.33)
Total	126	15(11.90)	16(12.70)	118	2 (1.69)	34 (28.81)	23	0 (0.00)	5 (21.74)	37	0(0.00)	6 (16.21)

RBPT: Fisher's exact test=10.805; df = 3; p = 0.007

SAT-EDTA: $X^2 = 11.445$; df = 3; p = 0.010

4.4 Prevalance of *Brucella* Antibodies by Sex

Of The 119 female horses examined, 1 (0.84%) was positive for *Brucella* antibodies using RBPT while 35 (29.41%) were positive by the SAT-EDTA test (Table 4.4). Similarly, of the 185 male horses, 16 (8.65%) and 26 (14.05%) were positive using RBPT and SAT-EDTA respectively. The seroprevalence by LGA showed that of the 93 female horses in Igabi LGA 1 (1.08 %) and 32 (34.41%) were positive by RBPT and SAT – EDTA respectively while among the 68 male horses in the LGA 1 (1.47%) and 12 (17.64%) were respectively positive by RBPT and SAT-EDTA. Of the 12 female horses from Sabon Gari LGA 1 (8.33%) was positive by the SAT-EDTA while none was positive by the RBPT. Similarly, of the 26 male horses from Sabon Gari LGA 1 (3.85%) and 4 (15.38%) were respectively seropositive by RBPT and SAT-EDTA. On their part, of the 14 female horses sampled in Zaria LGA, 2 (14.29%) were positive by SAT-EDTA and none was positive by RBPT while of the 91 male horses from this LGA 14 (15.38%) and 10 (10.99%) were respectively positive by RBPT and SAT-EDTA.

4.5 Prevalance of *Brucella* Antibodies in Horses by Age

The seroprevalence of horses by age is presented in Table 4.5 below. Horses between 1 and 5 years had seroprevalence of 8.33% and 12.50% by RBPT and SAT-EDTA respectively while those between 6 and 10 years had seroprevalence of 8.97% and 13.79% using RBPT and SAT-EDTA respectively. Similarly, horses in the 11 to 15 years old bracket had seroprevalence of 0.99% and 27.72% using RBPT and SAT-EDTA respectively while those above 15 years had seroprevalence of 2.94% and 35.29% respectively. As to the seroprevalence LGA, horses in Igabi LGA of age range of between the age of 1 and 5 years had seroprevalence of 20.00% by both RBPT and Sat-EDTA while those in Sabon Gari

LGA had seropositivity of 10.00% and 20.00% by RBPT and SAT-EDTA respectively and those in Zaria LGA had seroprevalence of 0.00% by both tests.

Horses of 6 to 10 age range in Igabi LGA had 1.89% and 18.87% Seropositivity by RBPT and SAT-EDTA respectively while those of the same age bracket in Sabon Gari LGA had 0.00% and 14.29% by RBPT and SAT-EDTRA respectively. Similarly, horses of this age bracket in Zaria LGA had 16.90% and 9.86% seroprevalence by RBPT and SAT-EDTAS respectively. As to the horses in the 11 to 15 years old bracket, horses in Igabi LGA had 0.00% and 31.51% seropositivity by RBPT and SAT-EDTA respectively while those in Sabon Gari LGA had 0.00% for both tests. The corresponding figures for horses in Zaria LGA for this age bracket are 4.76% and 23.81% respectively. The seroprevalence of Brucella antibodies for horses above q5 years in Igabi LGA were 0.00% and 36.67% by RBPT and SAT-EDTA while the corresponding figures for the same age bracket for horses from Sabon Gari LGA were 0.00% respectively for the two tests. As for the figures for horses in Zaria LGA, a seroprevalence of 25% by ach of the two tests was determined.

Table 4.4: Prevalence of *Brucella* antibodies by Sex of horses sampled in three LGAs of Kaduna State, Nigeria.

LGA	No Sampled	Sex				No Sampled	Male
		Female		Male			
		No Positive (%)		No Positive (%)			
		RBPT	SAT-EDTA	RBPT	SAT-EDTA		
Igabi	93	1 (1.08)	32 (34.41)	68	1 (1.47)	12 (17.64)	
Sabon Gari	12	0 (0.00)	1 (8.33)	26	1 (3.85)	4 (15.38)	
Zaria	14	0 (0.00)	2 (14.29)	91	14 (15.38)	10 (10.99)	
Total	119	1 (0.84)	35 (29.41)	185	16 (8.65)	26 (14.05)	
Chi-square		2.283*	0.130*	-	0.357*	0.050*	4.937
p-value		0.131	0.718	-	0.550	1.000	0.003

*Fisher's exact test

Table 4.5: Prevalence of *Brucella* antibodies by age of horses sampled in three LGAs of Kaduna State, Nigeria

Age	LGAs									Total	
	Igabi			Sabon Gari			Zaria			RBPT	SAT-EDTA
	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA		
1 – 5 years	5	1 (20.00)	1 (20.00)	10	1 (10.00)	2 (20.00)	9	0 (0.00)	0 (0.00)	2 (8.33)	3 (12.50)
6 – 10 years	53	1 (1.89)	10 (18.87)	21	0 (0.00)	3 (14.29)	71	12 (16.90)	7 (9.86)	13 (8.97)	20 (13.79)
11 – 15 years	73	0 (0.00)	23 (31.51)	7	0 (0.00)	0 (0.00)	21	1 (4.76)	5 (23.81)	1(0.99)	28 (27.72)
➤ 15 years	30	0 (0.00)	11 (36.67)	0	0 (0.00)	0 (0.00)	4	1 (25.00)	1 (25.00)	1 (2.94)	12 (35.29)
Total	161	2 (1.24)	45 (27.95)	38	1 (2.63)	5 (13.16)	105	14 (13.33)	13 (12.38)	17 (5.59)	63 (20.72)

Chi-square	2.837*	3.508*	-	1.769*	7.256*	3.240*
p-value	0.347	0.283	-	0.538	0.034	0.247

*Fisher's exact test

4.6 Prevalance of *Brucella* Antibodies in Horses by Activity

Of the 304 horses sampled 110 of the horses were ceremonial while 149 and 45 of them were respectively Polo and Racing horses (Table 4.6). Thirteen each (4.28 %) of the 110 ceremonial horses were respectively seropositive by RBPT and SAT-DTA while 3 (0.99%) and 39 (12.83%) of the Polo horses were respectively positive by RBPT and SAT-EDTA and 1(0.33%) along with 11 (3.62%) of the 45 racing horses were positive by RBPT and SAT-EDTA respectively.

Seroprevalence by use and Local Government Area indicated that of the 22 ceremonial horses from Igabi LGA, 3 (13.66%) were seropositive by SAT-EDTA and none of them was positive by RBPT (Table 4.6). Similarly, of the 122 Polo horses from this :LGA, 2 (1.64%) and 36 (29.51%) were seropositive by RBPT and SAT-EDTA while of the 17 racing horses from the LGA 7 (41.18%) were positive by SAT-EDTA and none was positive by RBPT.

As for the horses in Sabon Gari LGA, no ceremonial horse was tested for *Brucella* antibodies. However, of the 18 Polo horses in this LGA, 1 (5.56%) and 3 (16.67%) were positive for *Brucella* antibodies while 2 (10.00%) of the 20 racing horses were positive by SAT-EDTA and none was positive by RBPT (Table 4.6). Of the 88 ceremonial horses from Zaria LGA, 13 (14.77%) and 10 (11.36%) were respectively positive for *Brucella* antibodies using RBPT and SAT-EDTA while of the 9 Polo horses none was positive by both tests and of the 8 racing horses 1 (12.50%) and 2 (25.00%) were positive by RBPT and SAT-EDTA respectively.

