STUDIES ON BRUCELLOSIS IN CATTLE IN JIGAWA STATE, NIGERIA

By

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DECLARATION

I declare that the work reported in this dissertation titled **"*Studies on Brucellosis in Cattle in Jigawa State Nigeria*"** was carried out by me in the Department of Veterinary Medicine under the supervision of Prof. J.O.O. Bale, Prof. J.K.P. Kwaga, and Prof. U.S. Abdullahi. The information derived from the literature has been duly acknowledged in the list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

Farouk Umar MOHAMMED ---------- ----------
Signature Date

CERTIFICATION

This dissertation entitled "**STUDIES ON BRUCELLOSIS IN CATTLE IN JIGAWA STATE NIGERIA**" by Farouk Umar MOHAMMED meets the regulations governing the award of the degree of Doctor of Philosophy (VETERINARY MEDICINE) of Ahmadu Bello University, Zaria and is approved for its scholarly contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this dissertation to my parents:

Malami Babura and Rukayya Bello.

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ABSTRACT

A serological and bacteriological study of *Brucella* infection in cattle was carried out in Jigawa State Nigeria in order to determine the seroprevalence, identify isolates and assess factors responsible for the introduction, maintenance and spread of the *Brucella* infection in cattle herds in selected Local Government Areas of Jigawa State. The study employed Rose Bengal plate test (RBPT) and Serum Agglutination test (SAT) as screening tests and Complement Fixation test (CFT) as a confirmatory test. *Brucella* organisms were isolated using cultural methods and biochemical tests were used to identify *Brucella* species. Milk Ring (MRT) and Whey Agglutination tests (WAT) were used to screen milk samples. Questionnaires were used to identify presence of exposure factors to *Brucella* species. From the results of the study, 93 (4.98%), 83(4.44%) and 68(3.64%) out of the 1867 serum samples were positive with RBPT, SAT and CFT tests respectively. There was a strong degree of agreement between the screening and the confirmatory tests as indicated by Kappa value of 0.926 0.846 and 0.827. Prevalence of *Brucella* antibodies in the study herds indicated that 30 out of the 147 herds sampled were positive using RBPT, SAT and CFT tests, with an individual animal prevalence (IAP) ranging from 0 to 18.6% and a within herd prevalence (WHP) from 0 to 57.14% based on the CFT tests. The overall herd prevalence in the study based on the confirmatory test was 20.40 % (30/147). The results of Milk samples screened by MRT and WAT tests showed that 0.5% (5/869) and 0.72% (2/274) tested positive with the two different tests respectively. The results also showed that prevalence of *Brucella* infection was higher among female cattle 3.4% (70/2053) than males 0.52% (5/957) in all the study herds. The rate of seropositivity was highest among cattle of age group 37-72 months, 4.55% (37/813). Foreign breeds presented higher seropositivity of 3.2%

(8/249) than the local breeds 2.4% (67/2746). A total of 900 milk, 460 vaginal swabs, 325 prepucial swabs, 14 placentae, 11 stomach contents, 11 spleen and 11 liver samples were cultured for *Brucella* isolation. Two isolates were obtained, one each from placenta and vaginal swabs of Friesian and Bunaji cattle in the North Central and North West Senatorial Zones. Biochemical characterization of the isolates indicated that they were both *Brucella abortus*. From the study all the herd owners interviewed were aware of brucellosis as a disease entity with the local name Bakkale, but they differed in ways of recognizing it. The results also showed that 66 % (98/147) of herd owners used a local herb "Madaci" for treatment of *Brucella* infection, while 33.3% (49/147) utilize veterinary services. The study also indicated that none of the herds were vaccinated against brucellosis. Assessment of exposure factors responsible for introducing, maintaining and spread of *Brucella* infection between and within herds in the study areas indicated that there was statistical association between migration, hygiene practices, raising multiple species, housing, extensive husbandry system and natural breeding using bulls with prevalence of *Brucella* infection ($P< 0.05$). In conclusion the isolation of two *Brucella abortus* species and evidence of *Brucella* infection in the study herds has demonstrated that *Brucella* exists among cattle in the State. It was hereby recommended that cattle owners should be educated on the various methods of preventing and controlling brucellosis in the State.

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ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
CDC	Centres for Disease Control and Prevention
cELISA	Competitive Enzyme Linked Immunosorbent Assay
CFT	Complement Fixation Test
CI	Confidence Interval
CMI	Cell Mediated Immunity
CTL	Cytotoxic T lymphocyte
DNA	Deoxy Ribo Nucleic Acid
EIA	Enzyme Immuno Assay
ELISA	Enzyme Linked Immunosorbent Assay
ERIC	Entero Bacterial Repetitive Intergenic Concensus
FAO	Food and Agricultural Organization
FIA	Fluoroimmunoassay
HOOF	Hyper-variable Octameric Oligonucleotide Finger Print
ICFTU	International Complement Fixation Unit
IHP	Individual Herd Prevalence
JARDA	Jigawa Agricultural and Rural Development Authority
JRI	Jigawa Research institute
LPS	Lipopolysaccharide
LCR	Livestock Committee Report
MHD	Minimum Hemolytic Dose
MLST	Multi-locus Sequence Typing

MLSA	Multi-locus Sequence Assay
MLVA	Multi-locus Variable Number Tandem Repeat Analysis
MRT	Milk Ring Test
NCBI	National Centre for Biotechnology Information
OIE	Office International de Epizootics
O-PS	O-Polysaccharide
OR	Odds Ratio
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electroporesis
PS	Polysaccharide
RBC	Red Blood Cell
RBPT	Rose Bengal Plate Test
REP	Repetitive Entergenic Palindromic
RFLP	Restriction Fragment Length Polymorphism
RIA	Radioimmmunoassay
RNA	Ribo Nucleic Acid
r RNA	ribosomal Ribo Nucleic Acid
SAT	Serum Agglutination Test
SLPS	Smooth Lipopoly Saccharide
SNP	Single Nucleotide Polymorphism
SRBC	Sheep Red Blood Cell
ST	Sequence Typing

VBS or VBD Veronal Buffered Saline or Diluent

WAT Whey Agglutination Test

WHP Within Herd Prevalence

WHO World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Livestock plays a crucial role in the livelihood of the majority of Africans including the citizens of Nigeria (JARDA, 2010). It is one of the main sources of meat, milk, draught power and cash income. It also plays an important role in socio-cultural traditions of many African setting (McDermott and Arimi, 2002).

In Jigawa State, livestock and poultry are mainly kept by small to medium scale farmers in the semi-arid regions of the State (JRI, 2003). Livestock production sectors are defined in to three categories as small, ranging from 1 to 30 animals, medium 31 to 50 animals and large, more than 50 animals based on total number of cattle, goats and sheep (Livestock Committee Report, 2013).

Several documented bacterial diseases which are directly and indirectly transmitted occur commonly in Africa and are poorly controlled in both livestock and human populations (McDermott and Arimi, 2002; Smits and Cutler, 2004). These diseases such as brucellosis have a great socio-economic impact leading to low production due to abortion, reduced milk production, and loss of draft power, which ultimately have negative effects on cash income (Smits and Cutler, 2004). However, despite their economic and social importance, livestock management as well as programmes to control infectious diseases in Africa

including brucellosis has declined due to decreasing government support particularly for operational costs of disease control (McDermott and Arimi, 2002).

There is paucity of information on the occurrence and impact of zoonotic infections including brucellosis in many developing countries (Perry *et al*., 2002). In tropical Africa, *Brucella abortus* seroprevalence among cattle population was up to 40% and in some areas the prevalence was up to 60% (Akakpo and Bornarel 1987; OIE, 1987).

Bovine brucellosis has been reported in many countries, but has been eradicated from the livestock population of most European countries, Japan, Canada and the United States of America (Nicolette, 1980). Its occurrence and prevalence has been studied in Africa, Asia and Latin America (Mangen *et al*., 2002; Foggin *et al*., 1997; Arambulo, 1998). A high prevalence of 38.5% was reported in Uganda, Egypt, Sudan and Senegal (Faye *et al*., 2005). Moderate prevalence rates of 8.14% were reported in Nigeria, Tanzania, Ghana and Botswana (Chukwu, 1985; 1987), while a low prevalence of 1.6% was seen in Kenya (Kagumba and Nandokha, 1978), Djibouti (Chantel *et al*., 1994) and Eritrea (Omer *et al.*, 2000).

In Africa, brucellosis is considered to be one of the most serious disease problems facing the veterinary profession (McDermott and Arimi, 2002). The high prevalence is due to close human-animal contacts, food consumption customs (Nicoletti and Milward, 1985) and the fact that many countries have not yet started controlling or eradication schemes against diseases (Nicoletti and Milward, 1985). Brucellosis is perhaps the most widespread and economically important disease in tropical and sub-tropical regions (McDermott and

Arimi, 2002). The direct loss of meat as a result of abortion, infertility and weight loss in infected herds of cattle was estimated to be 15% while that of reduced milk production was 20% (Unger, 2002).

In many West African countries the disease is endemic (Unger, 2002) and has presented considerable impact on the economy through loss of milk and meat restriction in international livestock trade and diminished animal working power (McDermott and Arimi, 2002) The disease has major public health challenge, as animals are almost exclusively the source of infection to humans. It is often undiagnosed in both human patients and animal sources (Unger, 2002) and it is widely acknowledged that the epidemiology of brucellosis in humans is poorly understood, particularly in sub-Sahara Africa.

Bovine brucellosis was first reported in Nigeria by Earshaw and O' Brien (1926). From 1934 onwards, reports on brucellosis were documented (Anon, 1934). *Brucella abortus* is the specie that affects mainly cattle but it has been reported in other livestock species such as sheep and goats in Nigeria (Ocholi *et al*., 2005).

Reproductive failures due to a variety of infectious diseases occur worldwide where the most important cause has been recognized to be brucellosis (Young, 1991). It is an infectious disease affecting mainly sexually mature pregnant animals and is caused by members of the genus *Brucella* (Young, 1991; Adams, 2002; Santllano-Estrada *et al*., 2004; Ehizibolo *et al*., 2011).

Brucella species are Gram-negative, facultative intracellular bacterium that causes brucellosis in humans and many animals (Corbel, 1997). The brucellae are taxonomically placed in the alpha-2 subdivision of the class Proteobacteria. There are 10 species of *Brucella* based on preferential host specificity and they include *B. melitensis* (goats), *B. abortus* (cattle), *B. suis* (swine), *B. canis* (dogs), *B. ovis* (sheep), *B. neotomae* (desert mice), *B. ceti* (cetacean), *B. pinnipedialis* (seal), *B. microti* (voles), and *B. inopinata* (unknown) Ewalt *et al.,* 1994; Rose *et al.,* 1994; Cloeckaert *et al.,* 2004; O'Callaghan and Whatmore *et al*., 2005; Scholz, *et al*., 2008; Banai and Corbel 2010; Nymo *et al*., 2011.

Similarly, of the 10 recognized species of *Brucella*, *B. abortus, B. melitensis, B. suis*, and *B. canis* are pathogenic to humans. Human infections with *B. canis* are rare. *Brucella abortus, B. melitensis* and *B. suis* are the most pathogenic to humans and have been identified as agents amenable for use in bioterrorism, and are listed as category B priority pathogens by the United States Centers for Disease Control and Prevention (Younggun, 2012). The disease approximately affects over 500,000 humans worldwide annually (Anon, 1986; Hahn, 1991).

Brucellosis is a herd or flock problem and it spreads within the herd primarily by ingestion of contaminated material (Radostits *et al*., 2000). In animals, the presence and transmission of brucellosis is moderated by an interrelationship of factors including; climate, type of production system,(Nomadism/transhumance, extensive or intensive), herd size, livestock breeds and the age of the animal (Akakpo and Bornarel, 1987).

The spread of the disease between herds usually occurs by the introduction of asymptomatic chronically infected animal (Radostits *et al*., 2007). Transmission of infection to humans occurs through breaks in the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted foetuses or placentae (Nielsen and Duncan, 1990). An individual can also be infected following consumption of *Brucella* contaminated milk and cheese (Anon, 1986; Hahn, 1991).

Sources of transmission of bovine brucellosis are aborted foetuses, foetal membranes, vaginal discharges and milk from infected animals (Radostits, *et al*., 2000). The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, water and licking after birth, foetuses, and new born calves, all of which may contain a large number of the organisms and constitute a very important source of infection (Radostits *et al*., 2007).

The disease in cattle is characterized primarily by abortion in late pregnancy from the 5^{th} to the 8^{th} month of gestation and is frequently followed by retention of foetal membranes and endometritis which may be a cause of infertility (Radostits *et al*., 1994a). In bulls, the disease usually causes orchitis, epididymitis, seminal vesiculitis and sterility (Radostits *et al*., 1994b).

Despite the advances made in the diagnosis and therapy, brucellosis is still widespread in many developing nations. Conventionally, serological tests are used to screen for, or to confirm the disease. These screening tests are inexpensive, fast and highly sensitive but not necessarily highly specific (Alton *et al*., 1975). The most widely used serological tests for

the diagnosis of brucellosis in animals are Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Enzyme Linked Immunosorbent Assay (ELISA). The diagnostic value of these tests may be questionable on individual basis because of cross-reacting antibodies, but for screening of herds these tests remain ideal because an estimated prevalence of antibodies can be established (Akakpo and Bornarel, 1987).

Diagnosis of clinical brucellosis in humans and animals is initially made by use of appropriate serological or other immunological tests and confirmed by bacteriological isolation and identification of the agent (Alton *et al*., 1988; Nielsen, 2002). The disease occurs worldwide, except in those countries where bovine brucellosis (*B. abortus*) has been eradicated (Corbel, 1997). An evidence of eradication is usually defined as the absence of any reported cases for at least five years (OIE reports, 2002). These countries include; Australia, Canada, Cyprus, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden and the United Kingdom (OIE reports, 2002).

The Milk Ring Test (MRT) is often used as a herd test to determine the prevalence of *Brucella* infection and screening of herds. The MRT can also be used to test individual milk samples to identify the infected animal in the herd but, it may give false-positive results shortly after parturition, near the end of lactation and when mastitis is present (Alton *et al.*,1988). To overcome this problem Milk-ELISA is employed on individual milk samples to detect *Brucella* antibodies (Staak, 1990).

Isolation of the causative agent is the most accepted tool for confirmatory diagnosis of brucellosis. It has the advantage of detecting the organisms directly, but it is time

consuming since it takes about 10 days or longer for proper identification of the causative agents and it has reduced sensitivity in chronic infection (Alton *et al*., 1988). Besides, the culture materials must be handled carefully, as the organisms are class III pathogens (Alton *et al.*, 1988). For these reasons, genetic characterization using molecular DNA techniques has been pursued (Fekete *et al.*, 1992).

Numerous PCR based assays have been developed for the rapid identification of *Brucella*. These assays have been applied to detect *Brucella* DNA in varieties of clinical samples including tissues (mainly aborted foetuses and associated maternal tissues), blood, milk and semen (Fekete *et al.*, 1992; Leal-Klevezas *et al.*, 1995; Amin *et al.*, 2001; Kanani, 2007). Tests based on PCR are also found more sensitive than the traditional culture method (Amin *et al.*, 2001; Kanani, 2007). More recently, real-time PCR and multiplex PCR have been used for detection of *Brucella*, offering improvement in detection times and specificity (Kanani, 2007).

1.2 Statement of the Research Problem

Traditionally, livestock in Jigawa State are managed under extensive and semi-intensive husbandry systems which usually involve mixing of herds and sharing of grazing and water points (JARDA, 2001). Cattle movement, mixing and sharing of grazing points are very important factors that contribute to the epidemiology of brucellosis (Kadohiri *et al*., 1997).

Incidences of abortion and losses in cattle herds have been reported at the Jigawa State Veterinary Clinics over the years which tentatively were diagnosed as brucellosis and other

infectious diseases based on clinical presentation (JRI, 2003). There have been no proper investigations to ascertain the actual causes (JRI, 2003).

It is a habit of some farmers in parts of the State to consume milk raw while in some areas milk is kept overnight to ferment without boiling (Farouk, 2007). Consumption of contaminated raw and fermented milk could serve as a source of infection to humans (Radostits *et al.*, 2000).

Brucella antibodies were detected in milk and serum in some selected Fulani cattle herds in the State (Farouk *et al.*, 2011; 2013). This calls for further investigation of the disease in the State

There are reports on introducing foreign bulls (Friesian, Azawak and Semental) without testing and semen for artificial insemination (JRI, 2003). These have been documented as a source of transmitting *Brucella* (Radostits *et al*., 2007).

The State also shares borders with Niger Republic and transhumance pastoralists come in to the State during dry periods in search of pastures. They could serve as a source of introducing infection since their status regarding brucellosis is unknown, and they share and contaminate water and grazing points.

Serological investigations and reports have shown that brucellosis is endemic in Nigeria and evidence of infection has been demonstrated in cattle, dogs, sheep, pigs, goats, camels, wild rats and humans (Esuruoso, 1974a;1974b; Alausa and Osoba, 1975, Okoh *et al*., 1979;

Okoh,1980 ; Bale and Kumi-Diaka, 1981; Nuru, 1982; Bale and Nuru, 1985: Bale, 1991; Ajogi, 1997; Falade, 2002; Ocholi *et al.* 2005;Avong, 2006; Cadmus *et al.*, 2006; Junaidu *et al.*. 2006; Ofukwu *et al*., 2007; Bakari, 2010, Haladu, 2010; Mai *et al*., 2012).

Studies in Nigeria have indicated that *Brucella* has been isolated from goats (Falade, 1981), Pigs (Bale and Nuru, 1985), Horses (Bale and Kwanashie, 1985), Cattle (Ocholi *et al.,* 2004; 2005), So far, of the different biotypes of *Brucella* recognized, biotypes 1, 2, 3 and 4 have been isolated in Nigeria (Eze, 1978; Bale and Kumi-Diaka, 1981; Ocholi *et al.*, 2004; 2005: Bertu, 2014).

An investigation of a large brucellosis outbreak in the late 70s (Alausa, 1979) found that out of the 25 herds, comprising of 10 government, 3 private settled and 12 Fulani herds, 11 of the 12 Fulani pastoralist herds were serologically positive. Calf losses were reported especially in local nomadic herds, resulting in a shortage of meat and protein for the local populace. The outbreak coincided with a period of sahelian drought that saw a general reduction in cattle population in Nigeria (Alausa, 1983).

1.3 Justification for the Study

Livestock husbandry is one of the mainstay of agricultural activities in Jigawa State, Nigeria. Reports on problems and prospects of the livestock sub-sector for the year 2013 indicated that the State has a cattle population of 1.2 million heads (Livestock Committee Report, 2013).

Diagnostic survey report by Jigawa Agricultural and Rural Development Authority (2001) indicated that almost every rural household in the State keeps either cattle, sheep, goat, donkey or poultry which provide sources of income in terms of milk and meat or farm services (draft power).

Livestock and poultry production in the State is one of the major economic activities that contribute to the livelihood of the rural populace apart from crop farming (Jigawa Agricultural and Rural Development Authority, 2001). Losses due to brucellosis could lead to decrease in milk and meat output which subsequently could bring set back in the economy of the State or region.

The State has one of the major International cattle markets in Nigeria at Maigatari which supplies different parts of Nigeria with different species of animals whose status with regard to brucellosis is not known (JRI, 2003).

There is no documented information on the isolation of *Brucella* from the study areas to the best of my knowledge. There is lack of sufficient knowledge about the disease and the absence of effective prevention and management strategies amongst farmers in the State (Jigawa Research Institute, 2003).

There is the need to use both sensitive and specific diagnostic techniques for detection of the disease in the study areas based on screening and confirmatory tests. The antibody detection will be supported by more definitive tests such as cultural isolation and typing so that the status of the disease can be ascertained and documented.

1.4 Aim of the Study

The aim of the study was to determine the seroprevalence of brucellosis in Jigawa State, isolate and characterize *Brucella* and to assess the factors responsible for introducing, spreading and maintaining of the disease in the study herds.

1.5 Objectives of the Study

The objectives of this work were:

1. To determine the seroprevalence of brucellosis in cattle in Jigawa State using Rose Bengal Plate, Serum Agglutination and Complement Fixation tests.
2. To screen Milk using Milk Ring and Whey Agglutination tests
3. Isolate, identify, and characterize *Brucella* using Cultural and Phenotypic techniques
4. Assess exposure factors influencing introduction, spread and maintaining brucellosis in the study herds with close ended questionnaire

1.6 Research Questions

1. What is the current sero-prevalence of brucellosis in Jigawa State?
2. Is *Brucella* infection associated with age, sex and breed of animals?
3. What are the identifiable factors influencing introduction, spread and maintenance of brucellosis in the study herds?
4. Which species of *Brucella* are present in the study herds?

CHAPTER TWO

LITERATURE REVIEW

2.1 History of Cattle Brucellosis

Brucellosis (Bangs disease) of cattle was a disease of great economic importance to the farmers of Kansas and probably is responsible for greater loss estimated at $4,368,000 in 1937 to the cattle industry of the state than any other single disease (Abdilrahim, 2009).

Cows with brucellosis, produce fewer and weaker calves, produce 26-30% less milk, often become sterile and have a high percentage of retained placentae and problems of the reproductive organs (Radostits *et al*., 1994a). They are more subjected to mastitis, may have enlarged joints and lameness, increase the owners labour costs, necessitate keeping of an increased number of females, and reduce the value of all animals in the herd (Radostits *et al*., 2007).

In 1887, Dr. Bruce, an Englishman, isolated the germ that caused abortion in goats on the island of Malta and in 1897 Professor Bang of Denmark found it to be the cause of abortion in cattle (Corbel, 1998a). This disease of cattle was called Bang's disease until Dr. Evans of the Bureau of Animal Industry found that the germs causing abortion disease in swine, goats and cattle were very closely related (Corbel and Young, 1989b). Now the disease caused by this group of germs in any animal is called brucellosis in honourr of Dr. Bruce (McMahan, 1994).