Table 4.6 Prevalence of *Brucella* antibodies in horses by activity in three LGAs of Kaduna State, Nigeria

LGA	USES								
	Ceremonial			Polo			Racing		
	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA
Igabi	22	0 (0.00)	3 (13.66)	122	2 (1.64)	36 (29.51)	17	0 (0.00)	7 (41.18)
Sabon Gari	0	0 (0.00)	0 (0.00)	18	1 (5.56)	3 (16.67)	20	0 (0.00)	2 (10.00)
Zaria	88	13 (14.77)	10 (11.36)	9	0 (0.00)	0 (0.00)	8	1 (12.50)	2 (25.00)
Total	110	13 (4.28)	13 (4.28)	149	3 (0.99)	39 (12.83)	45	1 (0.33)	11 (3.62)

Chi-square	0.763	2.147	-	0.368	0.612	2.275
p-value	0.833	0.412	-	0.653	1.000	0.324

*Fisher's exact test

4.7 Respondents' Knowledge on Brucellosis

4.7.1 Groomers awareness and sources of information on brucellosis

A total of 40 respondents from Igabi, Sabon Gari and Zaria participated in the questionnaire responses (Table 4.7). Out of these 15 (37.50%) were aware of brucellosis while 25 (62.50%) were not. Furthermore, nine (22.50%) of these respondents reported that their sources of awareness on brucellosis was the media. Four (10.00%) others reported their fellow groomers as their sources of information while 2 (5.00%) others indicated professionals as their sources of information on brucellosis.

Out of these, 17 (42.50%) respondents were from Igabi LGA while 8 (20.00%) and 15 (37.50%) were from Sabon Gari and Zaria LGAs respectively. Seven (41.18%) of the respondents from Igabi were aware of brucellosis. Similarly 2 (25.00%) and 6 (40.00%) of those from Sabon Gari and Zaria respectively also said they were aware of the diseases.

On their sources of awareness on brucellosis, 4 (23.53%), 2 (11.76%) and 1 (5.88%) of the seven respondents from Igabi LGA respectively reported their sources of information on Brucellosis as the media, experienced groomers and professionals respectively. Similarly, 2 (25.00%) of the respondents from Sabon Gari reported their source of information to be the media and none indicated other groomers and professionals are being contributory to their knowledge on brucellosis. Furthermore, 3 (20.00%) of the 6 respondents from Zaria LGA indicated the media as their source of information on brucellosis while 2 (13.33%) and 1 (6.67%) of them respectively indicated other groomers and professionals as their sources of information on brucellosis.

Table 4.7: Groomers Awareness and Sources of information on Brucellosis in three LGAs of Kaduna State, Nigeria

LGA	No of Respondents	Awareness of Brucellosis		Sources of Awareness		
		Aware	Not aware	Media	Experienced groomers	Professionals
		No (%)	No (%)	No (%)	No (%)	No (%)
Igabi	17 (42.50)	7 (41.18)	10 (58.82)	4 (23.53)	2 (11.76)	1 (5.88)
Sabon Gari	8 (20.00)	2 (25.00)	6 (75.00)	2 (25.00)	0 (0.00)	0 (0.00)
Zaria	15 (37.50)	6 (40.00)	9 (60.00)	3 (20.00)	2 (13.33)	1 (6.67)
Total	40 (100)	15 (37.50)	25 (62.50)	9 (22.50)	4 (10.00)	2 (5.00)

Awareness: Fisher's exact test = 1.079, df = 2; p = 0.700

Source of awareness: Fisher's exact test = 3.362; df = 2; p = 0.913

4.7.2 Groomersknowledge on brucellosis transmission and its zoonotic nature

Of the 40 respondents, 6 (15.00%) reported brucellosis as being zoonotic while 34 (85.00%) did not (Table 4.8). Furthermore, 5 (12.50%) of them considered ingestion as a means of transmission of the disease while 1 (2.50%) of them regarded contact as a means of transmission of the disease. Five (12.50%) of the respondents considered night sweats as a sign of the disease while 3 (7.50%) of them regarded fever as the chief sign of the disease. As for the respondents in Igabi LGA, only 2 (11.76%) out of the 17 respondents indicated brucellosis being zoonotic and that two (11.76%) respondents reported ingestion as a means of transmission of the disease (Table 4.8). Furthermore, these two said that signs of the disease in man included fever and night sweats. As for respondents in Sabon Gari LGA, one (12.50%) of them indicated that brucellosis is zoonotic and that it is transmitted by ingestion. The respondent further indicated that the only clinical sign of the disease is night sweat. Of the 15 respondents from Zaria LGA, 3 (20.00%) responded that Brucellosis is zoonotic. Two (80.00%) and 1 (6.67%) of these respondents respectively said that the disease is transmitted by ingestion and contact respectively (Table 4.8). As to the sign of the disease, 2 (13.33%) and 1 (6.67%) respectively said that signs of brucellosis in man were night sweat and fever.

Table 4.8: Groomersknowledge on brucellosis being zoonotic and means of transmission of brucellosis in three LGAs of Kaduna State, Nigeria

LGA	No of Respondents	Brucellosis		Means of Infection		Recognition of Brucellosis		
		Zoonotic	Not Zoonotic	Ingestion	Contact	Night sweats	Fever	Others
Igabi	17 (42.50)	2 (11.76)	15 (88.24)	2 (11.76)	0 (0.00)	2 (11.76)	2 (11.76)	0 (0.00)
Sabon Gari	8 (20.00)	1 (12.50)	7 (87.50)	1 (12.50)	0 (0.00)	1 (12.50)	0 (0.00)	0 (0.00)
Zaria	15 (37.50)	3 (20.00)	12 (80.00)	2 (13.33)	1 (6.67)	2 (13.33)	1 (6.67)	0 (0.00)
Total	40 (100)	6 (15.00)	34 (85.00)	5 (12.50)	1 (2.50)	5 (12.50)	3 (7.50)	0 (0.00)

Zoontic brucellosis; Fisher's exact test = 0.322; df = 2; p = 0.921

Means of infection: Fisher's exact test = 0.354; df = 2; p = 0.925

Recognition: Fisher's exact test = 1.349; df = 2; p = 0.989

4.7.3 Respondents' knowledge on signs of brucellosis in horses and action taken

Responses on signs of brucellosis included abortion, poll evil and fistulous withers of which six (15.00%) of them indicated abortion while five (12.50%) and eight (20.00%) of them respectively pointed out poll evil and fistulous withers (Table 4.9). All the 40 respondents reported that they had never encountered any of these signs in their horses and as such there was no need to take any action.

4.7.4 Respondents' knowledge of brucellosis as a reproductive disease

Only two (5.00%) out of the 40 respondents indicated ever having abortion in their stables of which only one (2.50%) reported determining the cause of the abortion (Table 4.10). Furthermore, six (15.00%) of the respondents mentioned their stallions developing testicular swellings of which five (12.50%) of them reported to the clinic while the remaining one (2.50%) used traditional medicine to treat the condition.

One (5.88%) of the respondents that had abortion in their stables was from Igabi LGA while the second respondent was from Zaria LGA. Similarly, the respondent that reported determining the cause of abortion was from Igabi LGA while three (17.65%) of the respondents each that reported encountering testicular swellings were also from Igabi LGA and Zaria LGAs respectively. Of the five respondents that reported taking their horses with testicular swellings to the clinic, three (17.65%) and 2 (13.33%) were from Igabi and Zaria LGAs respectively (Table 4.10). The respondent who reported using traditional methods to treat testicular swellings was from Zaria LGA.

4.7.5 Respondents' Knowledge of brucellosis as a zoonosis

Six (15.00%) out of the 40 respondents in the study reported that they knew brucellosis as a zoonosis (Table 4.11). Three (50.00%) of these were from Igabi LGA while one (16.67%) and two (33.33%) were from Sabon Gari and Zaria LGAs respectively. Among those that indicated knowing the disease being zoonotic, 4 (10.00 %) indicated fever as one of its signs while 6 (15.00%), and two (5.00%) said that signs of the disease in man to include night sweat and headache respectively and none of the respondents from all the LGAs regarded abortion and weakness as signs of the disease in man (Table 4.11). Two (50.00%) of the respondents who said fever was one of the signs of brucellosis in man were from Igabi LGA while one (25.00%) each was from Sabon Gari and Zaria LGAs respectively. Similarly, 3 (50.00%) of the respondents who considered night sweat as being a sign of brucellosis were from Igabi LGA while one (16.67 %) and two (33.33%) were respectively from Sabon Gari and Zaria LGAs. One (50.00%) of the respondents from Igabi and Zaria LGAs considered headache as being a sign of brucellosis while none of those in Sabon Gari LGA indicated so.

4.7.6 Respondents' knowledge on transmission of brucellosis from animals to man

Of the 40 respondents, one (2.50%) of them said that the disease could be transmitted from animals to man through the consumption of contaminated meat while six (15.00%) others said transmission through milk was the main source of transmission of brucellosis from animals to man (Table 4.12).

Table 4.9: Respondents knowledge on signs and attitude towards brucellosis in horses in three of Kaduna State, Nigeria

LGA	Signs of brucellosis			Encountered cases		Action taken		
	Abortion	Wound location		Yes	No	Report to Clinic	Self Treatment	Do Nothing
		Poll	withers					
Igabi	5 (29.41)	0 (0.00%)	3 (17.65)	0 (0.00)	17(100.00)	0 (0.00)	0 (0.00)	0 (0.00)
Sabon Gari	0 (0.00)	0 (0.00)	2 (25.00)	0 (0.00)	8 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
Zaria	1 (6.67)	5 (33.33)	3 (20.00)	0 (0.00)	15(100.00)	0 (0.00)	0 (0.00)	0 (0.00)
Total	6(15.00%)	5(12.50%)	8(20.00%)	0(0.00%)	40(100.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)

Signs; Fisher's exact test = 9.703; df = 2; p = 0.05

Table 4.10: Groomersknowledge and attitude towards brucellosis as a reproductive disease in three LGAs of Kaduna State, Nigeria

LGA	Abortion in Stable		Determination of causes of abortion		Encountered testicular swellings		Action taken on enlarged testicles		
	Yes	No	Yes	No	Yes	No	Clinic	Traditional Medicine	Do nothing
Igabi	1 (5.88)	16(94.12)	1 (5.88)	16(94.12)	3 (17.65)	14(82.35)	3 (17.65)	0 (0.00)	0 (0.00)
Sabon Gari	0 (0.00)	8(100.00)	0 (0.00)	8 (100.00)	0 (0.00)	8(100.00)	0 (0.00)	0 (0.00)	0 (0.00)
Zaria	1 (6.67)	14(93.33)	0 (0.00)	15(100.00)	3 (20.00)	12(80.00)	2 (13.33)	1 (6.67)	0 (0.00)
Total	2 (5.00)	38(95.00)	1 (2.50)	39 (97.50)	6 (15.00)	34(85.00)	5 (12.50)	1 (2.5)	0 (0.00)

Abortion: Fisher's exact test = 0.723; df = 2; p =0.998

Causes: Fisher's exact test = 1.532; df = 2; p =0.977

Enlarged testicles: Fisher's exact test = 1.260; df = 2; p =0.702

Table 4.11: Respondents' knowledge of brucellosis as a zoonosis in three LGAs of Kaduna State, Nigeria

LGA	Brucellosis in man		Signs of brucellosis in man				
	Yes	No	Fever	Night sweat	Abortion	Weakness	Headache
Gabi	3 (50.00)	14(41.18)	2 (50.00)	3 (50.00)	0 (0.00)	0 (0.00)	1 (50.00)
Sabon Gari	1 (16.67)	7 (20.59)	1 (25.00)	1 (16.67)	0 (0.00)	0 (0.00)	0 (0.00)
Zaria	2 (33.33)	13(38.24)	1 (25.00)	2 (33.33)	0 (0.00)	0 (0.00)	1 (50.00)
Total	6 (15.00)	34(85.00)	4 (10.00)	6 (15.00)	0 (0.00)	0 (0.00)	2 (5.00)

Fisher's exact test = 0.372; df = 2; p = 0.921

Signs: Fisher's exact test = 1.564; df = 2; p = 0.899

4.7.6 Respondents' knowledge on transmission of brucellosis from animals to man

Of the 40 respondents, one (2.50%) of them said that the disease could be transmitted from animals to man through the consumption of contaminated meat while six (15.00%) others said that milk was the main source of transmission of brucellosis from animals to man (Table 4.12).

Table 4.12: Respondents' knowledge on transmission of brucellosis from animals to man in three LGAs of Kaduna State, Nigeria

LGA	No. Respondents	Method of Transmission		
		Meat Consumption(%)	Milk Consumption(%)	Do not Know(%)
Igabi	17	1 (5.88)	3 (17.64)	14 (82.35)
Sabon Gari	8	0 (0.00)	1 (12.50)	7 (87.50)
Zaria	15	0 (0.00)	2 (13.33)	13 (86.67)
Total	40	1 (2.50)	6 (15.00)	34 (85.00)

Fisher's exact test = 9.322; df = 2; p = 0.076

4.8 Respondents' Attitude to Brucellosis

4.8.1 Responses of groomers on ownership and breeding of mares

Of the 40 respondents, 17 (42.50%) reported having mares in their stables. Out of these six (15.00%) indicated that they bred their mares and that they had stallions in their stables (Table 4.13). None of the respondents indicated borrowing stallions while one (2.5%) of them reported he was in a habit of sending his mare to stallions in other stables for breeding. On the role of borrowing or sending mares to stallions in other stables, one each of the respondents (2.5%) indicated diseases that could be transmitted included brucellosis and trichomoniasis respectively.

4.8.2 Respondents' attitude on use of stable equipment

All the 40 (100%) groomers reported having grooming tools in their stables and that 21 (52.50%) of them were in the habit of lending out their grooming tools to others while 16 (40.00%) of them indicated that they occasionally borrowed grooming tools from others (Table 4.14). Furthermore, all the groomers observed that grooming tools could cause diseases. Among these diseases, 38 (95.00%) of them reported ulcerative lymphangitis while 29 (72.50%), two (5.00%) and three (7.50%) respectively reported ringworm, dermatophilosis and thrush as the diseases that can be transmitted by grooming tools.

4.8.3 Diseases spread through participation in tournaments

Among the 40 groomers, 27 (67.50%) of them reported ever participating in durbars (Table 4.15). Ten (25.00%) of them reported their horses coming down with ulcerative lymphangitis while 11 (27.50%) reported their horses encountering wounds from various

sources. Among those that reported their horses coming down with ulcerative lymphangitis, one (5.88%) was from Igabi LGA while two (25.00%) and seven (46.67%) were from Sabon Gari and Zaria LGAs respectively. As for those that reported coming down with wounds, seven (41.76%) were from Igabi LGA while three (37.50%) and one (6.67%) were from Sabon Gari and Zaria LGAs respectively. None of them reported their horses coming down with brucellosis, epizootic lymphangitis or other conditions through participation in tournaments.

Table 4.13: Respondents' practices on Breeding of Mares in three LGAs of Kaduna State, Nigeria

LGA	Ownership of Mares		Breeding of Mares		Ownership of Stallion		Borrowing of Stallion		Sending Mare to Stallion		Sexually transmitted diseases considered			
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	TB (%)	Brucellosis (%)	Trichomoniasis (%)	None (%)
Igabi	13 (76.47)	4 (23.53)	3 (17.65)	14 (82.35)	3 (50.00)	14 (82.35)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Sabon Gari	2 (25.00)	6 (75.00)	1 (12.50)	7 (87.50)	1 (12.50)	7 (87.50)	0 (0.00)	0 (0.00)	1 (12.50)	0 (0.00)	0 (0.00)	1 (12.50)	0 (0.00)	0 (0.00)
Zaria	2 (13.33)	13 (86.67)	2 (33.33)	13 (86.67)	2 (33.33)	13 (86.67)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (6.67)	0 (0.00)
Total	17 (42.50)	23 (57.50)	6 (15.00)	34 (85.00)	6 (15.00)	34 (85.00)	0 (0.00)	0 (0.00)	1 (2.50)	0 (0.00)	0 (0.00)	1 (2.50)	1 (2.50)	0 (0.00)

Table 4.14: Respondents' husbandry Practices in three LGAs of Kaduna state, Nigeria