The first cases to be recognized were in Iowa or Minnesota, where brucellosis became a major endemic problem in 1927 (Hench *et al*., 1948). The prevalence of the disease increased rapidly, but the extent of better diagnosis is uncertain. In 1930-1944, 36,513 cases of brucellosis were reported in the United States and 59% of the reported cases resurfaced seven years later (Angle, 1935; Hench *et al.*, 1948; cited at http/www. antimicrobe.org).

Brucellosis is an important disease among cattle and remains a major source of disease for humans and domesticated animals. In countries that do not implement high-quality and effective public health and domestic animal health programmes as well as a national brucellosis control and eradication programme, brucellosis will remain the most common form of an important zoonotic disease. Bovine brucellosis caused mainly by *B. abortus* has a major economic impact on developing countries (Unger, 2002).

The FAO, WHO and OIE consider brucellosis as the most highly spread zoonosis in the world (FAO, 2010). The importance of this highly contagious disease is due` to its economic impact on the animal industry, which affects adversely the supply of animal proteins, and the enormous danger to human health through either direct contact with infected animals or the consumption of contaminated milk and dairy products (Nikolaos, 1998).

Even though many countries have extensive eradication programmes, some even having eradicated the disease, brucellosis is still a serious disease challenging the veterinary and

medical professions. The reported incidences and the prevalence of the disease vary widely from country to country (Radostits *et al.*, 2000).

2.2 The Genus *Brucella*

Brucellae are Gram negative, facultative intracellular bacteria that cause brucellosis in humans and many animals (Corbel, 1997). The brucellae are taxonomically placed in the alpha-2 subdivision of the class Proteobacteria (Corbel and Morgan,1984). There are ten species of *Brucella* based on preferential host specificity namely *B. melitensis* (goats), *B. abortus* (cattle), *B. suis* (swine), *B. canis* (dogs), *B. ovis* (sheep), *B. neotomae* (desert mice), *B. ceti* (cetacean), *B. pinnipedialis* (seal), *B. microti* (voles), and *B. inopinata* (unknown) (O'Callaghan and Whatmore, 2011). Of the ten recognized species of *Brucella*, *B. abortus*, *B. melitensis*, *B. suis*, and *B. canis* are pathogenic to humans. Human infections with *B. canis* are rare. *Brucella. abortus*, *B. melitensis*, and *B. suis* have also been identified as agents amenable for use in bioterrorism and are listed as category B priority pathogens by the United States Centers for Disease Control and Prevention (Younggun, 2012).

At the microscopic level, *Brucella* species are smooth organisms that have lipopolysaccharide molecules containing a polysaccharide O-side chain made from a homopolymer of perosamine on their surface, while rough organisms lack this chain on their LPS (Caroff *et al*., 1984). Smooth and smooth-intermediate colonies are indistinguishable macroscopically. Rough colonies are usually less transparent than the smooth variants. They have a dull, granular surface and appear matte white, yellowish white/buff, or brown in colour. Mucoid colonies are similar to rough colonies except that they have a sticky glutinous texture. Observations at the DNA level are becoming more

helpful in further classifying *Brucella* organisms beyond conventional methods. These genetic markers are becoming the definitive means by which to identify one *Brucella* isolate from the other (Fynan *et al*., 1995).

2.2.1 Classification scheme

Isolates of *Brucella* species share greater than 90% DNA homology with one another. As a result, some investigators have suggested that all isolates be grouped under a single species of *Brucella* (Verger *et al.,* 1987). Currently, however, phenotypic characteristics are still used to divide the genus *Brucella* into species (Verger *et al.,* 2000).

The phenotypic properties used to characterize *Brucella* include, colony morphology, antigenicity, virulence, growth rate, CO_2-requirements, viability, phage susceptibility, salt sensitivity, antibiotic resistance, and resistance to dyes (Adams, 2002). The species have been further divided into biovars. The biovars are further divided to contain individual strains (Corbel, 1997).

The first major phenotypic classification, beyond host specificity is colony morphology (Corbel and Young, 1989b). The details of colony morphology (smooth vs. rough) have been previously described in descriptive features and can be used to determine if a colony is smooth or rough. Two highly definitive tests used in colony morphology determination utilize acriflavin and crystal violet (Alton *et al*., 1988). Auto-agglutination of colonies of bacteria in a 0.1% acriflavin solution indicates that the colonies are morphologically rough. A colony's ability to take up crystal violet stain further indicates rough colony morphology.

Another major classification scheme is based on antigens dominant within an isolate's O-polysaccharide (O-PS)(Corbel, 1989a). The two major antigens present in the O-PS are A- and M- antigens. A-dominant LPS is characterized as having 5 consecutive α-1,2 linked 4-formamido-4,6-dideoxymannose residues; M-dominant LPS is linked predominantly via α-1,2 with every fifth residue linked via α-1,3 linkages (Corbel, 1997). Smooth strains of *Brucella* are classified as either being A- or M- dominant based on the ability of the strain to auto-agglutinate in sera directed against either of these antigens (Corbel and Macmillan, 1998).

2.2.2 Genomics

Full genomic sequences have now been published for *Brucella abortus* (Sanchez *et al.*, 2001), *B. melitensis* (DelVecchio *et al.* 2002) and *B. suis* (Paulsen *et al.* 2002). As of October 11, 2011, the National Center for Biotechnology information (NCBI) genome sequence site has been found to contain 14 sequenced *Brucella* genomes. Furthermore, 25 additional *Brucella* genomes have been sequenced by the Broad Institute. Therefore, at least 39 *Brucella* strains have been sequenced. With the increasing number of sequenced bacterial genomes, it becomes possible to conduct a systematic comparative analysis of whole genomes of different *Brucella* strains and to dissect their genetic conservation and variability (Verger *et al*., 1987).

Each *Brucella* genome contains two circular chromosomes. The size of Chromosome I and II approximates 2.2 and 1.1 Mb, respectively. There are about 3200–3400 genes in each genome. Based on DNA–DNA hybridization studies, the genus *Brucella* is a highly

homogeneous group (>90% DNA identity among all nomen species (Verger *et al*., 2000). Ratushna *et al*., (2006) compared the genome sequences of *B. abortus* strain 9-941, *B. melitensis* strain 16 M, and *B. suis* strain 1330. They found that majority of the annotated genes in these three genomes share 98–100% sequence identity at nucleotide level.

The differentiating genes among these three species are located in large (~20 kb) regions (Ratushna *et al*., 2006). Whatmore *et al*., (2007) examined nine discrete genomic loci that correspond to 4396 bp of sequence from 160 *Brucella* isolates. A phylogeny analysis using the multilocus sequences showed that four classical *Brucella* species, *B. abortus, B. melitensis*, *B. ovis*, and *B. neotomae* were as well-separated clusters in the phylogenic tree structure. With the exception of biovar 5, *B. suis* isolates cluster together. *Brucella canis* isolates were located on a phylogenic branch closely related to *B. suis* biovars 3 and 4 isolates. Marine mammal isolates represent a distinct cluster (Whatmore *et al*., 2007). The major conclusion of the phylogenic tree analysis was verified by another maximum likelihood phylogenetic analysis of the 10 *Brucella* strains (Wattam *et al*., 2009). *Brucella. suis* as a single species, has been questioned since it has a broader host specificity but does not have any identified species-specific markers (Moreno and Moriyon, 2002). Since limited genome diversity exists among different *Brucella* species, a comparison of *Brucella* species whole genomes is a powerful tool to identify *Brucella* gene variability that is responsible for differences in host preference and virulence restriction.

The sequence insertion and deletion events may contribute to host specificity between different *Brucella* species. Rajashekara *et al*. (2004) used the complete genome sequence of

B. melitensis 16 M, a strain highly pathogenic to humans, to construct a genomic microarray. Hybridization of labeled genomic DNA from different *Brucella* strains to this microarray identified 217 open reading frames (ORFs) that were altered in five *Brucella* species, including *B. abortus, B. suis, B. canis, B. ovis*, and *B. neotomae*. Many of the ORFs are located in the 16 M genome in nine regions (genomics islands) ranging in size from 5 to 44 kb. Genomic islands lost in a given species are often restricted to that particular species (Rajashekara *et al*., 2004). The genomic islands missing in *B. ovis* are present in *Brucella* species pathogenic to humans. However, *B. neotomae*, a non-pathogenic species in humans and domestic animals, also possesses these islands. Interestingly, the genetic islands identified do not encode adhesins or secreted virulence factors that contribute to host specificity in other bacterial species (Moon *et al*., 1977; Tsolis *et al*., 1999; Inatsuka *et al*., 2005). It is likely that adhesins and secreted virulence factors are encoded in conserved loci where they are differentially expressed or inactivated by point mutations. As seen in *Bordetella* species gene inactivation or altered gene regulation may contribute to differences in host range and virulence of *Brucella* species in humans (Parkhill *et al*., 2003).

Comparative genomic analysis of *B. suis* and other members of the α-proteobacteria, in particular, belonging to the family *Rhizobiaceae*, has shown distinct similarities among these organisms both in genome structure and metabolic capabilities (Paulsen *et al.*, 2002; Tsolis, 2002). Of particular interest is the homology between two-component regulatory systems essential for intracellular replication (*BvrR/BvrS*), tumour formation in plants (*ChvG/ChvI*) and establishment of endosymbiosis (*ExoS/ChvI*) for *B. suis*, *Agrobacterium*

tumefaciens and *Sinorhizobium meliloti* respectively (Cheng and Walker 1998; Lopez-Goni *et al.,* 2002). Furthermore, the recent characterization of a type IV secretion system in *Brucella* that is essential for intracellular survival (O'Callaghan and Whatmore, 2011; Boschiroli *et al.,* 2002) and their paralogs in *A. tumefaciens* essential for tumour formation in plants (*virB* genes) has highlighted the possible common mechanisms of pathogenesis utilized by these microbes (Tsolis, 2002).

Scrutiny of genomes for classical virulence markers has shown a remarkable absence of these genes, such as capsules, fimbriae and toxins (Delrue *et al.,* 2004). Of surprise was the identification of genes encoding putative flagellin components (Halling, 1998). It was believed that these microbes were non-motile and whether these genes are functional and expressed is a subject of intense debate. It is possible that these genes may have a role *in vivo*, but this remains to be established. Technological advances have now made it possible to selectively purify total RNA from intracellular brucellae enabling detailed study of the trancriptome of these pathogens (Covert *et al.,* 2005).The extreme uniformity among the brucellae may offer an opportunity to elucidate both pathogenicity-related genes and discovery of reliable epidemiological markers through comparative genomic analysis (Rajashekara *et al.,* 2004).Whether these differences lie at the level of genes or single nucleotide polymorphisms (SNPs), the situation remains to be resolved.

2.2.3 Proteomics

Genomic approaches have much potential to identify differences in gene possession, or for the identification of SNPs. However, differential pathogenesis among these pathogens may result from post-translational modifications such as glycosylation (Paulsen *et al.,* 2002). To

date studies of the *Brucella* proteome have been limited. Recent investigations comparing proteomes of *B. melitensis* 16M and the vaccine strain, Rev1, have revealed differences in expression of the 31-kDa immunogenic outer membrane protein, regulatory proteins involved in the acquisition of iron, those utilized for sugar and amino acid binding and lipid degradation (Eschenbrenner *et al.*, 2002;). Insufficient data is currently available to conclude whether these differences are associated with the differences in virulence seen between these strains; however, it provides useful baseline information. As these studies were performed using organisms cultivated *in vitro*, the possible lack of expression of nonconstitutive virulence-associated genes must be considered.

As biotechnology advances, so does the possibility of assessing the proteomics of these microbes in intracellular environments. Investigation of proteomics of *Brucella* in cell lines derived from different tissues, and from susceptible and nonsusceptible host species, should shed light on these elusive mechanisms central to host–microbial interaction.

Today, it has become increasingly important to identify not only a particular species of *Brucella* but more importantly, to pinpoint what particular biovar or strain the isolate falls under. This is especially important when trying to determine if an isolate represents a wild-type, laboratory, or vaccine strain. Given the highly conserved nature within the genus, biochemical methods are usually an insufficient means of achieving a definitive classification of a particular isolate. In addition to the standard classification scheme, molecular methods are becoming the diagnostic method of choice. Methods such as restriction fragment length polymorphisms (RFLP) (Halling and Zehr, 1990), polymerase

chain reaction (PCR) (Fekete *et al*., 1992), and the use of nucleic acid probes (Clavareau *et al*., 1998; Grimmont *et al*., 1992) are being used to distinguish among strains of *Brucella*.

2.3 Epidemiology

Animals such as cattle, sheep and goats become infected after ingesting contaminated feeds, water or suckling from the dam or licking an infected placenta, new born or foetus (Corbel, 1997). The genitalia of infected animals that have aborted contain large concentrations of the organism, particularly the placenta and lochia (Godfroid *et al*., 2004a; b). Calves and lambs may also be infected in the uterus (Nielsen and Duncan, 1990; Bale and Nuru, 2001). Animals may also become infected by inhalation or through the conjunctiva. Human transmission has been reported through consumption of unpasteurized milk and milk products or by direct contact with infected carcasses and aborted materials, accidental inoculation during vaccination and also through laboratory exposure (Nielsen and Duncan, 1990).

The epidemiological variables which are considered to affect the initiation, spread, maintenance and/or control of brucellosis can be categorized into those related to the animal population, management, or biology of the disease (Nicoletti, 1980; Radositits *et al*., 1994a). The factors influencing the transmission of *Brucella* species in a geographical region can be classified into two categories namely those associated with transmission of the disease between herds (purchase of infected animals, proximity of infected herds to clean herds, sharing of pastures, dip tanks, watering points, and stray of infected animals into clean herds), and those influencing the maintenance and spread of infection within

herds (unvaccinated animals in infected herds, herd size, population density, method of housing and use of maternity pens) (Nicoletti, 1980; Radostits *et al*., 1994b).

Susceptibility of livestock to *Brucella* infection is influenced by the age (young animals are less susceptible to *Brucella* than older animal), sex and reproductive status of the individual animal (sexually mature pregnant animals) (Nicoletti and Milward, 1985). Placental trophoblasts produce erythritol in increasing amounts during the later stages of pregnancy which coincides with the period when pregnant cattle are more susceptible to infection with *Brucella abortus* (Nicoletti, 1980).

The preferential utilization of erythritol rather than glucose is characteristic of pathogenic *Brucella* strains (Sangari *et al*., 2000) Erythritol promotes the growth of some strains of *Brucella* and the metabolic pathway for degradation of erythritol has been described previously (Keppie *et al*., 1965). However, as *Brucella* has also been found in the reproductive tract of animals with no detectable levels of erythritol, the role of this sugar in the virulence of the organisms has been put into question (Sangari *et al*., 2000). *Brucella abortus* strain 19 is a spontaneous attenuated mutant widely used to vaccinate cattle. It is the only *Brucella abortus* strain that is inhibited by erythritol (Sangari *et al*., 2000).

2.3.1 Epidemiology of brucellosis in Europe, Middle East and North Africa

Bovine brucellosis has been eradicated from the livestock population of most European countries like, Albania, Bulgaria, United Kingdom, Croatia and Czech Republic (FAO/WHO/OIE, 1994).In Germany, France, Greece, Italy, Spain and Ireland low sporadic incidence due to *Brucella abortus* was reported. (FAO/WHO/OIE,1995). In Greece, despite

national brucellosis control programmes, the human incidence of the disease was recorded as 4.2 cases per 100,000 in 1998 (Jestapolulu *et al.*, 2008), with rates rising to between 17.3 and 1,110 per 100,000 in certain rural areas where pastoralism was an important part of livestock-keeping (Avdikou *et al*., 2005). Since pastoralists use an estimated 23% of land in the Middle East and North Africa, the persistence of brucellosis is important in this area of the world, especially due to the presence of *B. melitensis* in particular, biovar 3 in sheep and its transmission to humans (FAO, 2010).

Human brucellosis in Saudi Arabia is mostly caused by contact with sheep and goats (Cooper, 1992; Abass and Agab, 2002) in traditional Bedouin pastoral settings and is linked to the consumption of raw milk, as well as direct contact with animal secretions during parturition. Human brucellosis is also present in Jordan, Iraq, Iran, Egypt, Oman and Libya, where there are extensive nomadic pastoral communities (Abass and Agab, 2002).

In Jordan, the highest prevalence of brucellosis is found in cattle and sheep, whereas camels are the most important source of the disease in Iran, Egypt and Saudi Arabia, and goats are the primary contributor to human brucellosis in Iraq (Hotez *et al.*, 2012). These regions retain national disease surveillance and Health Service programmes, as well as large-scale therapeutic drug and vaccine campaigns. As these programmes generally control national disease levels in animals and humans, the main method of zoonotic brucellosis transmission is through the consumption of unpasteurised dairy products.

2.3.2 Epidemiology of brucellosis in Asia

As in the case of the countries mentioned above, human brucellosis is a notifiable disease in China. In Mongolia, brucellosis is a significant problem amongst nomadic herding people, with human incidence rates of 115 per 100,000 in 1992 (WHO and MOH, 1993; Foggin *et al*., 1997). Despite vaccination campaigns, which began in 2000, brucellosis has re-emerged as a major preventable disease in Mongolia, partly due to low vaccine coverage and a sharp increase in livestock populations (Roth *et al.,* 2003; Zinsstag *et al*., 2005). Data from the National Notifiable Disease Surveillance System for Inner Mongolia record a brucellosis prevalence of 29.2% among pastoral shepherds (Zhang *et al*., 2010). In this area, the rise of cases of human brucellosis over the past decade has been attributed to increases in the number of inexperienced workers in the agricultural sector in recent times. Similarly, (Bonfoh *et al*., 2012) found a representative human seroprevalence of 8%, which was statistically related to the seroprevalence of sheep brucellosis but not to that of goat or cattle brucellosis.

2.3.3 Epidemiology of brucellosis in Latin America

In Latin America, Peru, Mexico and Argentina are the main countries affected by brucellosis (Arambulo, 1998). Some 565 human cases were reported in Argentina in 1996, and the main regions affected were those with large rural goat populations. Human brucellosis due to *B. melitensis* also occurs in Bolivia, Brazil, Colombia, Costa Rica and the Dominican Republic (Benkirane, 2006).

Latin America is a very diverse area, with large differences in agriculture, climate, industry and livestock among its many countries. Despite routine vaccination measures in large

scale cattle operations, the disease is still present at low endemic levels throughout this region, a situation mainly attributed to remote small-ruminant herders (Samartino, 2002).

2.3.4 Epidemiology of brucellosis Sub-Saharan Africa

In global terms, the majority of human and animal brucellosis is found in Sub-Saharan Africa. With large pastoral communities, and the demand for meat and livestock products expected to double by 2050, brucellosis poses a major threat to this region and serious control efforts must be developed (Mangen *et al*., 2002.The prevalence of the infection has been recorded at herd level, within-herd level and individual animal level. Some measurements of human brucellosis have also been made (Unger, 2002).

In summary, persistent disease was observed in most countries in the Sahel, with Ethiopia, Chad, Tanzania, Nigeria, Uganda, Kenya, Zimbabwe and Somalia reporting brucellosis in humans attributed to domestic cattle, camels, goats and sheep (Mangen *et al*., 2002). A seroprevalence of 16.2% (a range of 10.2%-25.7%, with a 95% confidence interval) within cattle in sub-Saharan Africa was estimated, although this figure incorporates pastoral as well as other intensive, cattle-production systems (Mangen *et al*., 2002).

2.3.5 Situation of bovine brucellosis in Nigeria

The earliest occurrence of bovine brucellosis in Nigeria was contained in the Annual Report of the Veterinary Department for the year 1927 when ten cases of contagious abortion were reported. Earshaw and O'Brien (1926) reported cases of hygroma in the knee joint in indigenous cattle. Hall in 1934 diagnosed contagious bovine abortion at a government cattle ranch, where subsequently serological test revealed a positive reactor

rate of about 15% and at the same time field reports showed a low incidence of the disease in nomadic cattle. Records showed that from 1934 to 1944 there was no reference to animal brucellosis in the Veterinary Department Reports. From 1945 onward, brucellosis had been less regularly reported from Government livestock centres in Northern Nigeria, where the diseases became enzootic, on account of overcrowding.

Incidence of the diseases in the nomadic and semi nomadic herds which comprise over 90% of the cattle population that were mostly in the hands of the Fulani herdsmen was not determined (Banerjee and Bhatty, 1970). In the late 1940's despite the adoption of "Test and Slaughter" policy, the disease in most government herds was said to be on the increase as the Annual Report of the Veterinary Department of Nigeria for the years, 1946, 1947, 1948 indicated reactor rates of 9.2% (2,272 cattle), 30% (983 cattle) and 11.8% (1,735 cattle) respectively and three virulent *Brucella abortus* strains were isolated from cattle in Nigeria (Mettam, 1948).

The control of brucellosis in cattle by *B. abortus* Strain 19 vaccination recommended in 1946 was enforced with the production of the lypholised *B. abortus* S19 vaccine (Birkett, 1950) and this combined with culling of reactors proved effective in the disease control as the prevalence in the livestock farms had fallen to less than 5% (Banerjee and Bhatty, 1970).

In the nomadic and semi-nomadic Fulani cattle herds, though the existence of bovine brucellosis had long been recognized as indicated in the Annual Report of the Veterinary Department of Nigeria for the year 1934 (Anon, 1934), the disease had never been regarded

as a real public health or economic hazard. Wilson in 1955 reported an abortion rate of 20-24% in the local herds in Adamawa province. Though the exact cause of the abortion storms remained obscure (Wilson, 1955), subsequent serological tests carried out in the affected herds revealed the presence of 'a significant number of positive reactors' in Bauchi, Mambilla Plateau and Maiduguri (Macgregor; 1960; 1963; 1964). Brucellosis was confirmed serologically in the local herds with history of clinical abortion, joint lesions and infertility problems.