LGA	Grooming Tools		Lending Grooming Tools		Borrowing Grooming Tools		Caustion of Disease through Grooming Tools		Possible Disease through Grooming Tools			
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Ulcerative lymphangitis	Ringworm	Dermatop hilossis	Thrush
Igabi	17 (43.59)	0 (0.00)	3 (17.65)	14 (82.35)	3 (17.65)	14 (82.35)	17 (100.00)	0 (0.00)	17 (100.00)	16 (94.11)	1 (5.88)	0 (0.00%)
Sabon Gari	8 (20.51)	0 (0.00)	8 (100.00)	0 (0.00)	6 (75.00)	2 (25.00)	8 (100.00)	0 (0.00)	8 (100.00)	8 (100.00)	1 (12.5)	0 (0.00%)
Zaria	14 (93.33)	1 (6.67)	10 (66.67)	5 (33.33)	7 (46.67)	8 (53.33)	15 (100.00)	0 (0.00)	13 (86.67)	5 (33.33)	0 (0.00)	3 (100.00%)
Total	39(97.50)	1 (2.50)	21 (52.5)	19(47.50)	16(40.00)	24(60.00)	40 (100.00)	0 (0.00)	38 (95.00)	29 (72.50)	2 (5.00)	3 (7.50%)

Table 4.15: Respondents practices on disease spread through durbar participation in three LGAs of Kaduna State, Nigeria

LGA	Participation in Durbar		Disease Encountered				
	Yes (%)	No (%)	Ulcerative Lymphangitis	Epizootic Lymphangitis	Wounds	Brucellosis	Others
Igabi	7 (41.76%)	10(58.82%)	1 (5.88%)	0 (0.00%)	7 (41.76%)	0 (0.00%)	0 (0.00%)
Sabon Gari	5 (62.50%)	3 (37.50%)	2 (25.00%)	0 (0.00%)	3 (37.50%)	0 (0.00%)	0 (0.00%)
Zaria	15(100.00%)	0 (0.00%)	7 (46.67%)	0 (0.00%)	1 (6.67%)	0 (0.00%)	0 (0.00%)
Total	27 (67.50%)	13(32.50%)	10 (25.00%)	0 (0.00%)	11(27.50%)	0 (0.00%)	0 (0.00%)

Fisher's exact test = 1.349; df = 2; p = 0.989

4.9 Respondents' Practices with regard to brucellosis

4.9.1 Respondents' grazing practices with regard to brucellosis

Twenty (50.00%) of the respondents reported grazing their horses with other animals while one (2.5%) of the respondents indicated grazing his horses in areas where other animals were said to have previously aborted (Table 4.16). None of the respondents reported any abortion in his stable. Six (35.29%) of the respondents from Igabi LGA reported grazing their horses with other animals. Also, seven (87.50%) and seven (46.67%) each of the respondents from Sabon Gari and Zaria LGAs indicated doing so. Furthermore, the only respondent who reported grazing his horses where other animals aborted was from Zaria LGA.

4.9.2 Respondents' practices with regard to veterinary care

All the 40 respondents interviewed reported seeking veterinary assistance on the ailments of their horses (Table 4.17). These services were in connection with cases of babesiosis, wound management and cases of lameness. Thus, all the respondents sought for veterinary services in all their cases of babesiosis while 13 (32.50%) and 29 (72.50%) sought for veterinary care on wounds and lameness respectively (Table 4.17). All the respondents in the three LGAs routinely sought for veterinary attention on babesiosis while only three (17.65%), two (25.00%) and eight (53.33%) of the respondents from Igabi, Sabon Gari and Zaria respectively sought for veterinary attention on wounds in their horses. Furthermore, all the respondents from Igabi LGA sought for veterinary care on cases of lameness of their

horses while six (40.00%) each of the respondents from Sabon Gari and Zaria LGAs respectively sought for veterinary care for their horses.

Table 4.16: Groomers grazing practices in three LGAs of Kaduna State, Nigeria

LGA	Horse grazing with other animals		Grazing where other animals aborted		Any abortion by mares	
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
Igabi	6 (35.29)	11 (64.71)	0 (0.00)	17 (100.00)	0 (0.00)	17 (100.00)
Sabon Gari	7 (87.50)	1 (12.50)	0 (0.00)	8 (100.00)	0 (0.00)	8 (100.00)
Zaria	7 (46.67)	8 (53.33)	1 (6.67)	14 (93.33)	0 (0.00)	15 (100.00)
Total	20 (50.00)	20 (50.00)	1 (2.50)	39 (97.50)	0 (0.00)	40 (100.00)

Grazing: Fisher's exact test = 3.362; df = 2; p = 0.110

Other animals aborted: Fisher's exact test = 1.784; df = 2; p = 0.875

Abortion in mares: Fisher's exact test = 1.349; df = 2; p = 0.892

Table 4.17: Groomers practices with regard to veterinary care in three LGAs of Kaduna State, Nigeria

LGA	Vet medical care		Disease conditions routinely sought for care		
	Yes (%)	No (%)	Babesiosis(%)	Wound (%)	Lameness(%)
Igabi	17 (100.00)	0 (0.00)	17 (100.00)	3 (17.65)	17 (100.00)
Sabon Gari	8 (100.00)	0 (0.00)	8 (100.00)	2 (25.00)	6 (75.00)
Zaria	15 (100.00)	0 (0.00)	15 (100.00)	8 (53.33)	6 (40.00)
Total	40 (100.00)	0 (0.00)	40 (100.00)	13 (32.50)	29 (72.50)

Conditions: Fishers exact test = 4.332; df = 2; p = 0.084

CHAPTER FIVE

DISCUSSION

From the study, Arewa, Argentine, Sudanese and Talon horses were found to be kept in the study area by horse owners among whom are traditional rulership, polo and racing horse owners. This has also indicated the presence of exotic breeds of horses into the area and is quite understandable as Zaria is the traditional home for horse racing and polo activities (Musa, 2013). The presence of polo and racing horses has further shown that polo and racing are still popular in the study area as reported by Mshelia (2013) and Musa (2013).

The study has shown that the overall seroprevalence of *Brucella* antibodies in horses in the study area was 5.59% by RBPT and 20.07% by SAT-EDTA which is low compared with the seroprevalence study carried out by Ehizibolo *et al.* (2011) where they obtained 6.1% using RBPT. From the study, SAT-EDTA has been shown to be more sensitive than RBPT as it identified more horses with the antibodies. The finding of positive horses for *Brucella* antibodies in this study has indicated that the organism is circulating among horses in the study area since the grooms had reported that they were not vaccinating their horses against brucellosis. This finding is also significant as it brings challenges to polo and racing tournaments in the study area as it could lead to the spread of the infection to other horses from distant areas that might have come to the study area for tournament. It could also mean that, should horses from the study area go out for tournament in other areas, the infection could also spread that way.

The fact that the seropositivity was highest in the Arewa breed of horses was not surprising since they were found more in traditional owners' stables who use the horses more for

durbar, weekend riding by youths and sugar milling during the production of local sugar ('Mazalkyaila') as reported by Mshelia (2013) and Musa (2013). The near zero seropositivity for the Sudanese and Talon breeds of horses could be accounted for by their possible management practices since this study has demonstrated that they were giving their horses veterinary medical care.

From this study, the seroprevalence in males was higher than that in female horses. This is contrary to the findings of Kaltungo (2013) and Buhari (2014) who respectively reported higher seroprevalences in female ruminants (cattle and small ruminants) than in male animals. The higher seroprevalence in males as obtained in this study could be due to the fact that, unlike in small ruminants and cattle as reported by Kaltungo (2013) and Buhari (2014) respectively, more male horses are found among horse owners in the study area. Furthermore, many horse owners were in a habit of using geldings in preference to female horses.

The study has also demonstrated that seropositivity to *Brucella* antibodies in horses increased with age. This is in agreement with the findings of Aulakh *et al.* (2008), Abubakar *et al.* (2010), Kaltungo (2013) and Buhari (2014) who reported higher positivity in sexually matured small ruminants and cattle respectively in Kaduna State.

The seropositivity as seen in 1 to 5 years old horses in this study could be as a result of contaminated feeds during grazing as the respondents in this study reported grazing their horses where other animals grazed and among them is one who grazed his horses where other animals had previously aborted. Furthermore, their positivity could be through milk consumption during their young age as many mares were seropositive to *Brucella* antibodies

in this study. Another reason that can be advanced is that these horses were not sexually mature yet as to acquire the infection sexually through mating with other infected horses.

The fact that ceremonial horses were more seropositive for *Brucella* antibodies could be due to the less attention to veterinary care by the owners while the owners of polo and racing horses had more premium for it as they considered their activities as investments that required returns. Furthermore, the participation of ceremonial horses at weekend horse riding by youths and for marriage outing by grooms and their friends and well wishers could account for the extra sources of exposure for the *Brucella* organisms for this group of horses in the area.

From the study, many of the respondents were aware of brucellosis and they reported their sources of knowledge on the disease to the media, more experienced grooms among them and professionals. This is true as the Department of Veterinary Medicine in the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria used to organize training programmes on livestock diseases to livestock owners, horse owners and their grooms inclusive (Saidu *et al*, 1980; Saidu, 1986). Not only that, it was common to see grooms trimming the hooves of horses and even giving them traditional medications for some of their ailments. As to their mentioning professionals as their sources of information on horse diseases, most of the polo and racing horse owners organized routine veterinary medical care as documented from this study.

With regard to signs of brucellosis in horses, some of the respondents reported abortion and wound at the poll and withers. This seeming knowledge could be associated with or linked to their identification of the media and other sources of information as mentioned above.

Furthermore, their knowledge on the disease in humans could similarly be through these sources of information. However, it is to be understood that the fact that most of them did not regard the disease to be zoonotic could be translated to more problems as they can be exposed to the agents knowingly or ignorantly with attending consequences. The few that regarded the disease to be zoonotic were able to identify the signs of the disease in man and this can also be due to their exposure to the training programmes as mentioned above. Similarly, their association of means of transmission of the disease from animals to man could be from the sources mentioned above as well as the regular radio and television programmes by the National Agricultural Extension Research Liaison Service of the Ahmadu Bello University, Zaria. Furthermore, the Nigerian Veterinary Medical Association also carried out training programmes for livestock owners and professionals and information on the disease could similarly trickle down to them (Jahun, 2011).

The attitude of the respondents to brucellosis was captured through their breeding methods, use of grooming tools and participation in tournaments. Thus some (15%) of the respondents indicated lending out or borrowing stallions for breeding their mares or even sending their mares to stallions for breeding. This act is capable of spreading the disease, should any of them, mare or stallion be infected with *Brucella* organisms. Corbel (2006) reported that such acts are capable of transmitting the disease from herd to herd. With regard to borrowing or lending out grooming equipment, at least 40% of the respondents indicated doing so even though they knew that such acts were capable of leading to transmission of the disease in their stables. Their identification of diseases like ulcerative lymphangitis, ringworm, dermatophilosis and thrush as diseases that can be spread through lending or borrowing grooming tools did not stop them from doing so. This could be as

they had never had serious cases as a result of such habits or they could be disinfecting the tools in between use since there is evidence that they used to get routing training as observed earlier on. Their participation in tournaments has been shown to lead to diseases like ulcerative lymphangitis and wounds, though they reported never encountering brucellosis. This could be that during such tournaments no breeding programmes were arranged to warrant any horse acquiring infection through coitus.

From the study, the practices of the grooms with respect to grazing showed that they grazed their horses where other animals were being grazed and even in areas reported to be grazed by animals that had previously aborted. This is capable of spreading brucellosis, should the abortions be due to brucellosis. Bale (1980) reported that the system of husbandry greatly influences the spread of brucellosis. Furthermore, Hinton *et al.* (1977) reported that mares co-grazing with cattle could come down with brucellosis with subsequent abortion. In addition, the possibility of animals to come down with the disease is great since the FAO (2005) reported that the organisms can survive in pasture for up to 144 days at 20⁰C and even in urine for up to 40 days.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The study has determined that the seroprevalence of *Brucella* antibodies in the study area was 5.59% using the RBPT and 20.07% using the SAT-EDTA.
2. The seroprevalence of *Brucella* antibodies by breed was highest in the Arewa breed with a seroprevalence of 11.90% followed by the Argentine breed with 1.69% and least by the Sudanese and Talon Breeds with 0.00% seroprevalence respectively.
3. The seroprevalence of *Brucella* antibodies in horses was higher in males with a seroprevalence of 8.65% than in female who had a seroprevalence of 0.84% using the RBPT.
4. The seroprevalence of *Brucella* antibodies was seen to increase with age among all the horses with horses within the 6 to 10 year old bracket having the highest seroprevalence of 8.89% followed by those in the 1 to 5 year old bracket and least by those in the 11 to 15 year old bracket using the RBPT.
5. The seroprevalence of *Brucella* antibodies was highest in ceremonial horses (4.28%) followed by Polo (0.99%) and least in racing horses (0.33%) as used by the RBPT.
6. The Serum Agglutination Test (SAT-EDTA) was more sensitive than RBPT as it identified more animals positive than RBPT.
7. As many as (37.50%) of the respondents were aware of brucellosis and indicated their sources of knowledge as the media, other colleagues and professionals and even knew the signs of the disease in both animals and man and regarded it to also

be zoonotic as it is transmitted through the consumption of meat (2.5%) and milk (15.00 %).

8. The respondents were in the habit of lending out (52.50%) and borrowing (40.00%) grooming tools during durbar and tournaments even though they knew such acts were capable of spreading diseases like ulcerative lymphangitis (95.00%) and wounds (32.50%).
9. Respondents (50%) grazed their horses along with other animals or areas previously grazed by other animals known to have aborted and used Veterinary services for ailments in their horses.

6.2 Recommendations

1. There is the need to conduct further studies to determine the *Brucella* spp circulating among horses in the study area particularly that horses have been reported to graze with other animals and also to graze in areas reported have been grazed by animals that aborted in such areas.
2. There is the need to educate grooms and even horse owners and the general public in the study area on the public health significance of the disease, brucellosis, along with the epidemiology of the disease and other diseases with a view to avert these diseases as well as to prevent zoonoses.
3. There is the need to conduct further study on the role of grooming tools among Polo, racing and ceremonial horse handlers with a view to determine the actual roles these tools may play in the epidemiology of brucellosis.

4. There is also the need to examine stallions more closely on their possible carriage of *Brucella* organisms since they have been shown to be reservoirs brucellosis.
5. More search be made on Poll evil and fistulous withers among horses and other equidae in the study area since *Brucella* antibodies have been found to circulate among horses of various breeds and purposes in the study area in order to prevent possible transmission to humans since antibodies to the causative agent for these two diseases (*B abortus*) has been demonstrated in this study.

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APPENDICES

Appendix i

STRUCTURED QUESTIONNAIRE TO DETERMINE THE KNOWLEDGE, ATTITUDE AND PRACTICE OF HORSE OWNERS AND GROOMERS WITH REGARDS TO BRUCELLOSIS IN HORSES IN KADUNA STATE, NIGERIA

Department of veterinary Medicine, Ahmadu Bello University, Zaria, March, 2013

Researcher: Dr. Alhassan Yunusa BABA, MSc. Equine Medicine

Date: _____

Questionnaire No: _____

1. L.G.A _____
2. Ward _____
3. Name of Owner (optional) _____
4. Stable size _____
5. No. of mares in stable _____
6. No. of stallions in stable _____
7. No. of horses in stable _____
8. Purpose of horses: Ceremonial/polo/Racing

Stable Records

1. Horse breed: Arabian/Argentine/Sudanese/Arewa.
2. Do you have mares in your stable? Yes / No.
3. Do you breed your horses? Yes / No.
4. If the above question is yes, do you have your own stallion / mare? Yes / No.
5. If you borrow male / female for breeding your horse, do you consider sexually transmitted diseases? Yes / No
6. If the answer above is yes, which of these disease do you consider? T.B / brucellosis / trichomoniasis / none.