Within the past decades, observations on nomadic herds of cattle in the Northern states of Nigeria, and on many Government owned settled cattle herds in different parts of the country, have revealed that bovine brucellosis is widespread in Nigeria, with a pattern of low and high infection rates in specific areas (Esuruoso, 1965; Adams and McKay, 1966; Banerjee and Bhatty, 1970; Esuruoso and Hill, 1971; Esuruoso and Van Blake, 1972; Esuruoso, 1974a; Esuruoso and Ayanwale, 1980; Nuru and Dennis, 1975; Chukwu, 1987a; Ajogi, 1997). The infection rate was as high as 60% among breeding cows and heifers investigated in western states of Nigeria (Esuruoso, 1979).

Epidemiological investigation of bovine brucellosis in western Nigeria also revealed high rates of infertility and abortion among cattle under different management systems (nomadic or settled herds). An example is on a farm estate where abortion rate of 42% was reported among pregnant cows and serological evidence of bovine brucellosis remained at 50% (Esuruoso, 1974b).

In eastern Nigeria, (Chukwu, 1987) recorded a prevalence of 8.14% bovine brucellosis among slaughter cattle in Enugu and Nsukka.

In the Northern States of Nigeria, the infection rates from various herds have been generally low between 0-20 %. Extensive serological survey by Banerjee and Bhatty (1960) and Nuru and Dennis (1975) revealed prevalence of 8.06% and 7.1% respectively in cattle herds. A prevalence of 0.4% was recorded by Nuru, (1982) in slaughtered cattle in North Central States. Among the reasons given for the difference in the infection rates between cattle in the two areas of the country were that the cattle in nomadic herds in the North were constantly moving and therefore not likely to accumulate infection or spread it to each other as effectively as settled herds in the West and secondly in the intense Savannah heat of the North probably impose natural limit on the rate *B*. abortus infection in cattle (Alausa, 1983).

Trade cattle from the Northern states and those from across Nigerian borders from the Republics of Chad and Niger showed serological evidence of infection (Esuruoso, 1974b). The high prevalence of brucellosis among range cattle in many states of Nigeria can cause abortion and can affect fertility rate resulting to heavy economic loss to the livestock industry in the country (Alausa, 1983).

The present status of bovine brucellosis in Nigeria including its prevalence and isolation have been reviewed and brought into proper perspective (Halle and Ajogi, 1997; Nuru and Schnurrenberger, 1975; Eze 1977; Falade, 2002; Ocholi *et al*., 2004; Cadmus *et al*., 2006; Junaidu et *al*., 2006, Mai *et al*., 2012; Farouk *et al*., 2013; Bertu, 2014).

Reports of brucellosis from 1980 to date indicated that *Brucella* has been isolated from different livestock species in Nigeria (Bale and Kumi-Diaka, 1981; Ocholi *et al.*, 2005; Bertu, 2014), goats (Falade, 1981) and horse (Ocholi *et al.*, 2004, Bale and Kwanashie, 1985). From the studies, *B. abortus* biovars 1 and 3 were identified as the predominant strains (Ocholi *et al.*, 2004, Bertu, 2014). So far, of the different biotypes of *B. abortus* recognized, biotypes 1, 2, 3 and 4 have been isolated in Nigeria (Eze, 1978; Bale and Kumi-Diaka, 1981; Ocholi *et al.*, 2004, 2005; Bertu, 2014).

Proposals for a practical control approach for herds under intensive and extensive husbandry system (Rikin, 1988a) and the benefit-cost analysis for a brucellosis control programme in Nigeria has been presented (Rikin, 1988b). There is no established control programme in the country against the disease. Although efforts to determine the prevalence rate have been made by Rikin (1988a), reported cases bear little relation to the actual prevalence and incidence of the infection in the country (Alausa, 1983).

A review of the 127 accessible publications on brucellosis in Nigeria (Ducrotoy *et al.*, 2014) revealed only scanty and fragmented evidence on its spatial and temporal distribution in different epidemiological contexts. The few bacteriological studies conducted demonstrate the existence of *Brucella abortus* in cattle and sheep, but evidence for *B. melitensis* in small ruminants is dated and unclear. The bulk of the evidence consists of seroprevalence studies, but test standardization and validation are not always adequately described, and misinterpretations exist with regard to sensitivity and/or specificity and ability to identify the infecting *Brucella* species. Despite this, early studies suggest that

although brucellosis was endemic in extensive nomadic systems, seroprevalence was low, and brucellosis was not perceived as a real burden. Recent studies, however, may reflect a changing trend. Concerning human brucellosis, no studies have identified the *Brucella* species and most reports provide only serological evidence of contact with *Brucella* in the classical risk groups; some suggest brucellosis misdiagnoses as malaria or other febrile conditions.

2.4 Virulence and Pathogenicity

Brucella lacks well-known bacterial virulence factors such as cytolysins, capsules, exotoxins, secreted proteases, fimbriae, phage-encoded toxins, and virulence plasmids (DelVecchio *et al*., 2002; Paulsen *et al*., 2002).

Brucella can invade phagocytic macrophages and non-phagocytic epithelial cells (HeLa cells) *in vivo* and *in vitro* (Ko and Splitter, 2003; Kohler *et al*., 2003). *Brucella* virulence relies on the ability to survive and replicate in the vacuolar phagocytic compartments of macrophages.

Many *Brucella* virulence factors, such as lipopolysaccharide (LPS), type IV secretion system (T4SS);, and the BvrR/BvrS two-component system have been identified to be critical in the intracellular survival of *Brucella* inside macrophages (Guzman-Verri *et al*., 2002 ; Lapaque *et al*., 2005; Xiang *et al*., 2006; de Jong *et al*., 2008).

While these virulence factors may not directly mediate clinical manifestations of brucellosis, they are critical for *Brucella* to survive and replicate inside host cells. While

prolonged persistence of the brucellae in macrophages leads to the chronic infection, extensive replication of the bacteria in placental trophoblasts results in acute reproductive tract pathology and abortion in natural hosts (Roop *et al*., 2009).

Specifically, *Brucella* lifecycle contains two phases viz chronic infection of phagocytic macrophage leading to *Brucella* survival and replication, and acute infection of non-phagocytic epithelial cells leading to reproductive tract pathology and abortion (Moreno and Gorvel, 2004). The spleen and liver are the organs that contain many bacterial cells after *Brucella* invasion. After a majority of *Brucella* cells are killed *in vivo*, the remaining *Brucella* cells will persist and live for a long time *in vivo* (Hort *et al*., 2003).

Brucella invades hosts primarily by adhering and penetrating mucosal epithelium surfaces. Similar to the kinetic profile of *Brucella* inside macrophages, virulent *Brucella* have an initial adaption period followed by a replicative phase inside epithelial cells. Using cDNA microarray analysis, (Kohler *et al*., 2003) characterized the transcriptional profile of the intracellular pathogen *B. melitensis* following infection of HeLa cells. The study found that 161 and 115 *Brucella* genes were differentially expressed respectively, post-infection. Most of the genes expressed were involved in pathogen growth and metabolism. At the adaptation period, 126 (78% of 161) genes were down-regulated. At the replicative phase, 86 (75% of 115) genes were up-regulated.

2.5 Immunity and immune mechanisms

Although antibodies specific for the O-antigen (O polysaccharide or O-side chain) of the lipopolysaccharide can confer partial protection in some host species, cell-mediated

immunity (CMI) plays a critical role in protection against virulent *Brucella* infection (Macedo *et al*., 2008). The maturation and proinflammatory production of cytokines of dendritic cells is critical for controlling *Brucella* infections (Macedo *et al*., 2008). Recently it was found that *B. abortus* vaccine strain RB51 and *B. suis* vaccine candidate VTRS1 induce caspase-2-mediated apoptotic and necrotic macrophage cell death (Chen and He, 2009; Chen *et al*., 2011). The programmed cell death is inhibited by virulent *Brucella* strains. Caspase-2-mediated cell death induced by vaccine strain RB51 may promote an effective *Brucella* antigen presentation by a cross-priming mechanism (Bevan, 2006; Chen and He, 2009).

Passive transfer assays with mice suggest that both $CD4^+$ and $CD8^+$ T cells are important in protective immunity against brucellosis (Araya *et al*., 1989). To confer protection against *B. abortus* infection, immune $CD4^+$ T cells secrete many cytokines, including gamma interferon (IFN-γ) that stimulates the antimicrobial activity of macrophages (Jiang and Baldwin, 1993; Zhan and Cheers, 1993; He *et al*., 2001). A crucial role of IFN-γ in the resistance to *Brucella* infection was demonstrated in mice by *in vivo* antibody neutralization experiments and an IFN-γ knockout mouse study (Zhan and Cheers, 1993) $CD8^+$ cytotoxic T lymphocytes (CTL) are critical in killing *Brucella*-infected target cells (He *et al*., 2001).

Naturally infected animals and those vaccinated as adults with *B. abortus*, strain 19 remain positive to the serum and other agglutination tests for long periods. The antibody response to *Brucella* consists of an early lgA and lgM isotype response, the timing of which depends on the route of exposure, the dose of bacteria and the health status of the animal (Godfroid

et al., 2004a) The lgM response is followed shortly by production of lgG_1 antibody and later by lgG_2 (Nielsen, 2002).

The total concentration of lgG_2 increases with age. Most cross-reacting antibody, resulting from exposure to microorganisms other than *Brucella* specie consist of lgM, making serological tests which measure lgM not specific as false positive results occur, leading to low assay specificity (Saegerman *et al*., 2004). In the case of *Brucella* infection, the concentration of antibody, *Brucella* total lgG_1 increases with the level of antigen exposure. Therefore the monitoring of lgG_1 and lgG_2 *Brucella* antibody levels is relevant for detection of *Brucella*-infected cattle (Saegerman *et al*., 2004).

2.6 Pathogenesis

Application of novel techniques is being used to help unravel details of the host–microbial interactions for brucellae. As yet the details of how these facultative intracellular pathogens interact with the host cells remain poorly understood (Moreno and Moriyon, 2002). Most work has addressed the survival of *Brucella* within the macrophage where these bacteria can infect, persist and replicate for prolonged periods within their host. However, it must be remembered that these microbes have the ability to infect epithelial cell lines, professional and nonprofessional phagocytic cells, respiratory tissue, neurons and both male and female reproductive tissues to name but a few (Moreno and Gorvel, 2004). This extreme diversity of host–microbial interactions remains to be investigated.

Smooth brucellae gain entry into the host cells following interaction with cell surface microdomains known as lipid rafts (Watarai *et al.,* 2002). These lipid rafts contain

significant amounts of glycosylphosphatidylinositol anchored proteins, glycosphingolipids and cholesterol and are believed to play a significant role not only for internalization, but also for intracellular replication of brucellae (Roop *et al*., 2009). Investigation of the cellular interactions following internalization within macrophages has demonstrated that brucellae reside within the acidified phagosome (Rittig *et al.,* 2001). Indeed, the acidification of this environment is an important stimulus for expression of virulence genes of the *virB* operon, essential for expression of components needed for assembly of a type IV secretion system required for intracellular trafficking (Rouot *et al.,* 2003).

Once inside the host cell phagosome, and after being subjected to the initial oxidative burst, the internalized brucellae commence their subversion of the host phagosome through mechanisms as yet not fully elucidated. What is known is that the products of *vir*B operon interact with the endoplasmic reticulum in a way that the pH becomes neutral, nitrate ions may be utilized for anaerobic respiration and the brucellae undergo regulated multiplication (Celli *et al.,* 2005). This modified phagosome has been termed as the 'brucellosome' and as yet is poorly understood, however, cellular interaction through the type IV secretion system and host endoplasmic reticulum play a crucial role (Kohler *et al.,* 2003). One outcome of this cellular subversion is prevention of lysosomal fusion. The brucellae survive within this environment for prolonged periods, but must endure conditions of nutrient depletion and limited available oxygen (Moreno and Gorvel, 2004).

Interaction with placental trophoblasts suggests that the ability to acquire iron is vital as the brucellae enter their acute replicative stage with placental disruption resulting in foetal loss or birth of weak and infected offspring. It is interesting to note that iron acquisition is

altered in the attenuated vaccine strain of *B. melitensis*, Rev1 when compared with the virulent strain, 16M (Eschenbrenner *et al.*, 2002). Erythritol is believed to be important for determining tissue tropism for *B. abortus*. Indeed, *Brucella* uses this sugar alcohol in preference over alternatives and mutants unable to utilize erythritol are severely attenuated in ruminant hosts.

In vitro data suggests that *Brucella* metabolizing erythritol have a heightened requirement for iron, scavenged through siderophores such as 2,3-dihydroxybenzoic acid or brucebactin (Gonzalez Carrero *et al.*, 2002; Parent *et al.*, 2002). This may be linked with the requirement for effective iron acquisition for virulence in ruminant hosts. Novel iron regulatory systems have recently been proposed in other α-proteobacteriae, namely *Rhizobium*, but the role of these if any, among the closely related brucellae remains to be addressed (Chao *et al.*, 2004; Platero *et al.*, 2004; Yeoman *et al.*, 2004).

2.7 Pathology

Irrespective of the route of infection, the organism provokes a regional lymphadenitis which is characterized by reticuloendothelial cell and lymphoid hyperplasia, as well as infiltration of large numbers of mononuclear cells and some neutrophils, and few eosinophils and plasma cells (Moreno and Gorvel, 2004). Other lymph nodes in the body and the spleen may be affected later in the course of the infection but to a lesser degree (Bishop *et al*., 1994). There is considerable variation in the severity of the uterine lesions at abortion. As the disease progresses, lesions advance from acute to chronic endometritis (Roop *et al*., 2009).

Microscopically, the endometrium is infiltrated by lymphocytes and plasma cells and some neutrophils (Radostits *et al*., 2007). Microgranulomas may be scattered in the endometrium. The chorion is not uniformly affected and large parts may appear quite normal. The lesions in and at the periphery of the cotyledons, as well as those in the intercotyledonary area vary in extent, appearing to be most severe adjacent to cotyledons (Radostits *et al*., 2007). The affected cotyledons, or parts of them, are covered by sticky, odorless, brownish exudates, and are yellowish-grey as a result of necrosis. Parts of the intercotyledonary placenta are thickened, oedematous, and yellow-grey and may contain exudates on the surface. The udder in infected ruminants does not show any gross lesions, although supramammary lymph nodes may be somewhat enlarged (Godfroid *et al*., 2004a; b).

Some aborted foetuses have varying degrees of subcutaneous oedema and blood-tinged fluid in the thoracic and abdominal cavities, while the abomasal content is sometimes turbid, bright yellow and flaky (Nielsen and Duncan, 1990). Microscopically, most aborted foetuses reveal a multifocal bronchopneumonia, small foci of necrosis or microgranulomas in the liver, which may also occur in the lymph nodes, spleen and kidneys. In most aborted foetuses it is not possible, or it is very difficult, to demonstrate the organisms in tissue sections, notwithstanding that they may have been specifically stained for brucellae (Godfroid *et al*., 2004a).

In bulls, acute to chronic, uni- or bilateral orchitis, epididymitis, and seminal vesiculitis occasionally occur (Radostits *et al*., 2000). Orchitis is characterized by multifocal or diffuse necrosis of the testicular parenchyma, and a focal necrotizing epididymitis may occur. The scrotal circumference in these animals may be normal or severely increased (Bishop *et al*.,

1994). Strain 19 vaccine may also cause orchitis. Uni- or bilateral hygromas, especially of the carpal joints and other bursae, may be evident in some animals in chronically infected herds, or may occasionally follow inoculation of heifers with Strain 19 vaccine (Radostits *et al*., 2007). A progressive, erosive, non-suppurative arthritis of the stilfle joints has been reported in young cattle from brucellosis-free herds that were vaccinated with Strain 19 vaccines (Godfroid *et al*., 2004a).

2.8 Diagnosis

All abortions in cattle should be treated as suspicious for brucellosis and should be investigated, as the clinical signs are not pathognomonic. Diagnostic tests for brucellosis are sub-divided into three groups namely tests for the detection of *Brucella*, tests to detect immunoglobulins and those dependent on allergic reactions to *Brucella* (Alton *et al*., 1988: Saegerman *et al*., 1999; OIE Manual, 2004). There is no single available test that completely covers all requirements for diagnosing brucellosis. The ideal diagnostic test should detect infection early during the long and variable incubation period, not be influenced by the presence of non-specific antibodies, detect carriers, and differentiate between responses to vaccination and those to field infection (Alton *et al*., 1988; Nielsen, 2002).

2.8.1 Samples for the diagnosis of bovine brucellosis

The specimens of choice include; foetal membranes, lung, stomach content, liver and spleen of aborted foetuses and full-term calves and from live cows, uterine discharge, milk or colostrums. Other specimens which may be submitted include supramammary lymph nodes, retropharyngeal, mandibular, iliac, or pre-scapular and parotid lymph nodes, udder

tissue, fluid aspirated from hygromas, male sex glands, testes and semen or seminal plasma and external inguinal lymph nodes of infected bulls (Alton *et al*., 1998; OIE Manual, 2004).

2.8.2 Tests for the detection of *Brucella*

Because of variable incubation periods and the often subclinical nature of the disease in most animals, a definitive diagnosis should be based on isolation and identification of *Brucella* (Alton *et al*., 1988; OIE Manual, 2004). The isolation and characterization of the disease-causing agent by bacteriological methods is still considered the 'gold standard' for the diagnosis of brucellosis (Bricker, 2002). However, the method also has drawbacks. Due to the biological properties of *Brucella*, the cultural methods are time-consuming and when the level of infection is low they may fail (Gallien *et al*., 1998; Fekete *et al*., 1992). Thus, highly sensitive diagnostic tests for detection of *Brucella* based on the polymerase chain reaction (PCR) have been developed and reported and were reviewed by Bricker (2002).

2.8.3 Direct microscopic examination

Using Stamp's modification of Ziehl-Neelsen stain, *Brucella* colonies appear as red stained coccobacilli in smears (Stamp *et al*., 1950) While with Gram stain they appear as faintly sand appearance (Gram, 1884). However, other organisms such as *Coxiella burnetii*, *Chlamydophila abortus* and *Nocardia* species are also acid-fast.

2.8.4 Isolation

Brucella grows on serum dextrose agar, but growth from primary isolation is seldom clearly visible before 48 hours of incubation at which stage colonies are usually 0.5 to

1.0 mm in diameter (Alton *et al*., 1988). The use of selective media such as Farrell's medium (Brucella agar supplemented with 5% horse serum, and cocktail of 6 antibiotics) will substantially enhance the chances of isolation by inhibiting the growth of contaminants, although the growth of *Brucella* may be markedly slower Alton *et al*., 1996).For this reason the cultures should be incubated for five days or longer before being discarded as negative. Most wild strains are fastidious and slow-growing, and require supplementary carbon dioxide (5-10%) for primary isolation at an optimal growth temperature of 36-38^{O}C (Alton *et al*., 1988; Quinn *et al*., 1994).

2.8.5 Biochemical tests

For routine identification, a combination of growth characteristics, colonial and cellular morphology, staining properties, agglutinating antiserum and biochemical reactions allow an accurate identification (Quinn *et al*., 1994). *Brucella* is non-motile, catalase positive, and oxidase positive, give a rapid urease activity (except some *Brucella*, such as *B*. melitensis strains), reduce nitrate and are indole negative.

2.8.6 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is highly sensitive, very specific, rapid, and easily adapted to high volume demand diagnostic tool to detect slow growing bacteria including *Brucella* (Bricker, 2002). It can detect a few bacteria in a sample and is as sensitive as classical culture-based techniques (Bricker, 2003). Furthermore, it is possible to detect dead bacteria reducing the necessity of careful sample conservation before analysis (Ouahrani-Bettache *et al.,* 1996). As long as careful attention is given to avoid contamination in the

laboratory, the method is very reliable and usually highly reproducible at any properly equipped laboratory (Bricker, 2003).

Numerous PCR based assays for *Brucella* have been developed and published. The assays were designed to exploit a single unique genetic locus that was highly conserved in *Brucella* targeting BCSP31 or the 16S rRNA genes (Herman and De Ridder, 1992; Bricker, 2003). The advantage of these assays is that they tend to have a simple design and are very robust (Bricker, 2002). They are useful for screening or for identification when species or biovar designations are not critical (Bricker, 2002).

In order to differentiate *Brucella* species and/or biovars several PCR assays were developed (Bricker, 1994). These assays are directed toward genetic loci that are variable among species and/or biovars. Such targets are uncommon in *Brucella* since the genus is remarkably homogeneous and has been proposed to be a single species, while some large deletions and rearrangements have been reported within a specie or biovar. Most genetic differences consist of single nucleotide polymorphisms (Bricker, 2002). Differential PCR-based assays are particularly useful for epidemiological trace back, or for species-specific eradication programmes.

The different PCR assays that have been developed can be categorized as genus-specific and species-specific. Strategically, for the species-specific tests three approaches were reported (Bricker, 2002).

1. Assays designed with highly specific primers and stringent assay conditions.
2. Assays designed with semi-specific primers and mildly permissive assay conditions.

3. Assay based on amplification with random primers under very permissive conditions.

Brucella share a high degree of DNA homology (>90% for all species) on the basis of a DNA hybridization, thus *Brucella* was proposed as a monospecific genus (Verger *et al.*, 1987).The publishing of a complete genomic sequence of *Brucella melitensis* (Delvecchio *et al*., 2002), *Brucella canis* (Paulsen *et al*., 2002) and *Brucella abortus* (Sanchez *et al.*, 2001) have made it possible to search for regions of DNA sequence variability that made it useful as markers for identification and differentiation.

One of the genetic targets frequently used for strain identification and strain phylogeny is the rRNA operon, particularly the 16S rRNA gene (Weisburg *et al.*, 1991). These genes are highly conserved and diverge very slowly (Weisburg *et al.*, 1991). The DNA sequences from separate species within a genus will differ by only a few per cent. Sequence identity among 16S rRNA sequences is typically interpreted as indicating a single specie. The presence of the mobile genetic element IS*711* also known as IS*6501* (Halling and Young, 1994) has been a useful target for molecular characterization of classical *Brucella* species and biovars (Bricker and Halling, 1994) based on the number and distribution of IS*711* copies within the bacterial genomes.

Several techniques have been employed to find DNA polymorphism which enabled the molecular typing of *Brucella* species and their biovars. These include entero bacterial repetitive intergenic consensus (ERIC) sequence-PCR, repetitive intergenic palindromic (REP) (Mercier *et al*., 1996; Tcherneva *et al*., 2000), randomly amplified polymorphic

DNA-PCR or arbitrary primed-PCR (Fekete *et al.*, 1992; Tcherneve *et al.*, 2000) and pulse-field gel electrophoresis (PFGE).

Polymerase Chain Reaction has been a useful tool in combination with labelled probes to differentiate some *Brucella* species and biovars (Grimmont *et al*., 1992; Bricker and Halling 1995; Quahrani-Bettache *et al*., 1996; Fitch *et al*., 1996). The development of the technique known as Hyper-variable Octameric Oligonucleotide Finger prints (HOOF) by (Bricker, 2002) that exploits the occurrence of variable number tandem repeats differentiates among *Brucella* species, among previously characterized strains and among unrelated field isolates that could not be differentiated by classical methods.

The PCR, including the real-time format, provides an additional means of detection and identification of *Brucella* species (Bricker, 2002; Ocampo *et al*., 2005; Hinic *et al*., 2008). Despite the high degree of DNA homology within the genus *Brucella*, several molecular methods, including PCR restriction fragment length polymorphism (RFLP) and Southern blot, have been developed that allow, to a certain extent, differentiation between *Brucella* species and some of their biovars. Pulse-field gel electrophoresis has been developed that allows the differentiation of several *Brucella* species (Jensen *et al*., 1990; Michaux *et al*., 1997).

Brucella biotyping and distinguishing vaccine strains by PCR can be accomplished satisfactorily but there has been limited validation of the PCR for primary diagnosis. The first species-specific multiplex PCR assay for the differentiation of *Brucella* was described by Bricker and Halling (1994). The assay, named AMOS-PCR, was based on the

polymorphism arising from species-specific localization of the insertion sequence IS*711* in the *Brucella* chromosome, and comprised five Oligonucleotides primers that can identify *B. abortus*, biovars 1, 2 and 4 but could not identify *B. abortus* biovars 3, 5, 6 and 9. Modifications to the assay have been introduced over time to improve performance, and additional strain-specific primers were incorporated for identification of the *B. abortus* vaccine strains, and other biovars and species (Bricker and Halling 1994; 1995; (Bricker *et al*., 2003; Ocampo *et al*., 2005).

A new multiplex PCR assay (Bruce-ladder) has been proposed for rapid and simple one-step identification of all extant species of *Brucella* and vaccine strains (Garcia *et al*., 2006). The major advantage of this assay over previously described PCR is that it can identify and differentiate in a single step most *Brucella* species as well as the vaccine strains *B. abortus* S19, *B. abortus* RB51 and *Brucella, melitensis* Rev.1(Bricker and Halling, 1995). In contrast to other PCRs, Bruce-ladder is able to detect also DNA from *B. neotomae, Brucella pinnipedialis* and *B. ceti*. In addition, *Brucella abortus* biovars (Asarta, 1989; Bonet-Maury *et al*., 1954; Bosseray 1992; 1993; Bricker, 2002) and *B. suis* biovars (Angus and Barton 1984; Asarta, 1989; Ashford *et al*., 2004) can be identified by this new multiplex PCR. The only minor inconvenience of the Bruce-ladder is that some *Brucella canis* strains may be identified erroneously as *Brucella suis* (Macmillan and Cockrem, 1995)

2.8.7 Serological tests

Serological tests are widely used for detection of infected animals. However, no serological test is appropriate in each and every epidemiological situation. Consideration should

therefore be given to all factors that impact on the relevance of the test method and test results for a specific diagnostic interpretation or application (Alton *et al*., 1988). Most of the serological tests, particularly those using whole-cell suspensions as antigen, such as the serum agglutination test (SAT), Rose Bengal test (RBPT), complement fixation test (CFT), most enzyme-linked immunosorbent assays (ELISA) and milk ring test (MRT) have been developed against the O-polysaccharide (OPS) moiety of the smooth lipopolysacchartide (SLPS) (Nielsen, 2002).

2.8.7.1 Enzyme Linked Immunosorbent Assay

The utilization of enzymes as signal amplifiers in primary binding assays led to the production of enzyme immunoassays pioneered by Engvall and Perhman (1971; 1972). The original enzyme linked immunosorbent assay (ELISA) now represents only one of diverse group of assays collectively known as Enzyme Immunoassay (EIA) techniques. These assays give objective results and are extremely sensitive, reagents present less health hazards and they are stable and have long shelf-lives. Moreover the estimation of the results can either be visual or be made with rather simple spectrophotometer of the type found in most laboratories and are suitable for large application (Voller, *et al*., 1976; Nielsen and Duncan, 1990). This makes EIA an attractive replacement to Fluoroimmunoassay (FIA) and Radio immunoassay (RIA).

The ELISA has gained wide acceptance for serological diagnosis of bovine brucellosis because of its ability to detect antibody of all types. This is exemplified by the large number of reports of its use in brucellosis serology (Carlsson *et al*., 1976; Douglas and Kennedy, 1981). This allows its comparison with conventional tests (Van Aert *et al*., 1984).

It has been used in large-scale eradication of bovine brucellosis (Southerland, 1984), but it has two limitations for use directly related to smooth lipopolysaccharide (SLPS antigen). Cattle that are vaccinated with attenuated *B. abortus* strain 19 have residual antibody specific for SLPS which cannot be distinguished from that resulting from field strain infection (Anon, 1971). The second is that the SLPS of a bacteria such as *Yersinia enterocolitica* (Munoz *et al*., 2005; Nielsen *et al*., 2002), *Vibrio cholera*, *Escherichia coli* 0151, 0116 and *Pseudomonas maltophilia* (Nielsen and Duncan, 1990) and *Salmonella*, Kauffman-White group N (Corbel, 1997) cross-react with *B. abortus*.

The competitive ELISA (cELISA), was developed to increase specificity (Nielsen, (2002: Macmillan, 1990; Gall and Nielsen, 1994), by selecting a suitable monoclonal antibody to compete with antibody present in test serum, reactivity resulting from vaccine or cross-reacting antigens is virtually eliminated (Nielsen, 2002).

2.8.7.2 Rose Bengal Plate test (RBPT)

The test is highly sensitive for individual diagnosis, especially in cattle vaccinated with Strain 19 (Alton *et al*., 1988; Nielsen, 2002; OIE Manual, 2004) and can be performed in the field. The antigen stained with Rose Bengal stain, is buffered at a pH of 3.65 (Alton *et al*., 1988). At this level of activity, non-specific agglutinins are destroyed and lgG1 which is the most abundant antibody in the serum of infected animals agglutinates strongly (Alton *et al*., 1988; OIE Manual, 2004). This test is prescribed by the OIE for international trade in cattle.

The RBPT test has been described as a highly sensitive test that detects IgG antibodies. The test also reacts with antibodies of some microorganisms other than *Brucella* and also residual antibodies in animals vaccinated with strain 19 which would produce false positive reactions leading to false positive results.(Alton *et al*., 1975).

Blasco *et al*., (1994) described the use of Modified Rose Bengal Plate test in diagnosing *B. melitensis* infection in sheep. Modification of the test was done by increasing the volume of the serum by 3 folds (75 µl serum; 25 µl antigen). This was shown to increase the sensitivity of the test which could detect 96.6% of animals infected with *B. melitensis* biovar 3 and 92% of those infected with biovar 1

2.8.7.3 Serum Agglutination Test (SAT)

The SAT while not recognized as a prescribed or alternative test (OIE, 2004), has been used with success for many years in surveillance and control programmess for bovine brucellosis (OIE, 2004). Its specificity is significantly improved with the addition of EDTA to the antigen. The antigen represents a bacterial suspension in phenol saline (Nacl 0.85% w/v) and phenol (0.5% v/v). Antigen is delivered in concentrated state provided the dilution factor to be used is indicated on the bottle label.

The SAT, test is also a sensitive test that can quantify the level of antibody titre in serum, but it also produces false positive results due to non specific agglutinins (Alton *et al*., 1996). The test also produces false negative results with sera from some animals from which *Brucella abortus* can be isolated (Alton *et al*., 1988)

2.8.7.4 Complement Fixation Test (CFT)

The complement fixation test is very specific and sensitive and is regarded throughout the world as being the confirmatory test of choice for serological detection of infected animals (Blasco *et al*., 1994). According to Radostits *et al*., (1994a, b), the CFT rarely exhibits non-specific reaction and is useful in differentiating the titres of calf hood vaccination from those due to infection. The reactions to the CFT recede sooner than those to the serum agglutination test after calf hood vaccination with the Strain 19 vaccine. The test has been modified, standardized and adapted to microtitre systems (Alton *et al*., 1988). Results are expressed in International Units (IU) and a cut-off point of 20 IU has been defined, which is rigorously applied where Strain 19 vaccine has not been used for several years (Alton *et al*., 1988). The CFT has been a valuable asset in the control and eradication programmes as a confirmatory test and it is recommended by the OIE Manual as a test prescribed for international trade.

The CFT test is a reliable confirmatory test that gives a very good correlation between infection and positive reaction than any other agglutination test (Morgan, 1967). The test is extremely useful and reliable for the diagnosis of brucellosis in humans and animals most especially where the results of SAT and RBPT are either negative or inconclusive (Morgan, 1967). The test can also distinguish antibody due to natural infection and vaccination ((Nielsen, 2002). .

2.8.7.5 Milk Ring Test (MRT)

The test is used to detect antibodies in milk. The development of a positive reaction is dependent on two reactions namely; (a) aggregation or fat globules in the milk and (b)

stained *Brucella* cells (antigen) which are added to the milk, and are agglutinated by the *Brucella* antibody-fat globule complexes which rise to form a colored cream layer at the top. This is a screening test used on bulk milk to detect infected animals on a herd basis or to monitor clean herds. Herds for which the MRT is positive should be examined by serological tests to identify the infected animals (Alton *et al*., 1988). While it is a relatively insensitive test subject to wrong interpretation caused by various milk conditions such as mastitis, colostrums and milk at the end of the lactating cycle, it is recommended by the OIE as a screening test for bovine brucellosis.

2.8.7.6 Whey Agglutination Test (WAT)

Traum and Maderious in 1947 first reported the test and is indicated as an adjunct to other tests (MRT, MRT dilution, culture) or where for instance in cases of mastitis where it is not possible to run MRT. The test is less affected than the MRT by non-specific factors but is less sensitive.

2.8.8 Test to demonstrate an allergic reaction to *Brucella*

The brucellin skin test is an alternative immunological test which can be used to screen unvaccinated herds, provided that a purified, free, smooth LPS antigen is used Saegerman *et al*., 1999. This test has a very high specificity, such that serologically negative animals that are positive reactors to the brucellin test should be regarded as infected animals (Bercovich, 1998; Saegerman *et al*., 1999; Godfroid *et al*., 2004b). Results of this test may also aid the interpretation of serological reactions thought to be due to infection with cross-reacting bacteria, especially in brucellosis-free areas (Godfroid *et al*., 2004a). Although the brucellin intradermal test is one of the most specific tests for brucellosis (in non-vaccinated

animals), diagnosis should not be made solely on the basis of positive intradermal reactions given by animals in the herds, but should be supported by a reliable serological test (Alton *et al*., 1988).. The allergic test should be used as a flock or herd test rather than as a test for individual animals (Alton *et al*., 1988).

2.9 Molecular Epidemiology

2.9.1 Multi-locus variable–number repeat analysis

A highly discriminatory method for the genotyping of *Brucella* known as multiple-locus variable-number repeat analysis (MLVA) has become available (Le Flèche *et al*., 2006). This method makes use of various loci on the *Brucella* genome that are composed of repeats of short nucleotide sequences. These tandem-repeat units tend to occur in various numbers, and various alleles can be observed in different species and isolates. The recently published MLVA-16 assay, developed for the genotyping of *Brucella*, makes use of eight minisatellite loci for species identification, supplemented with a selection of eight more polymorphic microsatellite loci for the further characterization and differentiation of isolates (Al Dahouk *et al*., 2007; Le Flèche *et al*., 2006). Whereas the MLVA-16 assay can be used for the biovar classification of *B. abortus* and *B. suis*, no correlation between biovar and genotype has been observed for *B. melitensis* (Al Dahouk *et al*., 2007; Le Flèche *et al*., 2006).

The MLVA-16 typing of animal and human *Brucella* isolates has revealed that clusters of individual genotypes within species may show a distinct geographic distribution. For instance, human isolates of *B. melitensis* from Europe and North Africa can be divided according to their geographic origin into a West and an East Mediterranean cluster (Le

Flèche *et al*., 2006).Within the West Mediterranean cluster (which includes isolates from France, Switzerland, Tunisia, and Algeria), a clearly separate cluster originating from Italy can be identified (Al Dahouk *et al*., 2007). Genotypes are relatively stable, and isolates with identical MLVA patterns have been obtained from the same geographic area during a time span of almost three decades (Al Dahouk *et al*., 2007). A considerable number of distinct *B. melitensis* genotypes have already been identified (Al Dahouk *et al*., 2007). The MLVA typing additionally has some practical clinical applications, such as tracing sources of infections and discriminating relapse from re-infection (Al Dahouk *et al*., 2007; Marianelli *et al*., 2007; Smits *et al*., 2009).

2.9.2 Outer membrane protein typing

This typing approach has been used successfully for differentiation of microbial groups. The target genes with a high degree of diversity are digestion with restriction endonucleases to produce restriction fragment length polymorphism (RFLP) profiles for differentiation based on polymorphic alleles.

Several *Brucella omp* genes were considered as potential targets for the typing schemes. However, the *omp*2 locus [*omp*2a and *omp*2b] (Vizcaino *et al*., 2001), 36-kDa porin proteins (Vizcaino *et al*.,1997) show greatest polymorphism among the brucellae. Consequently, these could be utilized to differentiate brucellae. Vizcaino *et al*., 2004).Some additional value can be gained by including the more homogeneous *omp*25 gene (Cloeckaert *et al.*, 1996; Vizcaino *et al.* 2004). A further *omp* gene, *omp*31, is less useful as it is absent from all *B. abortus* biovars (Vizcaino *et al.*, 2001). However, polymorphism at

this *omp31* locus successfully enabled differentiation of *B. canis* from *B. suis* (Vizcaino *et al.*, 1997, 2004).

This method can differentiate isolates into nomenspecies, and in some cases, to biovar (Xiang *et al*., 2006). However, resolution provided by this method was not generally sufficient to reliably identify biovars (Cutler *et al.*, 2005). Furthermore, this assay is not entirely *Brucella*-specific, with *Ochrobactrum* generating a product with the *omp*25 PCR, albeit of smaller size. This method has proved itself of value for characterization of novel isolates recovered from marine mammals (Cloeckaert *et al.*, 2001; Vizcaino *et al.*, 2004). Typically, classical biotyping is unable to differentiate these isolates, though clear clustering is observed with the *omp* profiles of these strains.

2.9.3 IS711 Typing

Chromosomal indexing of DNA polymorphisms can be achieved through use of the variable number and location of copies of a mobile insertion sequence believed to be specific for *Brucella*. Genomic DNA is digested with restriction endonucleases such as *Eco*R1, and resulting fragments probed with labelled IS*711*. This insertion sequence is present in all strains of brucellae tested to date, with copy number varying from four or five to more than 20 copies seen among different nomenspecies and isolates of *Brucella*, whilst being absent from closely related bacterial species. To this end, IS*711* provides a specific target able to generate data on DNA polymorphisms among the brucellae (Cloeckaert *et al.*, 1993; 1995).

As with *omp* typing discussed above, IS*711* profiling of brucellae did not permit resolution of *Brucella* strains into the same groups as classical phenotype identification, although both methods, particularly when used in conjunction, are valuable tools for use in epidemiological studies (Cutler *et al.*, 2005). It could be argued that the IS*711* typing provides a more in depth method of indexing isolates as data is produced from the whole chromosome rather than a targeted gene. Although the lack of complete resolution may reflect biological reality it emphasizes the need for continued research and development into new epidemiological methods.

2.9.4 Amplified fragment length polymorphism

Amplified fragment length polymorphism (AFLP) has recently been heralded as a useful technique to differentiate numerous bacterial species. This technique provides a valuable method to index variation over the whole genome (Welsh and McClelland, 1990). The resolving power can be modified by the use of different combinations of enzymes and primers permitting assessment of varying levels of genomic diversity. The AFLP technique is based on the amplification of subsets of genomic restriction fragments using PCR. The DNA is cut with restriction enzymes and double stranded adaptors ligated to the ends of DNA fragments to generate template DNA for PCR amplification (Williams *et al*., 1990). The sequence of the adaptors and adjacent restriction site serve as primer binding sites for subsequent amplification of the restriction fragments. Selective nucleotides can be included at the 3′-ends of the PCR primers, which therefore prime DNA synthesis from only a subset of the restriction sites (Nadeau *et al*., 1992). Labelling of one of the primers with a fluorescent dye permits visualization of a banding pattern following electrophoresis on an acrylamide gel.

This method clearly identified isolates as *Brucella* and, with some minor exceptions, produced adequate resolving power to designate isolates to one of the currently recognized nomenspecies (Whatmore *et al.* 2005). On occasions, isolates could be assigned to particular biovars or groups of biovars. Unfortunately, resolution proved inadequate for epidemiological tracing purposes (Whatmore *et al.*, 2005). Although AFLP data may be of limited epidemiological significance, it should provide useful information on the taxonomy of *Brucella* and the relationship among the various nomenspecies and biovars.

2.9.5 Pulsed-Field gel electrophoresis

The PFGE may be used for genotyping or genetic fingerprinting Michaux *et al*., 1997). It is commonly considered a gold standard in epidemiological studies of pathogenic organisms. Subtyping has made it easier to discriminate among strains of *Listeria monocytogenes* and thus to link environmental or food isolates with clinical infections (Schwartz, and Cator, 1984).

Pulsed-field gel electrophoresis (PFGE) maps of the classical *Brucella* species genomes are composed of two circular chromosomes of approximately 2.1 and 1.2 Mbp (Michaux *et al*., 1993; 1997), with the exception of *B. suis* biovar 3, which has a single chromosome of 3.1 Mbp.

These PFGE studies revealed other differences, including a 640-kb inversion in the small chromosome of *B. abortus* 544 and a deletion in the small chromosome of *B. ovis*. The two chromosomes of brucellae differ in important ways (Paulsen *et al*., 2002). The origin of replication of the large chromosome (Chr I) is typical of bacterial chromosomes, while that of the small chromosome (Chr II) is plasmid like. Furthermore, most of the essential genes

are located on Chr I. The G+C content of the two chromosomes is nearly identical, consistent with the assertion that the assimilation and stabilization of a plasmid was an ancient event in brucellae.

2.9.6 Multi locus sequence typing (MLST)

The conventional MLST approach uses sequence divergence in housekeeping genes. About seven to nine housekeeping genes are commonly analyzed in order to obtain a reasonable balance between the acceptable identification power, time and cost for the strain typing (Whatmore *et al*., (2007). From each housekeeping gene, approximately 450 to 500 base pairs (bp) are amplified by PCR, followed by DNA sequencing and subsequent comparative sequence analysis.

Each unique sequence is assigned a specific allele number and alleles are combined into an allelic profile and further assigned to a specific sequence type (ST). New alleles result in a new combination and therefore in a novel sequence type. Because accumulated changes occur slowly and are regarded as selectively neutral, the MLST approach is a reliable tool for the overall characterization of microbial populations and the investigation of phylogenetic relationships. However, the slow molecular clock rate and the limited number of genes do not allow in-depth phylogenetic reconstructions and analysis of local epidemiological studies. Thus, unlike MLVA, MLST is of little value for outbreak investigations.

Whatmore *et al*. (2007) developed an MLST assay based on nine different loci. Of the nine loci, seven correspond to classical housekeeping genes. The remaining two loci target the omp25gene and an intergenic region, respectively, resulting in more discriminatory power. Nine loci are sequenced, totaling almost 4,400 bp, or 0.1% of the genome. Owing to clonal evolution and congruence of the phylogeny of individual loci, these data are usually analyzed as MLSA in which the sequence from all nine loci is concatenated.

Whatmore and colleagues investigated 160 *Brucella* isolates belonging to all known species and biovars (Whatmore *et al*., 2007). The assay grouped *B. abortus, B. melitensis, B. ovis* and *B. neotomae* as well-separated clusters according to their species affiliation. *Brucella suis* biovars 1 to 4 also clustered together but demonstrated higher intra-species heterogeneity when compared with the other species. *Brucella suis* biovar 5 formed a separated lineage. The ten *B. ovis* isolates, although from different geographical regions, were represented by a single sequence type.

The *Brucella canis* strains grouped in proximity to *B. suis* biovars 3 and 4 but were distinguishable by a different sequence type. *Brucella* isolates from marine mammals also grouped as a separated cluster. The results of that study confirmed the close proximity of *B. suis* biovars 3 and 4 with *B. canis* and the separate position of *B. suis* biovar 5, already suggested by phenotypic assays and molecular methods. On the basis of these results the authors concluded that this assay can be used to provide a strong phylogenetic backbone for the *Brucella* genus.