Husbandry Practices

1. Do you have grooming tools for your horses? Yes / No.
2. If yes, list such tools

3. Do you lend your grooming tools to other grooms? Yes / No.

4. If the answer is no, do you borrow grooming tools? Yes / No.
5. Do you think lending or borrowing grooming tools can lead to disease? Yes / No.
6. If the answer above is yes, which disease do you consider that can be transmitted with grooming tools? (i) _____ (ii) _____
_____ (iii) _____
7. Have you participated in durbar? Yes / No.
8. If the answer above is yes, did you encounter any disease in the process? Yes / No.
9. If the above answer is yes, which disease did you encounter? Brucellosis / wounds / ulcerative lymphangitis / others (specify)

10. Do you know brucellosis can affect animals? Yes / No.
11. If the above answer is yes, what was the source of your knowledge? Friend / Vet / Radio / TV.
12. What are the signs of brucellosis in horses: abortion / wound at poll / wound at withers
13. Have you encountered such signs in your horses? Yes / No.
14. If the above answer is yes, what did you do? Report to clinic / did local medication / did nothing.
15. Have you encounter abortion in your mares? Yes / No.
16. Did you determine the cause of the abortion? Yes / No
17. Have your male horses ever encountered enlarged testicles? Yes / No.
18. If the above answer is yes, what did you do to get the horse well? Vet clinic / Traditional treatment / nothing.
19. Can humans come down with brucellosis? Yes / No.
20. If the above answer is yes, what are the signs and symptoms of brucellosis in humans? Fever / light sweats / abortion / weakness / headache.
21. How can humans contract the disease brucellosis? Consumption of raw milk / Consumption of meat / Do not know.
22. Do your horses graze where other animals graze? Yes / No.
23. Have your horses ever grazed where other animals were seen to have aborted? Yes/No
24. If the above answer is yes, did you ever experience abortion in mares after that? Yes / No
25. Do you routinely seek for Veterinary medical attention for your horses? Yes / No
26. If the above is yes, what disease conditions do you seek for such help
(i) _____ (ii) _____ (iii) _____

No 27. Do you borrow or lend out horse bridle, mat or such other horse materials? Yes /

27. If the above answer is yes, have you ever encountered any disease as a result of such act Yes / No.

28. Do you consider borrowing or lending out horse materials as detrimental to horse

health? Yes / No.

30. If the above answer is yes, in what way? Transmission of disease / weakness / other

(specify) _____

THANK YOU

Appendix ii

Table 4.1: Distribution of horses sampled for *brucella* antibodies in three LGAs of Kaduna State, Nigeria.

LGA	No Sampled (%)	Breed of horses Sampled			
		Arewa	Argentine	Sudan	Talon
Igabi	161 (52.96)	37 (22.98)	118 (73.29)	3 (1.86)	3 (1.86)
Sabon Gari	38 (12.50)	11 (28.95)	0 (0)	5 (13.16)	22 (57.89)
Zaria	105 (34.54)	78 (74.29)	0 (0)	15 (14.29)	12 (11.42)
Total	304 (100)	126 (41.45)	118 (38.81)	23 (7.57)	37 (12.17)

Appendix iii

Prevalence of *Brucella* antibodies in horses sampled in three LGAs of Kaduna State, Nigeria

LGA	Number of Samples tested	Number (%) Positive by test type	
		RBPT (%)	SAT-EDTA (%)
Igabi	161	2 (1.24)	44 (27.33)
Sabon Gari	38	1 (2.63)	5 (13.16)
Zaria	105	14 (13.33)	12 (11.43)
Total	304	17 (5.59)	61 (20.07)

RBPT: Fisher's exact test=16.521; df = 2; p = 0.000

SAT-EDTA: $X^2 = 10.533$; df = 2; p = 0.005

Appendix iv

Table 4.3: Prevalence of *Brucella* antibodies in horses by breed sampled in three LGAs of Kaduna State, Nigeria.

LGA	Horse Breeds											
	Arewa			Argentine			Sudan			Talon		
	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA
Igabi	37	0 (0.00)	6 (16.22)	118	2 (1.69)	34 (28.81)	3	0 (0.00)	2 (66.67)	3	0(0.00)	1 (33.33)
Sabon Gari	11	1 (9.09)	1 (9.09)	0	0 (0.00)	0 (0.00)	5	0 (0.00)	0 (0.00)	22	0(0.00)	4 (18.18)
Zaria	78	14(17.95)	9 (11.54)	0	0 (0.00)	0 (0.00)	15	0 (0.00)	3 (20.00)	12	0(0.00)	1 (8.33)
Total	126	15 (11.90)	16 (12.70)	118	2 (1.69)	34 (28.81)	23	0 (0.00)	5 (21.74)	37	0 (0.00)	6 (16.21)

RBPT: Fisher's exact test=10.805; df = 3; p = 0.007

SAT-EDTA: $X^2 = 11.445$; df = 3; p = 0.010

Appendix v

Prevalence of *Brucella* antibodies by Sex of horses sampled in three LGAs of Kaduna State, Nigeria.

LGA	No Sampled	Sex				
		Female		Male		
		No Positive (%)		No Positive (%)		
		RBPT	SAT-EDTA	RBPT	SAT-EDTA	
Igabi	93	1 (1.08)	32 (34.41)	68	1 (1.47)	12 (17.64)
Sabon Gari	12	0 (0.00)	1 (8.33)	26	1 (3.85)	4 (15.38)
Zaria	14	0 (0.00)	2 (14.29)	91	14 (15.38)	10 (10.99)
Total	119	1 (0.84)	35 (29.41)	185	16 (8.65)	26 (14.05)

RBPT: Fisher's exact test=10.805; df = 3; p = 0.007

SAT-EDTA: $X^2 = 11.445$; df = 3; p = 0.010

Appendix vi

Prevalence of Brucella antibodies by Age of horses sampled in 3 LGAs of North Senatorial District of Kaduna State, Nigeria

Age	LGAs									Total	
	Igabi			Sabon Gari			Zaria			RBPT	SAT-EDTA
	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA	RBPT	SAT-EDTA
1 – 5 years	5	1 (20.00)	1 (20.00)	10	1 (10.00)	2 (20.00)	9	0 (0.00)	0 (0.00)	2 (8.33)	3 (12.50)
6 – 10 years	53	1 (1.89)	10(18.87)	21	0 (0.00)	3 (14.29)	71	12 (16.90)	7 (9.86)	13 (8.97)	20 (13.79)
11 – 15 years	73	0 (0.00)	23(31.51)	7	0 (0.00)	0 (0.00)	21	1 (4.76)	5 (23.81)	1 (0.99)	28 (27.72)
➤ 15 years	30	0 (0.00)	11(36.67)	0	0 (0.00)	0 (0.00)	4	1 (25.00)	1 (25.00)	1 (2.94)	12 (35.29)
Total	161	2 (1.24)	45(27.95)	38	1 (2.63)	5 (13.16)	105	14(13.33)	13 (12.38)	17 (5.59)	63 (20.72)
Chis-square	2.837*	3.508*	-	1.769*	7.256*	3.240*					
p-value	0.347	0.283	-	0.538	0.034	0.247					

Appendix vii

Prevalence of Brucella antibodies in horses by Use in 3 LGAs of Kaduna North Senatorial district of Kaduna State, Nigeria

LGA	USES								
	Ceremonial			Polo			Racing		
	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA
Igabi	22	0 (0.00)	3 (13.66)	122	2 (1.64)	36 (29.51)	17	0 (0.00)	7 (41.18)
Sabon Gari	0	0 (0.00)	0 (0.00)	18	1 (5.56)	3 (16.67)	20	0 (0.00)	2 (10.00)
Zaria	88	13 (14.77)	10 (11.36)	9	0 (0.00)	0 (0.00)	8	1 (12.50)	2 (25.00)
Total	110	13 (4.28)	13 (4.28)	149	3 (0.99)	39 (12.83)	45	1 (0.33)	11 (3.62)

Chi-square 0.763 2.147 - 0.368 0.612 2.275
p-value 0.833 0.412 - 0.653 1.000 0.324

*Fisher's exact test

Appendix viii

Groomers Awareness and Sources of information on Brucellosis in three LGAs of Kaduna State, Nigeria

LGA	No of Respondents	Awareness of Brucellosis		Sources of Awareness		
		Aware	Not aware	Media	Experienced groomers	Professionals
		No (%)	No (%)	No (%)	No (%)	No (%)
Igabi	17 (42.50)	7 (41.18)	10 (58.82)	4 (23.53)	2 (11.76)	1 (5.88)
Sabon Gari	8 (20.00)	2 (25.00)	6 (75.00)	2 (25.00)	0 (0.00)	0 (0.00)
Zaria	15 (37.50)	6 (40.00)	9 (60.00)	3 (20.00)	2 (13.33)	1 (6.67)
Total	40 (100)	15 (37.50)	25 (62.50)	9 (22.50)	4 (10.00)	2 (5.00)

Awareness: Fisher's exact test = 1.079, df = 2; p = 0.700

Source of awareness: Fisher's exact test = 3.362; df = 2; p = 0.913

Appendix ix

Grooms' knowledge on Brucellosis being Zoonotic and means of Transmission of Brucellosis in 3 LGAs of Kaduna North Senatorial District of Kaduna State, Nigeria.