2.10 Treatment

Brucellae are facultative intracellular bacteria, relapses after treatment usually appear (FAO, 2010). However, a practical and effective treatment regime could be used to treat valuable breeding animals and would benefit producers in countries which cannot afford to control the disease by the test and slaughter policy (Eaglesome and Garcia, 1992). Reports of unsuccessful attempts to treat bovine brucellosis involved the use of multiple injections of a combination of a long acting tetracycline and streptomycin (Nicoletti, 1980). The inadequacy of antibiotics regiments in controlling *B. abortus* may be due the failure of the antibiotic to penetrate cell wall barriers effectively.

As vaccines do not exist to protect people from *Brucella*, potentially one can take antibiotics as a measure to prevent infection, There is substantial evidence that mouse monoclonal antibodies, directed against the outer membrane proteins (Bowden *et al*., 1995; 2000), LPS (Cloeckaert *et al*., 1992, 1993) and polysaccharide (PS) (Mataraz *et al*., 1986; Phillips *et al*., 1989) of *Brucella* are protective in the mouse model against brucellosis. The latter antibodies provided the best protection against smooth species, to which humans are sensitive (Cloeckaert *et al*., 1991; 2002).

To date no approved antibodies are available for human use, as one cannot predict who will be exposed to *Brucella*, or when, if ever, the use of anti-*Brucella* antibodies to protect the military or civilians is an impractical countermeasure (Nicoletti and Milwars, 1985) It was also found that Brucellin, filter-sterilized culture filtrates of *B. abortus* or *B. melitensis*, reduced the severity and length of illness for patients (Huddleson and Johnson, 1933 cited by John, W.C., 2003). However, the optimum therapy for human brucellosis is still disputed despite extensive studies over the years (Corbel, 1997).

The treatment recommended by the World Health Organization (WHO) for acute brucellosis in adults is a combination of rifampicin and doxycycline daily for a minimum of 6 weeks (WHO, 1986). Several studies (Luzzi, *et al*., 1993; Arinze *et al*., 1992; Montejo *et*

al., 1993), suggested that the treatment with a combination of streptomycin and doxycycline may result in less frequent relapses than treatment with a combination of rifampicin and doxycycline. Infections with complications such as meningoencephalitis and treatment in children may require therapy of rifampicin and trimethoprim/sulphamethazolin (Lubani *et al*., 1989) while endocarditis may be treated with rifampicin, streptomycin and doxycycline for 6 weeks (Chan and Hardiman, 1993).

2.11 Control and prevention of brucellosis

Brucellosis is an infectious disease which has been controlled and eradicated in some countries (Goddfroid *et al*., 2004a). In sub-Saharan Africa, animal health services delivered by the public sector have greatly decreased over the last 20 years due to various factors such as decreasing government budgets, particularly for operational costs of disease control. Thus, programmes that require coordinated surveillance, information exchange and application of control measures are not implemented in many Sub-Saharan countries (McDermott and Arimi, 2002; Smits and Cutler, 2004). The main objectives for control and prevention of brucellosis are centred on the economic impacts of the disease and its public health consequences. Control activities mainly reported by countries include; surveillance of livestock movement, control within and outside herds, vaccination and test and slaughter programmess (Nicoletti, 1980; McDermott and Arimi, 2002; Godfroid *et al*., 2004b).

The elimination of the disease from the population is not the objective of a control, and it is implicit that some "acceptable level" of infection will remain in the population (WHO/ FAO/OIE, 2006). Control programmes have an indefinite duration and will need to be maintained even after the "acceptable level" of infection has been reached, so that the

disease does not re-emerge (WHO/ FAO/OIE, 2006). In many countries, methods for the control of brucellosis are backed by governmental regulations. Certain principles which can be used include;

1. The reduction of exposure to *Brucella* species.
2. Improving management practices in herds

These procedures may be further classified into test and slaughter, hygiene, control of animal movement and vaccination (WHO/FAO/OIE, 2006).

The development of an efficacious vaccine for brucellosis has been a challenge for scientists for many years. Despite the availability of two smooth live vaccine strains, S19 for cattle and Rev1 for small ruminants and a further rough attenuated strain, RB51 for cattle, the search for improved vaccines has continued. This is in part through the remaining virulence of the smooth vaccine strains in human hosts (Schurig *et al.,* 2002), their residual abortifacient potential in pregnant animals (Blasco, 1997) and their interference with conventional serological assays (Schurig *et al.,* 2002). In addition, the Rev1 vaccine has suffered from a lack of coordinated standardization in production methods leading to considerable variability in efficiency of different preparations (Blasco, 1997) and carries resistance to streptomycin, an antibiotic that is therapeutically useful in man (Cloeckaert *et al.,* 2002). Some of the advantages have been offered through the availability of the rough vaccine strain, RB51. This is an attenuated rough mutant of *B. abortus* that has provided promising data for vaccination of cattle. However, results from vaccination of wildlife have given less encouraging results with failure to protect species including bison, reindeer or elk (Cutler *et al*., 2005).

It is generally acknowledged that all of the available vaccines are only efficacious in specific hosts, and cross-protection is not readily achieved. Currently, no vaccine is available for protection of swine from brucellosis. However, this area is being actively addressed (Moriyon *et al.,* 2004). The RB51 vaccine has been assessed in swine, but with mixed results (Lord *et al.,* 1998; Edmonds *et al.,* 2001; Moriyon *et al.,* 2004). Efficacy testing in large, well-controlled groups will be essential in order to evaluate performance of these candidate vaccines in swine (Moriyon *et al.,* 2004). Despite the variable success of the RB51 rough vaccine, it may indeed have a valued role for booster immunization of livestock immunized during calf hood with smooth S19. Use in this manner would provide immunological stimulation, but without concomitant elevation of antibodies specific for the lipopolysaccharide (LPS) diagnostic antigen (Moriyon *et al.,* 2004).

Another area of interest is the potential of 'carrier' organisms to serve as a delivery vehicle for antigens of other microbes. Organisms considered as 'safe' such as *Lactococcus lactis*, have been engineered to encode *Brucella* vaccine candidate genes, with partial success (Pontes *et al.,* 2003). Despite the lack of detectable specific antibody a partial protective effect was demonstrable using this microbial vehicle.

To overcome these criticisms and induce a good Th1 stimulation, an essential prerequisite for immunity against intracellular pathogens, RB51 has been assessed for its ability as a multivalent vaccine controlling not only brucellosis, but also bovine tuberculosis (*Mycobacterium bovis*) and Johne's disease (*Mycobacterium avium paratuberculosis*) (Vemulapalli *et al.,* 2002). However, the less than optimal success of the current RB51 in wildlife would be a cause for concern if this live vaccine was shed for prolonged periods by vaccine recipients (Cook *et al.,* 2002; Elzer *et al.,* 2002).

One potential avenue for development of novel vaccines is 'nonliving vaccines'. Historically, successful vaccines against intracellular pathogens such as *Brucella* have consisted of live attenuated strains of the organism (Ko and Splitter, 2003). Although the protective response generated by live vaccination is both efficacious and sustained, there is a trade-off to be made in terms of bacterial persistence within target host and potential transmission to unintended recipients such as pregnant livestock or hosts in which the vaccine strains remain virulent and the possibility of reversion to virulence.

Nonliving brucellosis vaccines have been developed and used in the past, though with mixed results (Ko and Splitter, 2003). Vaccines utilizing killed preparations of *Brucella*, or antigenic fractions, had only limited success compared with the live attenuated strains. Vaccines based upon killed preparations of *Brucella*, have been shown to be protective but a single immunization is often insufficient to generate sustained protective immunity. Additional problems including local reactions at the inoculation site and production of antibodies complicating serodiagnosis, contributed to decline in the use of such vaccines (Schurig *et al.*, 2002).

Recent advances in immunological understanding, particularly regarding adjuvant technology and delivery systems may be able to address some of the problems encountered with the use of nonliving vaccines (Schurig *et al.*, 2002). Novel adjuvant and delivery systems could be utilized to both enhance and focus the immune response against subunit antigens. Use of poly-epsilon-caprolactone microparticles for the delivery of hot saline extracted antigens of *B. ovis* has shown protection against homologous challenge in the

mouse model. However the response was less effective against challenge with smooth strains of *Brucella* (Murillo *et al.,* 2002a; 2002b; 2002c).

DNA vaccination approaches offer the possibility of inducing both cellular and humoral responses (Onate *et al.,* 2003). Approaches have varied from use of a whole library from *B. abortus* (Leclercq and Oliveira, 2003), overcoming the need for prior knowledge and selection of specific antigens to selection of specific candidates and their subsequent evaluation as DNA vaccines against brucellosis. Various candidates have been explored for their value as DNA vaccines against brucellosis providing various levels of protective efficacy in the mouse model (Al-Mariri *et al.,* 2001a; 2001b; Leclerq and Oliveira, 2003; Onate *et al.,* 2003; Munoz *et al.,* 2005). Disadvantages of the DNA vaccination approach are the amount of DNA required to elicit the required response, and the often disappointing results obtained following assessment of the vaccines in the target animal (Babiuk *et al.,* 2003).

The availability of the genome data and the use of genomic approaches to identify potential vaccine candidate antigens, together with the expanding knowledge of the protective immune response may yet provide an efficacious non-living vaccine. Whether this type of vaccination approach could be cost-effective to deliver benefit where the disease has greatest impact, either following humanitarian upheavals or in areas of extreme poverty, remains an open question.

(Rikin, 1988a) proposed the control of brucellosis under an extensive and intensive management systems in Nigeria as follows; For a country desiring to control and eventually

eradicate the disease among cattle, the programme must be designed in agreement with the socio-cultural, economic, biological as well as political conditions prevailing in the country(Rikin,1988b Under the Nigerian condition, many special circumstances would have to be considered namely;

1. Restriction of cattle movement both within and from the neighbouring countries.
2. Recognize the symbiotic relationship between the nomadic system of cattle rearing, implementation and of course, the cooperation of herdsmen through:
    a) Settling the Nomads - Provide them with ready sources of water, adequate pastures to graze, elimination of cattle taxation and provision of quarantine areas.
    b) Education - Deployment of educators along their migration routes to indoctrinate them about modern method of management.
    c) Publicity - Promote public awareness through posters, information leaflets and films in languages understood by the nomads.
    d) Incentives - Free treatment of diseased cattle and distribution of supplementary feeds at subsidized rate as incentives.

Test all animals, quarantine and. remove reactors. Vaccinate all animals over three months of age with *B. abortus* strain 19. Remove reactors from herds that show infection rate of up to 20%. Retest every six months and remove additional reactors and vaccinate all animals above three months of age.. Increase the pace of testing, using the rapid plate agglutination test and confirm the positives with the card or equivalent test. Follow retesting schedule starting 120 days post-vaccination until the last reactor is removed Declare the farm brucellosis free after two to three tests have no reactors. The farm must then be kept under surveillance with milk ring test at quarterly intervals.

Test-and-slaughter policy with compensation should be implemented. Hygienic measures must be improved around grazing and watering areas. Effective prevention of entry at all international borders and at inter-state control posts must be instituted. All new additions must be screened for brucellosis status or vaccination before being allowed into a farm. Prevention of entry at all ports, of imported animals and semen, with the objective of intercepting brucellosis positive exotic cattle must be maintained.

2.12 Future Areas of Research in Brucellosis

2.12.1 Subunit vaccines

Identification of Subunit vaccine will be useful for development of medical counter measures against brucellosis. Description has been given that DNA encoding specific outer membrane proteins of *Brucella* are more effective, and cross-protective to more than one species of *Brucella*, than Omps by themselves (Cassataro *et al*., 2007). Also, a very low amount of single-dose of polysaccharide without adjuvant can give long-term cross-protective immunity against *Brucella spp*. in animal models (Cherwonogrodzky *et al*., 1995; 1996; Lord *et al*., 1998).

In the swine model, oral vaccination was as effective as intra-muscular injections and the polysaccharide thermostable vaccine did not require cold chain shipment (Lord *et al*., 1998). With regards to new subunit vaccine candidates, it was found that when either the supernatant or the cells are treated with hot (70^0C) phenol-water, both PS and an equivalent amount of glycosylated proteins (about two-thirds carbohydrate, one-third protein) were extracted. The latter fraction was found just as effective as polysaccharide for protecting mice (Cherwonogrodzky *et al*., 1996). Perhaps these glycosylated proteins are the best of both candidates, combining protective proteins and protective polysaccharide of *Brucella*.

2.12.2 Immunomodulators

Brucella polysaccharide either that of unusual composition such as the 4,6-dideoxy-4-formamido-D-mannose composition or that of unusual linkage such as the cyclic 1,2-beta-glucan, could double mouse macrophage activity against yeast zymosan (Castro *et al*.,

2007). Cyclic 1,2 beta glucan is both a common bacterial polysaccharide used for osmotic regulation and a virulence factor for some bacteria such as *Brucella* (Bundle *et al*., 1988; de Iannino *et al*., 2000; Chanput *et al*., 2012).The beta-glucans induces innate immunity in part by stimulating cytokines (Lashkarbolouki *et al*., 2008; Noss *et al*., 2013;) which in turn play a role in clearance of this bacterium (Vitas *et al*., 1996). In situations Where *Brucella* has commandeered the mammalian cell, it may be possible to override the cell's incapacitation with potent immune stimulating compounds (Vitas *et al*., 1996).

2.12.3 Serum components other than antibodies

The body's defenses are far more diverse and robust than serum components. Although *Brucella* appear to have evolved a resistance to serum cationic peptides called defensins (Gazi *et al*., 2011), with its polysaccharide coating composed of mannose derivatives, the bacterium has been found to be sensitive to the mannose binding lectin in the blood which also activates complement lysis (Morgan and Poland, 2011). On a similar theme, although the mechanism has been resolved with a fungal model, when the macrophage has been exposed to mannose polymers, the receptor lectin-1 can induce the macrophage mannose receptor to be shed, bind to the pathogen and enhance its destruction (Fox *et al*., 1993). These serum components should be investigated further to determine if they play a role in long term immunity against *Brucella.*

2.12.4 Antibody therapy

There have been different descriptions and categories of anti-*Brucella* antibodies. One antibody group reported was Type 1 in that it bound to the length of the polysaccharide and was produced in high titres in infected animals (Rubio *et al*., 2002). The other group was Type 2 which bound to the tip of the polysaccharide and was produced in vaccinated animals (Cherwonogrodzky *et al*., 1990; Monreal *et al*., 2003; Rubio *et al*., 2002). It is unclear whether agglutinating/non-protective and

non-agglutinating at neutral pH protective antibodies are Type 1 and Type 2 antibodies respectively (Cherwonogrodzky *et al*., 1990; Hoffman and Houle, 1995; Joller *et al*., 2010; Wang *et al*., 2011). The latter may have protective or therapeutic value as medical counter measures.

2.12.5 Liposome encapsulated antibiotics and vaccines

Once the bacterium has invaded the cell and tissues, it is difficult to clear (Paulsen *et al*., 2002) In order to deliver the antibiotic in high concentration directly into the cell, liposome encapsulation has been evaluated (Castro *et al*., 2007). It was found that positively charged pluri-lamellar vesicles were the most effective for delivery of antibiotics into mouse monocytes (Singha *et al*., 2011). The usefulness of liposomes has been extended for enhanced delivery of antigens and DNA vaccines (Ireland *et al*., 2010; Cherwonogrodzky and Nielsen, 1988).

2.12.6 Cross-reactions and cross-protection

Recently it was found that vaccination with *B. suis* 145 PS protected mice from *B. suis*, *B. melitensis* and *B. abortus* (Hur *et al*., 2011). During the course of testing this vaccine candidate in mice, it was noticed that a few of the unvaccinated control mice acted as vaccinates, having 1000-fold less bacteria in their spleens than their littermates after *B. suis* challenge (Hur *et al*., 2011). It was likely that the unvaccinated but protected mice had been inadvertently exposed to the latter. Regardless, if surrogate markers of protection from brucellosis could be determined, it would be a very useful area that can be explored in future research.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study Area

Jigawa State lies between latitude $10^0$57N and 13^0 03N and longitude $8^0$08E and $10^0$37E and covers a total land area of about 22,410 sq.km. The State has 27 Local Government Areas (LGAs). It also has three senatorial zones namely North Central, North East and North West (Fig 3.1). The State was estimated to have a cattle population of about 1.2 million heads, with an International cattle market situated at Maigatari local Government area (Livestock Committee Report, 2013).

The climate is semi-arid, characterized by a long dry season and variable rainy season which vary considerably over the years and are erratic. The mean annual temperature is about 25^0C in the coolest month and 39^0C in the hottest month.

3.2 Research Materials

Materials used for the research include, Syringe (20mills) 18G hypodermic needle, Swabs, Universal sample bottles without anticoagulant, Ice box, Ice pack, Swab sticks, Rose Bengal antigen, Serum Agglutination antigen, Milk Ring antigen, Casein enzyme, Normal saline, Phenol saline, Test tubes, Racks, Ceramic tile, Microtitre Pipette, Microtitre plates, Veronal buffered diluents, Stock solution of sodium chloride (42.5g), barbituric acid (2.875g), sodium diethyl barbiturate (1.875g), magnesium sulphate (1.018g), and calcium

chloride (0.147g), 1 litre of distilled water, 0.04% gelatine solution, 3% suspension of fresh sheep red blood cells (SRBC) sensitized with an equal volume of rabbit anti-SRBC incubator, plastic culture plates, blood agar base media, modified tryptone soya broth, 1% peptone, 5% horse serum, 1% dextrose and ready mixed antibiotic supplement at the following concentrations; bacitracin (25 iu), polymyxin B (5 iu), cycloheximide (100 µg), nalidixic acid (5 µg), nystatin (100 iu) and vancomycin (20 µg), Gas pack, Inoculating loop, Stomacher, Basic Fuchsin and Thionin dyes, Mono specific anti-sera A and M Christensens urea media, Serum dextrose agar, Control strains, Crystal violet, Lugols iodine, Acetone, acetic acid, microscope slides, Microscope, and Oil immersion

3.3 Sample Size Determination

In order to calculate the required number of herds to be sampled for this study, for estimating the prevalence of *B. abortus* infected herds, an expected herd prevalence (P_{exp}) of 40% desired absolute precision (d) of 5 % and a confidence interval of 95% was applied using the formula $n = 1.96^2 P_{exp} (1- P_{exp})/d^2$ (Thrusfield, 1995).

This equals = $\frac{1.96 \text{ X } 1.96 \text{ X } 0.4 \text{X } 0.6}{0.01}$ = 92 herds

Using the multistage cluster sampling, the design effect (D) of the survey was calculated using the formula D = 1 + (b-1) *roh* (Bennett *et al*., 1991) where b is the average number of samples per cluster and *roh* is the rate of homogeneity equivalent to the intra-cluster correlation coefficient (*P*) in single stage cluster sampling. It was decided to sample 7 herds per local government area (b = 7). An intra-cluster coefficient of p = 0.09 was reported for *B. abortus* in cattle (Otte and Gumm, 1997). A high value of 0.09 was used for the *roh*.

The design effect was calculated to be D = 1+ (7-1) X 0.09 = 1.54

The number of herds resulted to 92.19 × 1.54 = 141.97 herds and in the course of the survey 147 herds were eventually sampled. Pregnancy wastage samples were also collected from two abattoirs and a slaughter slab from the three Senatorial Zones.

3.4 Study Design

An assumption was made that 50% of the cattle from the 27 Local Governments could be infected with brucellosis since there was no reported prevalence from the Local Governments Areas. The cattle population in the LGAs range from 21000-45000 heads (LCR, 2013). A total of 21 LGAs were selected out of 27 in the State by simple random method (Micheal, 2005) using balloting. Furthermore using the multi-stage sampling, wards were randomly selected from each selected Local Government Areas. The LGAs have an average of 10-11 wards, where 7 wards were selected from an LGA. A herd with at least 10 matured animals was selected per ward from a sampling frame compiled by the Zonal Veterinary Officers from the 3 Zones. Animals sampled from each herd included breeding bulls, matured females, heifers and calves of greater than six months of age. Pregnant cows and heifers, cows with observed brucellosis clinical signs or previous history of abortion, in the last 3 month before sample collection were also sampled.