LGA	No of Respondents	Brucellosis		Means of Infection		Recognition of Brucellosis		
		Zoonotic	Not Zoonotic	Ingestion	Contact	Night sweats	Fever	Others
Igabi	17 (42.50)	2 (11.76)	15 (88.24)	2 (11.76)	0 (0.00)	2 (11.76)	2 (11.76)	0 (0.00)
Sabon Gari	8 (20.00)	1 (12.50)	7 (87.50)	1 (12.50)	0 (0.00)	1 (12.50)	0 (0.00)	0 (0.00)
Zaria	15 (37.50)	3 (20.00)	12 (80.00)	2 (13.33)	1 (6.67)	2 (13.33)	1 (6.67)	0 (0.00)
Total	40 (100)	6 (15.00)	34 (85.00)	5 (12.50)	1 (2.50)	5 (12.50)	3 (7.50)	0 (0.00)

Zoontic brucellosis; Fisher's exact test = 0.322; df = 2; p = 0.921

Means of infection: Fisher's exact test = 0.354; df = 2; p = 0.925

Recognition: Fisher's exact test = 1.349; df = 2; p = 0.989

Appendix x

Respondents knowledge on signs of brucellosis in Horses in 3 LGAs of North Senatorial District of Kaduna state, Nigeria

LGA	Signs of Brucellosis			Encountered cases		Action taken		
	abortion	Wound location Poll withers		Yes	No	Report to Clinic	Self Treatment	Do Nothing
Igabi	5 (29.41)	0 (0.00%)	3 (17.65)	0 (0.00)	17 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
Sabon Gari	0 (0.00)	0 (0.00)	2 (25.00)	0 (0.00)	8 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
Zaria	1 (6.67)	5 (33.33)	3 (20.00)	0 (0.00)	15 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
Total	6 (15.00)	5(12.50)	8(20.00)	0(0.00)	40(100.00)	0 (0.00)	0 (0.00)	0 (0.00)

Signs; Fisher's exact test = 9.703; df = 2; p = 0.05

Appendix xi

Grooms' Knowledge of brucellosis as a Reproductive disease in 3 LGAs of North Senatorial District of Kaduna state, Nigeria

LGA	Abortion in Stable		Determination of causes of abortion		Encountered testicular swellings		Action taken on enlarged testicles		
	Yes	No	Yes	No	Yes	No	Clinic	Traditional Medicine	Do nothing
Igabi	1(5.88)	16(94.12)	1(5.88)	16 (94.12)	3(17.65)	14(82.35)	3(17.65)	0 (0.00)	0(0.00)
Sabon Gari	0(0.00)	8(100.00)	0(0.00)	8 (100.00)	0 (0.00)	8(100.00)	0 (0.00)	0 (0.00)	0(0.00)
Zaria	1(6.67)	14(93.33)	0(0.00)	15(100.00)	3(20.00)	12(80.00)	2(13.33)	1 (6.67)	0 (0.00)
Total	2(5.00)	38(95.00)	1(2.50)	39(97.50)	6(15.00)	34(85.00)	5(12.50)	1 (2.5)	0(0.00)

Abortion: Fisher's exact test = 0.723; df = 2; p =0.998

Causes: Fisher's exact test = 1.532; df = 2; p =0.977

Enlarged testicles: Fisher's exact test = 1.260; df = 2; p =0.702

Appendix xii

Respondents' Knowledge of brucellosis as a zoonosis in 3 LGAs of North Senatorial District of Kaduna state, Nigeria

LGA	Brucellosis in man		Signs of brucellosis in man				
	Yes	No	Fever	Night sweat	Abortion	Weakness	Headache
Gabi	3 (50.00)	14(41.18)	2 (50.00)	3 (50.00)	0 (0.00)	0 (0.00)	1 (50.00)
Sabon Gari	1 (16.67)	7 (20.59)	1 (25.00)	1 (16.67)	0 (0.00)	0 (0.00)	0 (0.00)
Zaria	2 (33.33)	13(38.24)	1 (25.00)	2 (33.33)	0 (0.00)	0 (0.00)	1 (50.00)
Total	6 (15.00)	34(85.00)	4 (10.00)	6 (15.00)	0 (0.00)	0 (0.00)	2 (5.00)

Fisher's exact test = 0.372; df = 2; p = 0.921

Signs: Fisher's exact test = 1.564; df = 2; p = 0.899

Appendix xiii

Respondents' knowledge on Transmission of brucellosis from animals to man in 3 LGAs of North Senatorial District of Kaduna State, Nigeria

LGA	No. Respondents	Method of Transmission		
		Meat Consumption	Milk Consumption	Do not Know
Igabi	17	1 (5.880)	3 (17.64)	14 (82.35)
Sabon Gari	8	0 (0.00)	1 (12.50)	7 (87.50)
Zaria	15	0 (0.00)	2 (13.33)	13 (86.67)
Total	40	1 (2.50)	6 (15.00)	34 (85.00)

Fisher's exact test = 9.322; df = 2; p = 0.076

Appendix xiv

Respondents' practices on Breeding of Mares in three LGAs of Kaduna State, Nigeria

LGA	Ownership of Mares		Breeding of Mares		Ownership of Stallion		Borrowing of Stallion		Sending Mare to Stallion		Sexually transmitted diseases considered			
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	TB (%)	Brucellosis(%)	Trichomoniasis (%)	None (%)
Igabi	13 (76.47)	4 (23.53%)	3 (17.65%)	14 (82.35%)	3(50.00)	14 (82.35%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Sabon Gari	2 (25.00%)	6 (75.00%)	1 (12.50%)	7 (87.50%)	1 (12.50%)	7 (87.50%)	0 (0.00%)	0 (0.00%)	1 (12.50%)	0 (0.00)	0 (0.00%)	1 (12.50%)	0 (0.00%)	0 (0.00)
Zaria	2 (13.33%)	13 (86.67%)	2 (33.33)	13 (86.67%)	2 (33.33)	13 (86.67%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (6.67%)	0 (0.00)
Total	17(42.50%)	23(57.50%)	6 (15.00%)	34(85.00%)	6 (15.00%)	34 (85.00%)	0 (0.00)	0 (0.00)	1 (2.50%)	0 (0.00)	0 (0.00)	1 (2.50%)	1 (2.50%)	0 (0.00)

Appendix xv

Respondents' husbandry Practices in three LGAs of Kaduna state, Nigeria

LGA	Grooming Tools		Lending Grooming Tools		Borrowing Grooming Tools		Causation of Disease through Grooming Tools		Possible Disease through Grooming Tools			
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Ulcerative lymphangitis	Ringworm	Dermatoph ilosis	Thrush
Igabi	17 (43.59)	0 (0.00)	3 (17.65%)	14 (82.35%)	3 (17.65)	14 (82.35%)	17 (100.00%)	0 (0.00%)	17 (100.00%)	16 (94.11%)	1 (5.88%)	0 (0.00%)
Sabon Gari	8 (20.51)	0 (0.00%)	8 (100.00%)	0 (0.00%)	6 (75.00%)	2 (25.00%)	8 (100.00%)	0 (0.00%)	8 (100.00%)	8 (100.00%)	1 (12.5%)	0 (0.00%)
Zaria	14 (93.33%)	1 (6.67%)	10 (66.67%)	5 (33.33%)	7 (46.67%)	8 (53.33%)	15 (100.00%)	0 (0.00%)	13 (86.67%)	5 (33.33%)	0 (0.00%)	3 (100.00%)
Total	39(97.50%)	1 (2.50%)	21 (52.5%)	19(47.50%)	16(40.00%)	24(60.00%)	40 (100.00%)	0 (0.00%)	38 (95.00%)	29 (72.50%)	2 (5.00%)	3 (7.50%)

Appendix xvi

Respondents practices on disease spread through durbar participation in three LGAs of Kaduna State, Nigeria