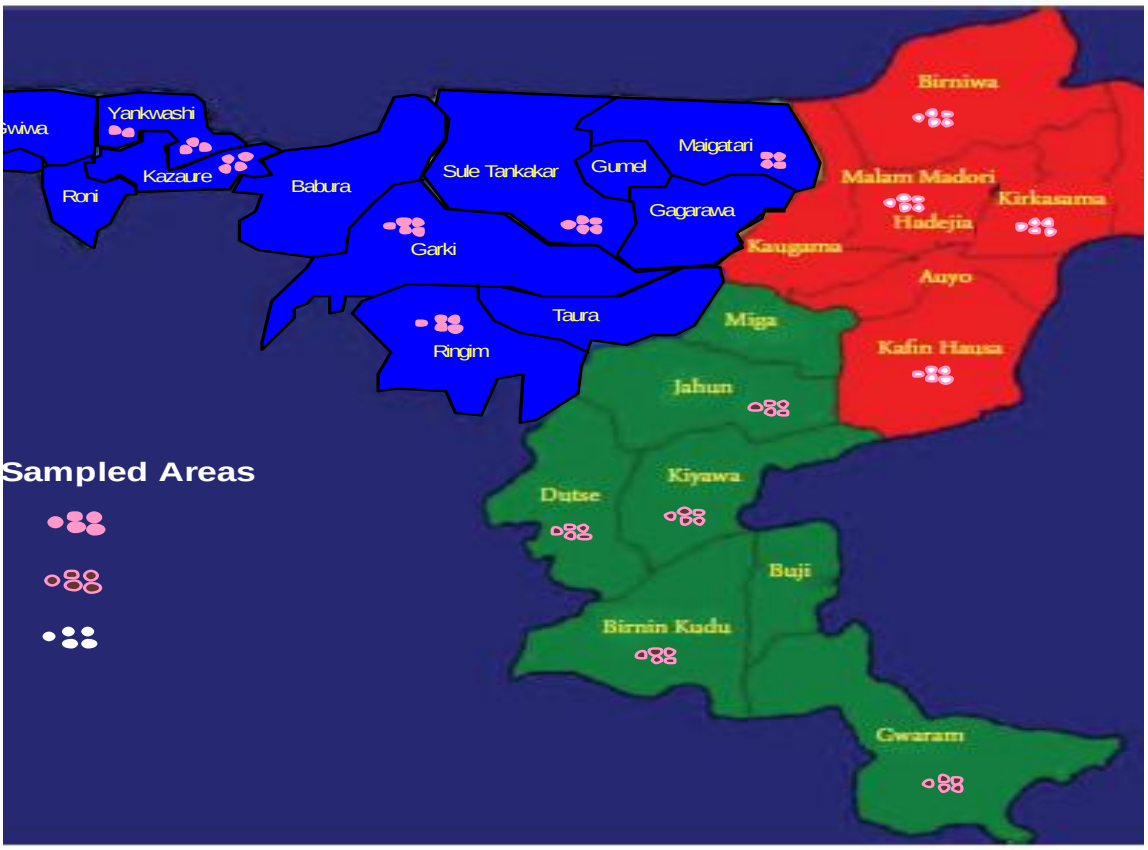

Fig 3.1: Map of Jigawa State showing 27 local government areas, 3 Zones and sampling locations.

Source; www.Jigawa-states.blogspot.com)

Key

- **Northeast Senatorial Zone**
- **Northwest Senatorial Zone**
- **Northcentral Senatorial Zone**

3.5 Sample Collection

3.5.1 Herd samples

Samples collected from the study herds included blood for serum from breeding bulls, dry cows, heifers and calves. Depending on the herd size a range of between 10 -24 animals per herd were bled. Cattle of minimum age of 6 months and above were considered for blood collection in order to minimize maternal antibody from interfering with results of serological tests and the selection of an animal was done systematically as described by Thrusfield, (1995). Animals were restrained with the help of the cattle owners and from each animal, using sterile 10 ml syringe and 18 G needles, 10 millilitres of blood was collected from the jugular vein and was transferred into sterile universal bottles without anticoagulant and kept in slanting position to enable decanting of sera.

Milk samples (20mls) only were collected aseptically from the four quarters of a lactating cow from each selected herd. The milk samples were divided into two aliquots. Half was stored in universal bottles containing 1% peptone while the remaining half was stored in sterile universal bottles. The milk samples were tested using Milk Ring Test. Second sampling was done one year after the first with milk collected from the same herds but different lactating animals from the three Senatorial Zones. The milk samples were tested using Whey Agglutination test. Blood samples for serum only were collected from all lactating cows that tested with either Milk Ring test or Whey Agglutination test in order to check agreement between results of milk and serum.

Swabs from the prepuce and vagina were collected from breeding bulls, pregnant cows and heifers and cows and heifers that had recent abortions that occurred not more than two

months prior to sample collection. Selection of animals for swab collection was done systematically and swab sticks were inserted into the vaginal and prepuce and swabs were taken. All swab samples were stored directly into universal bottles containing 1% peptone water which was used as transport medium.

Foetal membranes were collected using hand gloves from herds where cases of retained placenta were encountered during sampling. The placenta samples were removed using forced extraction and stored in sterile polythene bags and transported on ice to the laboratory for enrichment.

Samples of pregnancy wastage such as stomach contents, liver and spleen were collected from abattoirs and slaughter slabs when encountered during visitation that was done once in two weeks. About 2 grams each of liver and spleen samples were cut and collected fresh in sterile containers and transported on ice to the laboratory for further processing. Stomach content samples was collected using sterile syringe and needle where 5 millilitres of the contents was aspirated and transported on ice to the laboratory.

3.6 Serological Tests Procedure

The sera were screened for *Brucella* antibodies using Rose Bengal Plate test (RBPT), Serum Agglutination Test (SAT) and Complement Fixation Test (CFT).The RBPT and SAT were performed at the Microbiology Laboratory at National Animal Production Research Institute, Shika while the CFT was performed at the *Brucella* Reference Laboratory in Zaragoza, Spain, courtesy of Dr Clara M. Marin.

The RBPT was carried out according to the standard methods of Alton *et al.* (1975 and as described by Blasco *et al.*(1994) and OIE, (2004).

3.6.1 Procedure for Rose Bengal Plate Test

1. One drop of (30 µl) of serum was placed on a test area of clean glass plate.
2. One drop or 30 µl of Rose Bengal *Brucella abortus* stained antigen was also placed adjacent to the test serum.
3. The serum and the antigen were mixed gently with a tooth pick
4. The glass plate was then rocked slowly to and fro for four minutes.
5. Any degree of agglutination was observed in the wet state after 4 minutes

Control - Known positive and negative sera were used as controls.

The antigen and the controls were sourced from Central Veterinary Laboratory Weybridge United Kingdom.

The results of the RBPT test were interpreted as either negative (-) or no agglutination and positive (+) for any degree of agglutination.

3.6.2 Procedure for Serum Agglutination Test

The test was carried out in accordance with the OIE Manual (2002) and as described by Bakari (2010). Five tubes were arranged serially per sample.

1. About 0.8 ml of phenol-saline solution was added into the first tube and 0.5 ml was added into the remaining four tubes using a pipette.
2. Similarly, 0.2 ml of the serum to be tested was added into the first tube containing phenol-saline and mixed thoroughly without frothing. About 0.5 ml of the mixed contents in the first tube was carried over to the second tube from which after mixing,

0.5 ml was transferred to the third tube and the process was repeated up to the 5^{th} tube from which after mixing 0. 5ml of the serum dilution was discarded.

3. To each tube 0.5 ml of the antigen diluted in 1:10 normal saline was added and mixed thoroughly which resulted to a serum dilution of 1:10 1:20 1:40 1:80 1:160
4. The tubes were then incubated at 37^0C for 24 hours

Results interpretation:

The degree of agglutination was assessed on the amount of clearing that has taken place in the tubes as compared to the set up standard. The tubes were examined without being shaken, against a black background with a source of light coming from above and behind the tubes. Sera with serum agglutination of a dilution of 1:40 (50 international units) and above were considered as positive reactor samples (Alton *et al*., 1975).

3.6.3 Procedure for Complement Fixation Test

The test was carried out in accordance with the OIE Manual (2004) using standard 96-well microtitre plates with round (U) bottoms. The serum, antigen and complement were incubated at 37^0C for 30 minutes.

Test method

1. The serum samples were diluted in ratio 1:5 with Veronal Buffered Diluent (VBD) and the complement was inactivated in a water bath at 57^0C for 30 minutes.
2. Using standard 96-well U-bottom microtitre plates, volumes of 50 µl of the diluted sample was added to wells in column 1 and column 11.
3. 25 µl of VBD was added to all wells of column 2 to 10 and column 12.
4. 25 µl of the sample was transferred from column 1 to 2 until column 10 from which 25 µl was removed and discarded.

5. 25 µl of the sample was transferred from column 11 to 12 and 25 µl was removed from column 12.
6. The antigen was diluted 1:32 in VBD.
7. 25 µl of diluted antigen was added to all wells of column 1 to 10.
8. 25 µl of VBD was added to all wells of column 11 and 12.
9. The complement was diluted in ratio 1:40 in VBD.
10. 50 µl of diluted complement was added to each well of the microtitre plate.
11. The plates were incubated for 60 minutes at 37^0C in a humid chamber.
12. 50 µl of the indicator system was added to each well of the microtitre plate.
13. Control wells containing diluents only, complement and diluents, antigen and complement plus diluents were set up to contain 75 µl volumes in each case.
14. The plate were closed with adhesive tape and the results read after the plates have been left incubated in a water bath at 37^0C for 30 minutes

Interpretation of CFT results

The results were expressed in international CFT units (icftu) and sera giving positive fixation at a titre equivalent to 20 iu or greater were considered to be positive. The test was examined visually: sedimentation of the sensitized RBC = Positive result. Complete lysis of the sensitized RBC = Negative result.

3.6.4 Procedure for Milk Ring Test

The milk ring test was carried out as described by Hamilton and Hardy (1950) and the results interpreted as per Cruikshank *et al*. (1975).

1. One ml of fresh milk was pipetted into narrow test tubes (Khan tubes)
2. One drop (0.03 ml) of milk ring antigen (Hematoxylin stain) was added.

3. The contents were then mixed gently but properly.

4. The samples were then incubated at 37^0C for one hour.

The colour of cream ring and colour of milk column were observed and interpreted as follows; when the colour of the cream ring is definitely blue and colour of milk column white (+3), definitely blue and slightly white (+2), definitely blue and definitely blue (+), same colour as the milk column and definitely blue (±), white and definitely blue (—).

The milk ring antigen was sourced from Central Veterinary Laboratory Agency Weybridge United Kingdom.

3.6.5 Procedure for Whey Agglutination Test (WAT)

Milk samples that were collected were subjected to whey agglutination test as described by Traum and Maderious (1957).

1. About 1g of casein enzyme was measured and added to each of the 5 ml labelled milk samples
2. The milk samples to which casein enzyme was added were incubated at 37^0C for 1 hour
3. Using a pipette, 1 ml of the clear whey was removed from the defatted milk.
4. 0.2ml of the clear whey from each sample was subjected to Whey tube agglutination test below

Five tubes were arranged serially per sample

1. To the first tube, 0.8 ml of normal-saline solution was added. Furthermore, 0.5 ml of the normal saline was added into each of the remaining four tubes using a micro pipette.

2. 0.2 ml of the whey sample was added to the first tube containing normal-saline and mixed thoroughly without frothing. Half a milliliter of the contents in the first tube was transferred to the second tube from which after mixing the process continued to the 5th tube from which after mixing 0.5 ml of the whey dilution was discarded.
3. To each tube, 0.5 ml of the antigen diluted in 1:10 was added and mixed thoroughly which resulted in whey dilution of 1:10, 1:20, 1:40, 1:80 and 1:160
4. The tubes were then incubated at 37^{0}C for 24 hours

Interpretation of WAT results

The degree of agglutination was assessed on the amount of clearing that toke place in the tubes as compared to the set up standard. The tubes were examined without being shaken against a black background with a source of light coming from above and behind the tubes. Whey showing agglutination at a dilution of 1:40 (50 international units) and above was considered as positive reactor samples (Alton *et al*., 1975).

The SAT antigen was sourced from Central Veterinary Laboratory Agency Weybridge United Kingdom.

Degree of clearance results

Complete agglutination and sedimentation with clear supernatant is recorded as ++++, near complete agglutination and clearing +++, marked agglutination and clearing ++ some sedimentation and clearing +, no clearing as ─

3.7 Culturing Samples for *Brucella* Isolation

3.7.1 Media preparation

Farrells modified medium (Farrell and Robinson, 1972) was prepared by supplementing blood agar base with 5% horse serum, 1% dextrose and ready mixed antibiotic supplement

at the following concentrations; bacitracin (25 iu), polymyxin B (5 iu), cycloheximide (100 µg), nalidixic acid (5 µg), nystatin (100 iu) and vancomycin (20 µg).

Modified Tryptone Soya broth was used to enrich tissue samples and was prepared by mixing 30 g of dehydrated mTSB (Thermo Fisher Scientific) in 1litre of distilled water. It was mixed and sterilized by autoclaving at 121^0C for 15 minutes.

Peptone broth was prepared by dissolving 15g of peptone base media in 1litre of purified water. It was mixed thoroughly making a 1% w/v and autoclaved at 121^0C for 15 minutes. The broth was allowed to cool and I milliliter was dispensed in to each sample bottle intended for collection of swabs, and milk samples.

3.7.2 Culture of Milk, Swabs, Tissue Samples and Stomach Contents

Milk and Swab samples were cultured for *Brucella* isolation at the Bacterial Zoonosis Laboratory in the Department of Veterinary Public Health and Preventive Medicine and Microbiology laboratory at the National Animal Production Research Institute Shika.

About 1 µg of casein enzyme was added to each of the labelled milk samples and incubated for 1 hour. The whey was then removed from each sample and about 20 µl of the whey from each sample was spread and streaked on plate of Farrell modified medium. Furthermore each labelled swab stick was also streaked onto Farrell modified medium. All plates were incubated under microaerophilic condition using gas pack at 37^0C for 3-7 days for *Brucella* like colonies (Alton *et al.*, 1988). Plates were discarded if no growth was evident after 7-10 days of incubation.

About 2 grams each of Liver, spleen and placenta of aborted foetuses collected from the abattoirs and slaughter slab were homogenized using stomacher and loopful were spread on plates of Farrell modified medium and streaked. Using sterile Pasteur pipette, 3 drops of stomach contents from each sample was also spread and streaked on plates of Farrell modified medium. All the plates were incubated under microaerophilic condition using gas pack at 37^0C for 3-7 days for *Brucella* like colonies according toe method of Alton *et al*. (1988). Plates were discarded if no growth was evident after 7-10 days of incubation.

Suspected *Brucella* colonies were purified by sub-culturing on serum dextrose agar and the pure isolates were stained using Gram and Modified Ziehl-Neelsen staining methods and viewed microscopically. The isolates were transported on ice to Aminu Kano Teaching Hospital Kano and preserved on skim milk at -80^0C. They were later transported on ice to National Veterinary Research Institute, Vom, for further biochemical identification and characterization.

3.8 Gram Staining

Suspected colonies were identified according to the method of Gram (1884)

1. A Smear was made on a clean, dried glass slide and fixed over the burnsen flame

2. The smear was then covered with crystal violet stain for 30 seconds and then washed off with water.

3. The smear was then covered with Lugols iodine for 30 seconds and washed off with water

4. Acetone was poured to decolorize iodine (5 seconds) and rapidly washed off

5. The slide was covered with carbol fuchsin for 30 seconds and washed off with water

6. The slide was air dried and observed using microscope under oil immersion lens (x100).

3.9 Modified Ziehl-Neelsen staining

Suspected colonies were identified by Ziehl-Neelsen stain according to the method of Stamp et *al***.** 1950**).**

1. Smears were made on a grease-free, clean, dried glass slide and fixed over the burnsen flame.
2. The smears were flooded with a 1:10 dilution of carbol Fuchsin for 10 minutes.
3. It was washed in tap water.
4. 0.5% acetic acid was added for 30 seconds.
5. It was washed again in water
6. 1% methylene blue was used to counter stain the smear
7. The slide was air dried and observed under oil immersion lens(x100).

3.10 Biochemical Characterization of *Brucella* Isolates

The isolates were taken to National Veterinary Research Institute, Vom where characterization of the isolates was done as described by Alton *et al*. (1988). The following biochemical tests were performed;

3.10.1 Growth in the presence of Carbon dioxide (CO_2)

A suspension of *Brucella* colonies was sub-cultured onto serum dextrose agar plates in duplicate. One plate was incubated in an anaerobic jar containing carbon dioxide generating

pack while the other was incubated aerobically at 37^0C for 24 hours and growth was observed.

310.2 Urea hydrolysis

The Christensen's urea media was used to check for urease activity. A slope of the media was made with addition of 20% urea solution and was inoculated with suspected *Brucella* cultures and incubated at 37^0C for 24 hours. Change of colour of the urea media to purple pink was indicative of urease activity and positive reaction.

3.10.3 Hydrogen sulphide production

A colony of the suspected isolate to be tested was inoculated onto serum dextrose agar slope and a strip of lead acetate paper was placed in the tube in a straight position so that it did not touch the inoculated media in the tube. It was then incubated at 37^0C and observed daily for 24-48 hours for colour change of the lead acetate paper. Blackening of the lead acetate paper at its tip down to its length indicated positive result.

3.10.4 Agglutination with monospecific antisera A and M

A drop of diluted anti-A and anti-M sera were placed on a clean glass slide, a loopful of the suspected *Brucella* culture was emulsified with each of the sera. They were rocked gently for one minute and observed for agglutination. Presence of agglutination with any of the anti-sera indicated positive reaction.

3.10.5 Sensitivity to thionin and basic fuchsin dyes

Thionin and basic fuchsin dyes (stock concentration) were prepared by mixing 1.0gm of the stock solution per litre of deionized water at a pH of 7.5 and temperature of 25^0C.

Three sets of labelled plates containing blood agar base media were prepared and 3 ml, 6 ml and 12 ml of thionine were added to the three media to give three different concentrations of 1:100,000, 1:50,000 and 1:25,000. Two sets of labelled plates containing blood agar base media were also prepared with the addition of 3 ml and 6 ml basic fuchsin dye giving two different concentrations of 1:100,000 and 1: 50,000. The plates were arranged from the highest concentration to the lowest concentration plates for each dye so that inoculation was started from the lowest to the highest.

A suspension of the isolate was made by suspending a loopful of a 24 hour culture in 0.5 ml of saline in a sterile tube and covered with its sterile cover. Using a sterile cotton swab soaked in bacterial suspension a horizontal line was streaked across the media using same side of the swab for the same type of dye but changing to another side for a different dye. Five different control strains (*Brucella abortus* S19, *Brucella melitensis* 16 M, *Brucella suis* 1330, *Brucella ovis* (Bow) and *Brucella melitensis* Rev1) were also inoculated in a similar manner. The plates were incubated at 37^0C in atmosphere of 5-10% carbon dioxide and another set of plates of the same dyes concentration. Growth and absence of growth on any of the dye media at any concentration was observed. Absence of growth on any of the dye media at any concentration was considered a negative reaction while the presence of growth was regarded as positive reaction.

3.10.6 Biotyping of *Brucella* isolates

Biotyping of isolates was carried out using Tbilisi reference phage at the National Veterinary Research Institute Vom. A suspension of the isolates and the control (*B. abortus* strain 19) organisms was made using McFarland standard. A looful of each organism was placed onto each of the two nickel-sized areas of a fresh dry *Brucella* agar. The inoculated areas were allowed to dry and one loopful of Tbilisi (Tb) phage that was diluted was placed at the center of one spot of inoculums. To the other spot was added the Tb phage at a dilution of 10^4 and the plates were allowed to dry thoroughly, inverted and incubated at 37^0C under microaerophilic condition and examine for plaque formation at 24 and 48 hours interval.

3.11 Questionnaire Analysis

Questionnaires with closed ended questions were administered to a herd owners in all herds where samples were collected. Profile of each herd owner was obtained using the check list on the questionnaire. Information such as owner's name, sex, age, occupation and level of education were obtained. Total of 147 questionnaires were administered. The questionnaires were designed to assess knowledge of herd owners on brucellosis and to also assess exposure factors responsible for introduction, spread and maintenance of brucellosis in the study herds

3.12 Data Analysis

Prevalence *was* calculated at the level of Zones, Herds, Sex, Age and Breed. Individual and herd prevalence were also calculated in order to determine the number of individual

positive reactors among each group of cattle tested and also to see the difference in herd prevalence from the three zones and the overall herd prevalence in the study areas.

$$\text{Individual Animal Prevalence} = \frac{\text{Number of individual positive animals}}{\text{Number of animals tested in the herd}}$$

$$\text{Within Herd Prevalence} = \frac{\text{Number of positive reactors}}{\text{Number of serum samples tested from the herd}}$$

$$\text{Herd Prevalence} = \frac{\text{Number of herds with at least one positive reactor}}{\text{Number of herds tested}}$$

The data were analyzed using Epi Info v 3.2.2, 2000 and Minitab for windows v12, 1998. The data collected from the questionnaire survey were analyzed using descriptive statistics. Association between a potential exposure factor and proportion of positive herds and negative herds was calculated using Chi-square and Fishers exact tests. Odds Ratio (OR) with 95% confidence intervals of Odds ratio was also calculated using a 2 by 2 contingency table.

CHAPTER FOUR

RESULTS

4.1 Samples Collected from the Three Senatorial Zones of Jigawa State Nigeria.

Samples collected include 1867 blood for serum, 1143 milk, 460 vaginal swabs, 325 prepucial swabs,11 samples each of liver spleen and stomach contents and 14 placentae (Table 4.1).

4.2 Prevalence of *Brucella* antibodies in Serum samples from the Three Senatorial Zones of Jigawa State Nigeria based on RBPT, SAT and CFT Tests

In the North Central Zone, the prevalence was found to be 5.61% (35/623) and 4.65% (29/623) with the Rose Bengal and Serum Agglutination Tests respectively, while with the Complement Fixation Test it was 3.69% (23/623). In the North East Zone, the prevalence was 4.66% (29/622) and 4.34% (27/622) using the RBPT and SAT tests while with the CFT test it was 3.69% (23/622). The prevalence in the North West Zone was 4.66% (29/622) and 4.34 % (27/622) with the RBPT and SAT tests while the CFT test confirmed 3.53% (22/622) (Table 4.2).

Table 4.1: Samples collected from the Three Senatorial Zones of Jigawa State Nigeria

Zones	Serum	Milk	Swabs		Tissues			Stomach contents
			Vaginal	Prepucial	Liver	Spleen	Placenta	
Northcentral	623	381	160	115	7	7	8	7
Northeast	622	381	150	105	4	4	5	4
Northwest	622	381	150	105	0	0	1	0
Total	**1,867**	**1143**	**460**	**325**	**11**	**11**	**14**	**11**

Table 4.2: Prevalence of *Brucella* Antibodies in Serum samples from the Three Senatorial Zones of Jigawa State Nigeria based on RBPT, SAT and CFT Tests

Zone	Type of test	Number tested	Number positive	Prevalence (%)
North Central	RBPT		35	5.61
	SAT	623	29	4.65
	CFT		23	3.69
North East`	RBPT		29	4.66
	SAT	622	27	4.34
	CFT		23	3.69
North West	RBPT		29	4.66
	SAT	622	27	4.34
	CFT		22	3.53

4.3 Degree of Agreement between RBPT, SAT and CFT Tests based on Kappa values

Kappa test was used to measure the degree of agreement between the three tests ran in parallel in the study. The results showed there was an almost perfect agreement among RBPT, SAT and CFT tests (Table 4.3).

4.4 Overall Prevalence of *Brucella* Antibodies in Serum samples in Jigawa State based on the Screening tests (RBPT and SAT) and the Confirmatory test (CFT)

The overall prevalence of *Brucella* antibodies in serum in the State showed that 4.98% (93/1867) tested positive with RBPT test, 4.44% (83/1867) with SAT test and 3.64% (68/1867) with CFT test as indicated in Table 4.4.

4.5 Individual Animal Prevalence based on RBPT, SAT and CFT in the North Central Zone

The individual animal prevalence in the North central zone was 16.6% (16/96) in Dutse, 11.1 % in Birnin kudu (14/96), Gwaram 9.8% (9/91), and Buji 8.9% (8/89) based on RBPT. The SAT results was 14.4% (14/96) in Dutse, 13.3% (12/96) in Birnin kudu, while in Gwaram it was 7/91 (7.6%) and Buji 7.8% (7/89). In Dutse the CFT results were 11.1% (14/96) in Birnin kudu 10% (9.96), Gwaram 7.6% (7/91). There were no positive reactors recorded in Kiyawa, Jahun and Miga (Table 4.5).

Table 4.3: Degree of agreement between RBPT SAT and CFT Tests based on Kappa values

Type of test	Number positive	Number negative	Kappa value
RBPT	93	1774	0.932
CFT	52	1815	
SAT	83	1784	
CFT	68	1799	0.846
RBPT	93	1774	
SAT	83	1784	0.827

Kappa Interpretation

0 < = ≡ less than chance agreement

0.01-0.02 ≡ Slight agreement

0.21-0.40 ≡ Fair agreement

0.41-0.60 ≡ Moderate agreement

0.61-0.80 ≡ Substantial agreement

0.81-0.99 ≡ Almost perfect agreement

Table 4.4: Overall Prevalence of *Brucella* Antibodies in Serum samples in Jigawa State based on the Screening tests (RBPT and SAT) and Confirmatory test (CFT).

Type of Test	Overall Number tested	Overall Number Positive	Overall Prevalence
RBPT	1867	93	4.98
SAT		83	4.44
CFT		68	3.64

Table 4.5 Individual Animal Prevalence (IAP) based on RBPT, SAT and CFT in the North Central Senatorial Zone

Herds	No. of Animals tested	RBPT Positive	Individual animal prevalence (%)	SAT Positive	Individual animal prevalence	CFT Positive	Individual animal prevalence
Dutse	96	16	16.6	14	14.5	14	14.5
Kiyawa	82	0	0	0	0	0	0
B/Kudu	96	14	15.5	12	13.3	9	10
Gwaram	91	9	9.8	7	7.6	7	7.6
Jahun	88	0	0	0	0	0	0
Buji	89	8	8.9	7	7.8	7	7.8
Miga	87	0	0	0	0	0	0
Total	**623**	**47**		**40**		**37**	

4.6 Individual Animal Prevalence based on RBPT, SAT and CFT in the North East Senatorial Zone

Individual animal prevalence in Hadejia was 21.6% (21/97), and 20.65% (19/92) in Auyo based on RBPT. The prevalence with SAT was 19.58% (18/97) in Hadejia and 19.56% 181/92) in Auyo. In Hadjia the CFT result was 18.5% (15/97) and 18.47% (15/92) in Auyo. There were no positive reactors in Guri, Kirikasamma, Kaugama and Mallam Maduri (Table 4.6).

4.7 Individual Animal Prevalence based on RBPT, SAT and CFT in the North West Senatorial Zone

Individual animal prevalence in Yankwashi, Kazaure and Maigatari based on RBPT was 14.1% (13/92), 13.3% (12/90) and 10.3% (9/87) respectively, while the prevalence based on SAT test was found to be 14.1% (13/92) in Yankwashi, 12.2% (11/90) Kazaure and 7.04% (7/87) in Maigatari. The CFT results was 10.86% (10/92) in Yankwashi 12.2% (11/90) in Kazaure and 7.04% (7/87) in Maigatari.There were no positive reactors recorded in Garki, Ringim, Taura and Gumel (Table 4.7).

Table 4.6 Individual Animal Prevalence (IAP) based on RBPT, SAT and CFT in the North East Senatorial Zone

Herds	No. of Animals tested	RBPT Positive	Individual animal prevalence (%)	SAT Positive	Individual animal prevalence	CFT Positive	Individual animal prevalence
Hadejia	97	21	21.6	18	19.58	16	18.55
Guri	86	0	0	0	0	0	0
KKM	88	0	0	0	0	0	0
Birniwa	90	0	0	0	0	0	0
Auyo	92	19	20..65	18	19.56	15	18.47
Kaugama	83	0	0	0	0	0	0
M/M	86	0	0	0	0	0	0
Total	**622**	**40**		**36**	**3.4**		**31**

Table 4.7 Individual Animal Prevalence (IAP) based on RBPT, SAT and CFT in the North West Senatorial Zone

Herds	No. of Animals tested	RBPT Positive	Individual animal prevalence (%)	SAT Positive	Individual animal prevalence	CFT Positive	Individual animal prevalence
Yankwashi	92	13	14.1	13	14.1	10	10.86
Kazaure	90	12	13.3	11	12.2	11	12.2
Maigatari	87	9	10.3	7	8.04	7	8.04
Garki	87	0	0	0	0	0	0
Ringim	89	0	0	0	0	0	0
Taura	87	0	0	0	0	0	0
Gumel	90	0	0	0	0	0	0
Total	**622**	**34**		**31**		**28**	

4.8 Within Herd Prevalence (WHP) Based on RBPT, SAT and CFT in the North Central Senatorial Zone

An overall within herd prevalence (WHP) of 71.4% (15/21) based on RBPT was observed in a herd coded as DT1. The WHP was reduced to 57.14% (12/15) by both SAT and CFT tests. It was a semi-intensive herd that rear Friesian, and Sokoto Gudali breeds of cattle which were sourced from Maizube farms in Niger State and also from Takwasa farms in Kazaure Jigawa State. It was one of the herds where abortion occurred and the placenta was retained for more than 48 hours and *Brucella* abortus was isolated from the placenta of the Friesian cow.

In Birnin Kudu a within herd prevalence of 21.4% (3/14) was observed in herd coded BK1 using the RBPT and SAT tests, while with the CFT test the WHP was 14.2% (2/14). Herd BK3 in Birnin Kudu had a prevalence of 40% (10/25) based on RBPT test with the SAT tests the prevalence was 36% (9/25) while with the CFT test it was 28% (7/25) This herd was a transhumance herds that travel to different parts of Nigeria during the dry season period in search of pastures. While in Gwaram a within herd prevalence of between 8.3-25% was recorded. In Buji the WHP ranged between 6.6-29.4%. These herds also migrate as far as Niger State during dry season. No positive reactors were recorded in Jahun, Miga, Kiyawa and Taura (Appendix VII).

4.9 Within Herd Prevalence (WHP) Based On RBPT SAT and CFT in the North East Senatorial Zone of Jigawa State

Herds from this zone showed within herd prevalence (WHP) based on CFT ranging from 0-40%. They usually move to surrounding states like Bauchi and Gombe during the dry season in search of pastures. The highest WHP was observed in a herd coded as HJ1 35% (7/20) based on RBPT test, while with the SAT and CFT tests it was 30% (6/20) respectively. In Auyo the within herd prevalence ranged from 15.3% to 30% based on CFT. Among the herds in Auyo only the herd coded as AU3 migrates as far as Plateau State in search of pastures. There were no positive reactors recorded in Birniwa, Guri, Kirikasamma, Kaugama and Mallam Maduri (Appendix VIII)

4.10 Within Herd Prevalence (WHP) Based on RBPT SAT and CFT in the North West Senatorial Zone

Within herd prevalence in Yankwashi, Kazaure and Maigatari based on RBPT SAT and CFT tests was found to be 6.6% to 35%. The highest WHP was observed in Yankwashi 35% (7/20) in herd coded as YK4 which managed Rahaji and Bunaji breeds of cattle. The second isolate obtained from this study was from a cow in this herd which delivered a weak calf that died a week later. *Brucella abortus* was isolated from the vaginal swab of the Bunaji cow in herd YK4. In Maigatari,a herd coded as MG5 had a WHP of 28.5% (4/14). The herd manages Rahaji cattle which were normally sourced from neighbouring Niger towns or at the International cattle market in Maigatari.There were no positive reactors recorded in Garki, Ringim, Taura and Gumel (Appendix IX).

4.11 Overall Herd Prevalence of *Brucella* Infected Herds in the Three Senatorial Zones of Jigawa State Nigeria

The overall herd prevalence in the North Central Zone was 22.4.3% (11/49) while in the North East Zone 18.36% (9/49) and in the North West Zone the prevalence was 20.4 %, (10/49) as shown in Table 4.8, there were no statistically significant difference in herd prevalence between the North Central, North East and North West ($P > 0.05$). The overall herd prevalence in Jigawa State was 20.4% (30/147).

4.12 Prevalence of *Brucella* Antibodies in Milk in the 3 Senatorial Zones of Jigawa State based on Milk Ring Test (MRT)

The prevalence in the North Central Zone using MRT was 1% (3/300), in the North West Zone 0.6% (2/300). There were no positive reactors in the North East Zone as shown in Table 4.9.

4.13 Prevalence of *Brucella* Antibodies in Milk in the 3 Senatorial Zones of Jigawa State based on Whey Agglutination Test (WAT)

The prevalence in the North Central Zone was 2.12% (2/94), while there were no positive reactors in the North East and North West Zones (Table 4.13).

Table 4.11: Overall Herd Prevalence of *Brucella* Infected Herds based on CFT in the Three Senatorial Zones of Jigawa State Nigeria

Location	Herds tested	Positive herds	Herd prevalence (%)
North central	49	11	22.4
North East	49	9	18.36.
North West	49	10	20.40
Total	**147**	**30**	**20.4**

Table 4.12: Prevalence of *Brucella* Antibodies in Milk in the 3 Senatorial Zones of Jigawa State based on Milk Ring Test (MRT)

Location	Type of Test	Number tested	Number positive	Prevalence (%)
North Central	MRT	290	3	1.03
North East	MRT	290	0	0
North West	MRT	289	2	0.69
Total		**869**	**5**	**0.5**

Table 4.13: Prevalence of *Brucella* Antibodies in Milk in the 3 Senatorial Zones of Jigawa State based on Whey Agglutination Test (WAT)

Location	Type of Test	Number tested	Number positive	Prevalence (%)
North Central	WAT	91	2	2.12
North East	WAT	91	0	0
North West	WAT	92	0	0
Total		**274**	**2**	**0.72**

4.14 Seroprevalence of *Brucella* antibodies in Cattle in Jigawa State Nigeria based on Age, Sex and Breed

Seropositivity among male and female cattle tested in the study indicated that the prevalence was higher among females 3.4% **(**70/2053) than males 0.52% (5/952).

The prevalence among seropositive cattle among the age groups showed that cattle of age 6-12 month recorded a prevalence of 0.21% (1/456) followed by 13-24 months with a prevalence of 1% (7/697).The age group 25-36 month had a prevalence of 2.87% (30/1044) while 37-72 month had a prevalence of 4.55% (37/813). Comparing the age groups 6-12month with 13-24 month, age group 13-24 months with 25-36 month and between age group 25-36 months with 37-72 month no statistical association was observed with prevalence of brucellosis except between age groups 25-36 month ($p< 0.05$).

Breeds seropositivity among the cattle tested in the study indicated that the prevalence among the local breeds was 2.4% (67/2746) while with the foreign breeds the prevalence was 3.2% (8/249), there were no positive reactors recorded among the cross bred.

4.15 Seropositivity among herds with absence or presence of brucellosis clinical signs in the study areas

Seropositivity among herds with absence or presence of brucellosis clinical signs indicated that herds with reported cases of abortion had a prevalence of 14.2% (6/42), while herds with no abortion histories had a prevalence of 22.8% (24/105). Herds that presented clinical signs of retained placenta had a prevalence of 14.2% (2/14), while those that did not have cases of retained placenta had a prevalence of 21.1% (21/133).Herds with weak calves

presented a prevalence of 11.1% (2/18) while those with no weak calves had a prevalence of 21.7% (28/129). Herds that had cases of still birth presented a prevalence of 7.5% (3/40) while those without still birth had a prevalence of 25.2% (27/107).

Table 4.14: Seroprevalence of *Brucella* antibodies in cattle in Jigawa State Nigeria based on Age, Sex and Breed

Variable	Number tested	Number Positive	Number negative	Prevalence (%)	95% CI OR	P- value
Sex						
Male	957	5	952	0.52	0.06-0.38	0.0000041[b]
Female	2053	70	1983	3.40		
Age (months)						
6-12	456	1	455	0.21		
13-24	697	7	690	1.00	0.01-1.74	0.156835[a]
25-36	1044	30	1014	2.87	0.14-0.82	0.013133[b]
37-72	813	37	776	4.55	0.37-1.04	0.72230[b]
Breed						
Local	2746	67	2679	2.4	0.34-1.72	0.5922022[b]
Foreign	249	8	241	3.2		
Cross	15	0	0	0		

[a]Fishers exact test

[b]Chi-square

Table 4.15: Sero-positivity among herds with absence or presence of brucellosis clinical signs in the study areas

Brucellosis clinical signs	Number of Herds tested	Positive herds	Prevalence (%)	Odds ratio	95%CI	P-value
Abortion						
Absent	105	24	22.8			
Present	42	6	14.2	1.78	0.62-5.34	0.3480554[b]
Retained placenta						
Absent	133	28	21.1			
Present	14	2	14.2	1.60	0.31-11.01	0.7355721[a]
Weak calves						
Absent	129	28	21.7	2.22	0.45-14.87	0.3685858[a]
Present	18	2	11.1			
Stillbirth						
Absent	107	27	25.2	4.16	1.10-18.44	0.0320068[b]
Present	40	3	7.5			

[a]Fishers exact test

[b]Chi-square

4.16 Isolation of *Brucella* Species from Milk, Swabs, Tissues and Stomach Contents

Results of *Brucella* isolation from samples cultured yielded one isolate each from placenta and vaginal swab. Thus only two isolates were obtained from two animals in the study areas. The isolate obtained from placenta was from a Friesian cow in a semi-intensive farm in Dutse that managed both local and exotic breeds. History revealed that the animal was sourced from a known intensive farm from Adamawa State and was brought to Duse with pregnancy. The animal had still birth on calving and had placenta retained for more than 48 hours. The second isolate was obtained from a Bunaji cow from an extensive farm in Yankwashi, which managed local breeds only. History also revealed that the cow was sourced from a cattle market in Falgore Kano State. The cow delivered a weak calf which subsequently died after one week.

4.17 Identification of *Brucella* isolates

4.17.1 Gram staining and Modified Ziehl-Neelsen staining

The two suspected *Brucella* isolates appeared faintly stained, coccobacilli on Gram staining while with the Modified Ziehl-Neelsen they appeared as red stained coccobacilli (Plate 4.1).

4.17 .2 Biochemical Tests

The two isolates grew under microaerophilic condition of 5% CO_2. They were urease positive, H_2S positive, sensitive to Thionin and Basic Fuchsin dyes and also agglutinated with Monospecific Antisera A antiserum characteristic of *Brucella abortus* species.

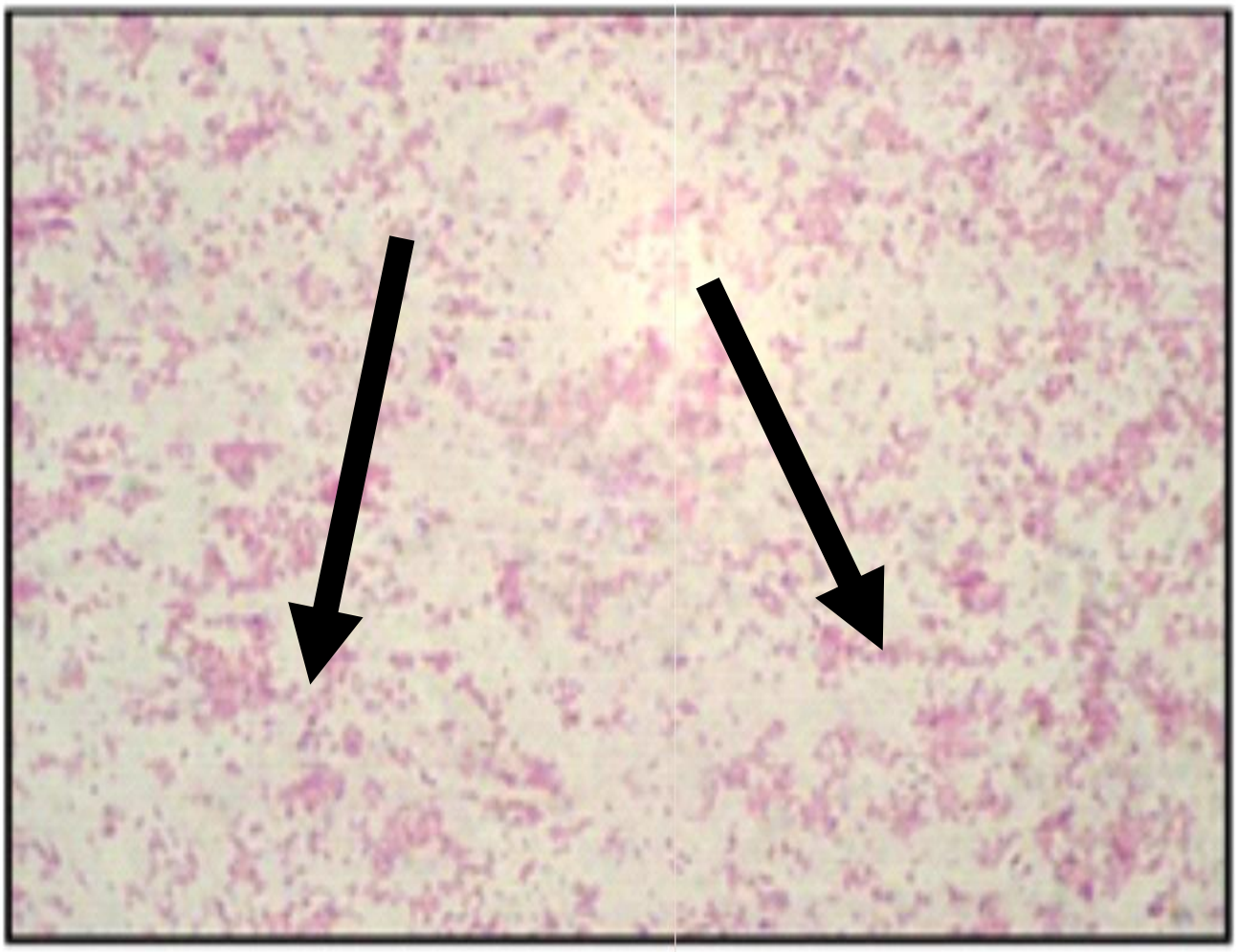

Plate 4.1: Modified Ziehl-Neelsen Staining of *Brucella* isolates (1000x)
Arrows showing colonies of *Brucella*

4.18 Questionnaire analysis

4.18.1 Demography of herd owners

Majority of the herds were managed by adults 98.6 % (145/147) while the remaining 1.4% (2/147) were managed by teenagers. Most of the herds were owned by farmers/pastoralists who constituted 70.06% (103/147), while 10.2% (15/147) of the herds were owned by public servants and 19.72% (29/147) by businessmen. In terms of education, 75.5% (36/147) of the herd owners who responded had informal education while 24.5% (36/147) had formal education (Table 4.18.1)

4.18.2 Herd information

Breed characterization of the herds showed that 76.19% (112/147) kept local breeds only, 1.36% (2/147) managed purely foreign breeds while 22.4% (33/147) kept foreign, locals and their crosses (Table 4.18.2). Information obtained in the study indicated that 23.12 % (34/147) were managed under semi-intensive system, while 76.9% (113/147) were under extensive husbandry system.

Majority of the herds 82.3% (121/147) bred their animals using natural mating system, while 17.68% (26/147) used artificial insemination. Furthermore 79.9% (117/147) sourced their animals from open markets, while 20.41% (30/147) sourced from known farms (Table 4.18.2).

Herd size categories in the study indicated that 7.48% (11/147) were small sized herds (10-30), 78.71% (116/147) were medium size herds (30-50), while 13.6% (20/147) were large herds (50-100) as indicated in (Table 4.18.2).In terms of housing, majority 78.9%

(116/147) of the herds were mainly pastoralist herds that were kept outside ``while the remaining 21.1% (31/147) were farms with cattle pens. (Table 4.18.2)

4.18.3 Herd owners knowledge/awareness on brucellosis

All the herd owners knew brucellosis with the local name (Bakkale or Ciwon guiwa), but they differed in ways of identifying it. About 34.01% (50/147) of them identified the disease based on abortion, 14.21% (21/147) recognized the disease based on hygroma, 19.04% (28/147) identified the disease based on weak calves, while 32.65% (48/147) identify brucellosis based on stillbirth (Table 4.18.3).

Majority of the herd owners 92% (135/147) believed the disease could be transmitted through ingestion of contaminated feed and water, while the remaining 8% (11/147) believed that the disease is airborne. Half of the respondents, 50.34% (74/147) accepted that the disease caused reduction in milk. None of the herd owners knew that the disease could result in infertility. Furthermore none of the herd owners had information on the disease in humans (Table 4.18.3).