LGA	Participation in Durbar		Disease Encountered				
	Yes (%)	No (%)	Ulcerative Lymphangitis	Epizootic Lymphangitis	Wounds	Brucellosis	Others
Igabi	7 (41.76%)	10(58.82%)	1 (5.88%)	0 (0.00%)	7 (41.76%)	0 (0.00%)	0 (0.00%)
Sabon Gari	5 (62.50%)	3 (37.50%)	2 (25.00%)	0 (0.00%)	3 (37.50%)	0 (0.00%)	0 (0.00%)
Zaria	15(100.00%)	0 (0.00%)	7 (46.67%)	0 (0.00%)	1 (6.67%)	0 (0.00%)	0 (0.00%)
Total	27 (67.50%)	13(32.50%)	10 (25.00%)	0 (0.00%)	11(27.50%)	0 (0.00%)	0 (0.00%)

Fisher's exact test = 1.349; df = 2; p = 0.989

Appendix xvii

Grooms' grazing Practices in 3 LGAs of North Senatorial District of Kaduna State, Nigeria

LGA	Horse grazing with other animals		Grazing where other animals aborted		Any abortion by mares	
	Yes	No	Yes	No	Yes	No
Igabi	6 (35.29)	11 (64.71)	0 (0.00)	17 (100.00)	0 (0.00)	17 (100.00)
Sabon Gari	7 (87.50)	1 (12.50)	0 (0.00)	8 (100.00)	0 (0.00)	8 (100.00)
Zaria	7 (46.67)	8 (53.33)	1 (6.67)	14 (93.33)	0 (0.00)	15 (100.00)
Total	20 (50.00)	20 (50.00)	1 (2.50)	39 (97.50)	0 (0.00)	40 (100.00)

Grazing: Fisher's exact test = 3.362; df = 2; p = 0.110

Other animals aborted: Fisher's exact test = 1.784; df = 2; p = 0.875

Abortion in mares: Fisher's exact test = 1.349; df = 2; p = 0.892

Appendix xviii

Grooms' practices with regard to Veterinary care in 3 LGAs of North Senatorial District of Kaduna State, Nigeria

LGA	Vet Medical Care		Diseases conditions routinely sought for care		
	Yes	No	Babesiosis	Wound	Lameness
Igabi	17 (100.00)	0 (0.00)	17 (100.00)	3 (17.65)	17 (100.00)
Sabon Gari	8 (100.00)	0 (0.00)	8 (100.00)	2 (25.00)	6 (75.00)
Zaria	15 (100.00)	0 (0.00)	15 (100.00)	8 (53.33)	6 (40.00)
Total	40 (100.00)	0 (0.00)	40 (100.00)	13 (32.50)	29 (72.50)

Conditions: Fishers exact test = 4.332; df = 2; p = 0.084

Appendix xix

Showing some of the positive samples



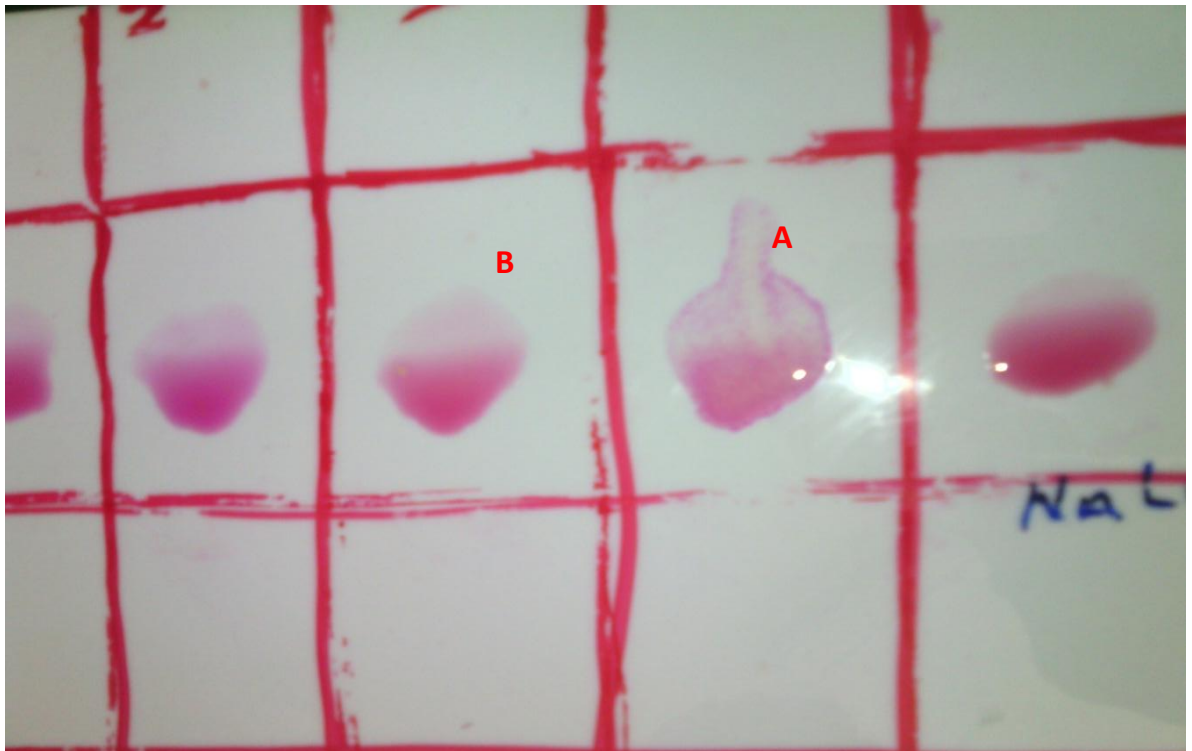
Appendix xx

Plate I showing the RBPT antigen from Onderstepoort Research Institute, South Africa



Appendix xxi

Plate II showing positive (A) and negative (B) RBPT



Appendix xxii

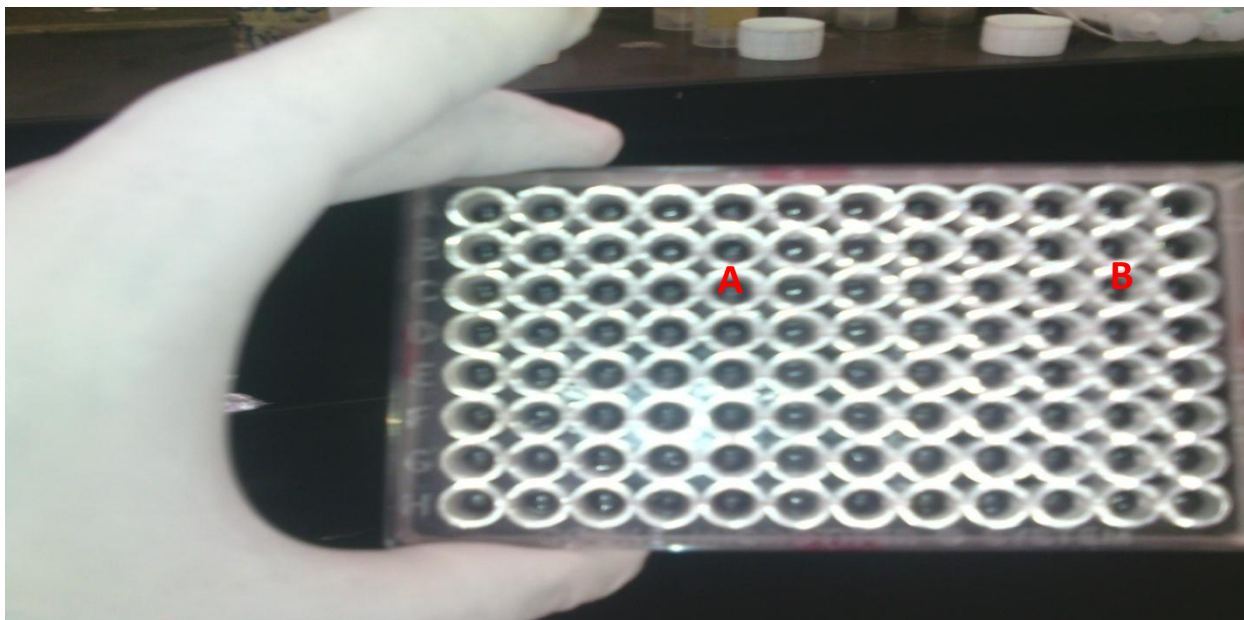


Plate III showing the positive (A) and negative (B) SAT-EDTA results