Among the respondents, 33.3% (49/147) of the herd owners called the attention of a Livestock Assistant when they observe a case of brucellosis in their herds, while the remaining 66.6% (98/147) applied local treatment (Madaci). Herd owners did not have information on vaccination against brucellosis. However 32.7% (48/147) participated in the annual vaccination against diseases like CBPP, PPR and Rabies that was organized by the Jigawa State Ministry of Agriculture and Natural Resources. (Table 4.18.3)

Table 4.18.1: Demography of Herd Owners in selected herds in Jigawa State, Nigeria

Variable	Proportion	Percentage (%)
Owners sex		
Male	147	100
Female	0	0
Age		
Adult	145	98.6
Teenager	2	1.4
Occupation		
Farming	103	70.06
Public servant	15	10.20
Business men	29	19.72
Educational status		
Formal	36	24.5
Informal	111	75.5

4.18.2.: Herd information in selected cattle herds in Jigawa State Nigeria

Variable	Frequency	Percentage (%)
Breed		
Locals	112	76.19
Foreign	2	1.36
Cross	33	22.4
Husbandry system		
Extensive	113	76.9
Semi-intensive	34	23.12
Breeding program		
Natural	121	82
Artificial insemination	26	17.68
Sources of animals		
Open market	117	79.9
Known farm	30	20.41
Herd size		
Small (1-30)	11	7.48
Medium (30-50)	116	78.9
Large (50-100)	20	13.60
Farm/Herd type		
Fulani herd	116	78.9
Farm	31	21.1

4.18.3: Herd owners knowledge/awareness on brucellosis (N=147)

Variable	Proportion	Percentage (%)
Disease identification		
Name of disease		
Local name (Bakkale)	147	100
Means of Recognition		
Abortion	50	34.01
Hygroma	21	14.28
Retained placenta	0	0
Weak calves	28	19.04
Stillbirth	48	32.65
Transmission		
Ingestion	135	92
Aerosol	11	8
Reduction in milk		
Yes	74	50.34
No	73	49.65
Disease recognition in		
Humans		
Yes	0	0
No	0	0
Vet service		
Yes	49	33.3
Local treatment	98	66.6
Vaccination		
S19	0	0
Annual vaccination	147	100

4.18.4: Assessment of factors influencing the introduction of brucellosis into the study herds based on CFT

From the study, majority of the herds 93.19% (137147) do not institute quarantine in herds, while 6.08% (10/147) institute quarantine in their herds. Seropositivity of brucellosis among herds that do not practice quarantine was 21.89% (30/137). No positive reactors were recorded among herds that practiced quarantine (Table 4.18.4).

Among the herds under study, 17.68% (26/147) breed through artificial insemination while 82.21% (121/147) breed using bulls. Seropositivity of brucellosis among herds that breed using artificial insemination and natural mating was found to be 3.84% (1/26) and 23.9% (29/121) respectively. There was association between brucellosis and breeding using bulls as observed in the study herds $p<0.05$

Majority of the herds 99.31% (146/147) introduced new animals frequently in to their herds while only one herd introduce not frequently 0.68.3% (1/147).Seropositivity among herds that introduce new animals frequently was found to be 20.5% (30/146) (Table 4.18.4).

Herds that sourced their animals from cattle markets constituted 79.59% (117/147), while those that sourced from farms represent 20.40% (30/147). Seropositivity among herds that purchased animals from cattle market was 22.2% (26/117) while among herds that purchase from farms it was 13.3% (4/30). There was a statistical association between sourcing of animals from cattle market and brucellosis in the study herds $p<0.05$.

Table 4.18.4: Assessment of factors influencing the introduction of brucellosis into the study herds based on CFT

Variables	Herd Tested (%)	Positive herd	Negative herd	Prevalence (%)	Odds ratio	95% CI OR	P-value
Quarantine Practice							
Practice quarantine	10(6.8)	0	10	0	0	0.0-2.	0.21459[a]
Don't Practice	137(93.19)	30	107	21.89			
Breeding Practice							
Artificial Insemination	26(17.68)	1	25	3.84	0.13	0.01-0.95	0.041212[b]
Natural Method	121(82.31)	29	92	23.9			
Animal Introduction							
Frequently	146(99.31)	30	116	20.5			1.000000[a]
Not frequently	1(0.68)	0	1	0			
Animal Sourcing							
Cattle market	117(79.59)	26	91	22.2	1.86	0.55-6.92	0.410033[a]
Farms	30(20.40)	4	26	13.3			

[a]Fishers exact test; [b]Chi-square

4.18.5 Assessment of factors influencing the maintenance of brucellosis within the Study Herds based on CFT

In this study, herders that reared animals by semi-intensive system constituted 30.16% (45/147), while those that manage under extensive system represents 69.38% (102/147). Seropositivity among herds that were either managed under semi-intensive or extensive systems was found to be 6.6% ((3/45) and 26.47% (27/102) respectively. There was significant association between extensive systems of husbandry with brucellosis as observed in the study herds $p<0.05$ (Table 4.18.5).

Majority of the herd owners interviewed were pastoralists which constituted 78.91% (116/147), while 21.08% (31/147) were semi-intensive farms owned by business men. Seropositivity of brucellosis among pastoralist herds was found to be 24.13% (28/116), while in farms it was 6.45% (2/31).

Herd sizes in this study were categorized into three 10-30 (small) 30-50 (medium) and 50-100 (large). Small herds constituted 4.08% (6/147), medium herds constituted 91.15% (134/147) and large herds 6.80% (10/147). Seropositivity among the three different herd sizes showed 20.14% (27/147) among medium herd size and 30% (3/10) among large herds. There were no positive reactors recorded among small herds (Table 4.18.5).

Of the 147 herd owners interviewed, 2.04% (3/147) dispose placenta by burying while 97.95% (144/147) dispose placenta by throwing within farm premises. Seropositivity among herds that dispose placenta by burying was found to be 33.3% (1/3) while herds those that dispose within herd premises was 20.13% (29/144).

Manure removal was used to asses hygiene practice among the herds interviewed. Among the 147 herds, 38.09% (56/147) remove manure regularly, while 61.90 % (91/14) don't remove manure regularly. Seropositivity among herds that remove manure regularly was found to be 5.35% (3/56) while it as 29.67% in littered herds. There was significant association between brucellosis and unhygienic herds in the study herds $P< 0.05$ (Table 4.18.5).

Herds that provided tap water to their livestock constituted 18.36% (/27147) while those that provided well water constituted 81.63% (120/147). Seropositivity among herds that provide tap water was found to be 11.1% (3/27) while it was 22.5% (27/120) among herds that provide well water as shown in (Table 14.18.5).

4.18.6 Assessment of factors influencing the spread of brucellosis within the Study Herds based on CFT

Herds that migrate constituted 41.49% (61/147) while those that do not migrate constitute 58.5% (86/147). Seropositivity of brucellosis among herds that migrate was found to be 40.98% (25/61) while in those that don't migrate it was 5.81% (5/86).A statistical association was observed between brucellosis and cattle migration $P< 0.05$ (Table 4.18.6).

Raising mixed species was common with majority of the herds 89.8% (132/147), while those that did not raise mixed species constituted 10.2% (15/147). The prevalence was 21.21 % (28/132) among herds that reared multiple species than those that kept single

specie (Table 4.18.6). There was strong association between herds that kept multiple species with brucellosis $P < 0.05$

Herds that shared common water and grazing points constituted 76.8% (113/147) with prevalence of 20.35% (23/113), while those that did not share water and grazing points constituted 23.12 % (34/147) with prevalence of 20.58% (7/34) (Table 4.18.6).

Table 4.18.5: Assessment of factors influencing the maintenance of brucellosis within the Study Herds based on CFT

Variables	Herd Tested (%)	Positive herd	Negative herd	Prevalence (%)	Odds ratio	95% CI OR	P-value
Husbandry system							
Semi-Intensive	45(30.61)	3	42	6.6	0.28	0.04-0.72	0.011611[b]
Extensive	102(69.38)	27	75	26.47			
Housing							
Pastoralist system	116(78.91)	28	88	24.13	4.61	0.98-29.86	0.054904[b]
Farms	31(21.08)	2	29	6.45			
Herd size							
10-30	6(4.08)	0	6	0	0.00	0.00-4.00	0.596039[a]
30-50	134(91.15)	27	107	20.14	0.59	0.12-3.1	0.434695[a]
50-100	10(6.80)	3	7	30	0.00	0.00-4.11	0.250000[a]
Placenta disposal							
Burying	3(2.04)	1	2	33.3	1.98	0.0-29.39	0.4984624[a]
Farm premises	144(97.95)	29	115	20.13			
Hygiene Practice							
Remove manure	56 (38.09)	3	53	5.35			
Littered premises	91(61.90)	27	64	29.67	0.13	0.03-0.50	0.000834[b]
Source of water							
Tap water	27(18.36)	3	24	11.1			
Well water	120(81.63)	27	93	22.5	0.43	0.10-1.67	0.288051[b]

P<0.05 significant; [a]Fishers exact test; [b]Chi-square

Table 4.18.6: Assessment of factors influencing the spread of brucellosis within the Study Herds based on CFT

Variables	Herd tested (%)	Positive herd	Negative herd	Prevalence (%)	Odds ratio	95% CI OR	P-value
Migration							
Yes	61(41.49)	25	36	40.98	11.25	3.69-36.69	0.000006[b]
No	86(58.5)	05	81	5.81			
Raising mixed species							
Multiple species	132 (89.79)	28	104	21.2	5.79	1.26-36.08	0.0183226[b]
Single specie	15(10.20)	02	43	4.4			
Sharing of water point							
Share	113(76.87)	23	90	20.35	0.99	0.35-2.85	0.8313627[b]
Don't Share	34(23.12)	07	27	20.58			
Sharing grazing point							
Share	113(76.87)	23	90	20.35			
Don't Share	34(23.12)	07	27	20.58	0.99	0.35-2.85	0.831362[b]

$P < 0.05$ significant
[a]Fishers exact test
[b]Chi-square

CHAPTER FIVE

5.1 DISCUSSSION

The overall prevalence of *Brucella* antibodies in bovine sera in Jigawa State based on the CFT test was 3.64%. There was no statistically significant difference in prevalence between the three Zones as indicated by the three different tests. It was observed that in all the three different Zones, cattle were managed by a common husbandry practice which was either Extensive or Semi- intensive system. Most of the herds usually graze inside the demarcated grazing reserves within the Zones during the dry season. During the wet season they graze around communal pastures near their settlements and thus spread of infection among and within herds may occur in almost the same pattern.

A strong agreement was observed between the screening tests and the confirmatory test. The degree of agreement observed between the three different tests was because they are all sensitive tests and they apply same principle of antibody detection based on agglutination. In addition the CFT test can specifically differentiate antibodies due to *Brucella* and other antibodies due to cross reacting bacteria or vaccination.

The prevalence of 3.64% based on CFT found in this study was low when compared to previous work by Mai *et al*. (2012) who reported a prevalence of (26.3%) in some cattle herds in nearby States like Kano and Kaduna. It was also lower than the 8.9% reported by Magaji in 2014 in a Research Farm in Shika, Zaria. The high prevalence observed in their studies was attributed to the higher number of animals tested compared to the present study.

In contrast the 3.64% reported in this study was higher than what was reported in some West African countries like Gambia (1.1%) and Senegal (0.6%) using RBPT and CFT (Unger, 2002), which may be attributed to differences in geographical location which may influence prevalence of a disease.

From the study, there were differences in individual animal prevalence in all herds from the three different zones. Cattle concentrations in the North Central and North West Zones were less when compared to the North East Zone, where cattle concentrate due to availability of pastures and rice straws. The highest individual animal prevalence of 18.5% found in the North East Zone was probably due to contact with other animals and sharing of grazing points.

Based on the confirmatory test, there were differences in within herd prevalence rates in the study herds among the three different zones. A within Herd Prevalence (WHP) ranging between 0 and 57.14% was observed. The highest within herd prevalence of 57.14% was found in Dutse in a herd coded as DT1 due to the abortion which occurred in the herd which resulted to contamination of herd premises with uterine and vaginal discharges. The placenta also serves as a source of disseminating *Brucella* following licking by herd mates. *Brucella abortus* was isolated from the placenta of a Friesian cow in the herd.

There was variation in WHP among herds in Birnin-Kudu, Gwaram Buji, Miga, Jahun and Kiyawa in the North Central Zone. The within herd Prevalence ranged from 0-29.4% and interestingly all these herds were transhumance herds that move within Nigeria and in some cases to neighbouring countries. The difference in the WHP observed was due to the large

number of animals that tested positive in the herds due to infection they have acquired following contact with herd mates and also sharing of resting points and grazing areas.

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Based on the confirmatory test the within herd prevalence (WHP) in the North East Zone ranged from 0-20%. The difference in WHP found in the study may be due to difference observed in management practice in the different herds. The herds were also migratory herds and they could have acquired the infection as a result of sharing communal pastures.

In the North West Zone the Within Herd Prevalence (WHP) range from 0-35% and some of the herds in this zone are migratory herds. The highest WHP was found in a herd coded Yk4 in Yankwashi. *Brucella abortus* was isolated from the vaginal swab of a Bunaji cow in herd Yk4. The high prevalence recorded from the herd could due to shedding of the bacteria from the infected cow to other herd mates.

The WHP found in this study from all the three Zones was higher than the 14.6% prevalence reported by Bakari (2010), in some selected herds in Kura local Government Areas of Kano State due to differences in management practice.

The overall herd prevalence found in the study was 20.48% and at least one CFT positive herd was identified from each Zone. There was no statistically significant difference in herd prevalence between the different herds in the three different zones. Most of the herds under study presented similar patterns of housing for their cattle and they also used the same type of feeding and drinking troughs. They also, utilized well as source of water for their animals and they rested in the same areas contaminated with urine and faeces. Spread of

infection between herds occurs in almost the same pattern. However some herds move from North West Zone to the North Central and North East Senatorial Zones for grazing because of availability of pastures and rice straws.

The overall herd prevalence in this study was lower than the 55% herd prevalence reported by Bertu (2014) in Kachia grazing reserve in Kaduna State. The high herd prevalence observed by Bertu was attributed to the settlement of pastoralists with their herds from different parts of Nigeria inside the reserve and usually many herds with unknown status of *Brucella* infection settled in clusters or close proximity inside the reserve and likelihood of spread among the herds was possible.

The prevalence of *Brucella* antibodies in milk in the North Central and North West Zone was found to be 1% and 0.6% with no reactors detected in the North East Zone based on MRT. With the WAT the prevalence was 2.12% in the North Central Zone with no reactors were detected from the North East and North West Zone. There was no statistically significant difference in prevalence between the three different Zones based on the two different tests which were attributed to the similarity in production system in the Zones. Comparing the prevalence found in this study with the 18.6% reported by Cadmus *et al* (2008) the prevalence was low and that could be attributed to differences in climate, location and management practices which may have influence on spread and rate of *Brucella* infection.

In all the herds sampled, prevalence rates were higher among females than males and this was due to the large number of females sampled compared to males in each herd.

Pastoralists keep more female animals in their herds because of offsprings they got and also milk. Brucellosis has been described as a disease that primarily affects sexually matured pregnant females (Radostits *et al*., 2007). However other studies reported that there is equal chance of both sexes getting infected (Crawford *et al*., 1990). Also, Keppie *et al*. (1965) described the capacity of erythritol in enhancing multiplication of *Brucella* in the placenta and uterus due to its availability in the tissues during pregnancy and this could increase susceptibility of females to brucellosis than males.

The results of the study also indicated that a higher prevalence was observed among cattle older than 37 months of age when compared with the age groups 6-12 months, 13–24 months and 25- 36 months. The higher prevalence rate in older cattle found in this study was attributed to constant exposure over time to the infectious agent thus older animals presented higher prevalence. Comparing the findings of this work with other research work showed some differences. Cadmus *et al*. (2008) observed no difference between cattle older than 36 months and 12–36 month old, whereas Matope *et al*. (2011) reported decreased frequency of brucellosis with increasing age, with 24–48 months old cattle having higher odds of being seropositive compared to those older than 84 months. They concluded that some older cows may not exhibit detectable antibody titres possibly due to latency or self-limiting infection which is common in chronic brucellosis

The prevalence among breeds in this study was found to be higher among local breeds (Bunaji and Rahaji) than foreign breeds (Friesian). This was attributed to the larger number of locals tested compared to the foreign. However some studies have shown that foreign breeds usually present higher prevalence compared to locals due to constant confinement of

the foreign breeds in unhygienic herds. On the other hand other studies have also shown that local breeds are constantly moving and are therefore not likely to accumulate or spread infection to each other as effectively as the foreign breeds (Nuru and Dennis in 1975). Similarly, Magaji (2014) reported 13.1% prevalence in Bunaji breeds which was higher than in crosses in a research farm in Zaria probably due to confinement since the farm was an intensive farm. In general differences in breed susceptibility are uncommon with brucellosis and breed susceptibility may depend on management system (Radostits *et al*., 2007)

In the course of the study it was observed that herds with cases of abortion and retained placenta encountered during sampling had the highest prevalence of 14.2% each, followed by those with weak calves 11.1% and still birth 7.5%. As documented in the literature, once an abortion occur contamination ensues within that herd from uterine and vaginal fluids, large concentrations of *Brucella* are also disseminated during this process. Animals may also lick aborted foetus and placenta resulting to serious spread within that herd. This could have been responsible for the higher prevalence observed in herds with cases of abortion and retained placenta. Radostits *et al*. (2000) documented that abortion in the last trimester, retained placenta, birth of weak calves and stillbirth constitute some of the cardinal signs of brucellosis. He further highlighted that in some herds usually the animal aborts once but will continue to shed the organism throughout its life. Herds with retained placenta are always considered as suspect for brucellosis.

An attempt to isolate *Brucella* from milk, swabs, placenta, spleen, liver and stomach contents was made. *Brucella abortus* was isolated from the placenta of a Friesian cow and

vaginal swab of a Bunaji cow. The placenta contains large amount of *Brucella* due to presence of erythritol which enhance its multiplication. The bacterium also survives within the lumen of the vagina before ascending to the uterus. Thus the placenta and the high vaginal swab samples were positive for *Brucella* isolation. The inability to isolate from milk could be due to absence of the organism in milk even though antibodies were present demonstrating presence of infection.

Isolation of *Brucella abortus* species from a Friesian and Bunaji cattle in the study area was suggestive of presence of brucellosis in the study herds. Furthermore the disease can spread within and neighbouring herds if adequate control measures are not put in place which could subsequently raise the prevalence rate and establish the presence of the disease in various parts of the State. Previous work indicated that *Brucella abortus* biotypes 1, 2, 3 and 4 were isolated from cattle in Nigeria (Bale *et al*., 1981; Ocholi *et al*., 2005; Bertu, 2014). However, the complete characterization of the two *Brucella abortus* species found in this study to determine or identify the biotypes using the Tbilisi reference phage was incomplete because the phages did not react with both the control strain 19 and the field isolates. Thus the isolates were stored in the isolate bank at National Veterinary Research Institute Vom, Nigeria.

An assessment of the 147 questionnaires from the herds under study revealed that 70.06% of the herds were owned by pastoralists. Traditionally cattle serve various purposes to the pastoralist communities in Jigawa State. They serve as a source of livelihood through provision of milk, meat, and offspring. The pastoral communities knew brucellosis with the local name Bakkale and they have different ways of identifying it. Thus they apply a herb

called madaci to treat cases of brucellosis. Madaci was regarded by the pastoralist to have some anti-bacterial activity which may help an animal during recovery process.

Vaccination against brucellosis was not carried out in all the herds under study. They don't know that cattle are vaccinated against the disease. However some of the herd owners allow their animals to be vaccinated against diseases like Contagious Bovine Pleuro Pneumonia (CBPP) and Peste de Petits Ruminants (PPR) during the annual vaccination exercise organized by Jigawa State Government. Calf hood vaccination was shown to minimize losses due to brucellosis (Radostits *et al*., 2007). In Nigeria currently, vaccination against brucellosis is done on request and not practiced as a routine (Abdullahi, U.S, personal communication 2014). However some African countries like South Africa, Botswana and Namibia vaccinate against brucellosis routinely (Brucella Workshop in Africa, 2013).

Among the herds under study, no positive reactors were found in herds that practiced quarantine while herds that do not practice quarantine recorded a prevalence of 21.89%. Herds that do not practice quarantine can introduce infected animal in to their herds unknowingly while those that practice quarantine minimize risk of introducing infection in to their herds and that could be the reason why no reactors were recorded.

Largely, most of the herds under study bred their animals by natural mating method with local or foreign bulls, while small percentages breed through artificial insemination (AI). The prevalence was found to be higher among herds that breed using bulls (23.9%) than herds using artificial insemination (3.84%). Semen used for AI purpose are usually certified free of *Brucella* hence chances of transmission using AI is very rare. Breeding bulls are not

usually tested against brucellosis before use on farms in Jigawa State except in some enlightened herds/farms (JRI, 2003). Documented studies have shown that diseased bulls and contaminated semen serve as means of transmitting brucellosis in a herd (Radostits *et al*., 2000).

Herds that sourced their animals frequently and from cattle markets have higher prevalence rate to *Brucella* infection than those that don't source regularly and from farms. Risk of introducing infection was higher among herds that source from cattle market because the status of such animals on brucellosis is unknown and they don't have records thus serving as sources of infection. On the other hand animals sourced from farms may have records and possibly may be screened against brucellosis before introduction. The findings in this study were similar to what was reported by Al-majali (2005), in Jordan, while Reviriego *et al*. (2000) described addition of new animals from open market as a very important risk for introducing brucellosis in a herd or flock.

The system of husbandry practiced by most of the farmers in the study was extensive which involved movement of animals in search of pastures. The prevalence was higher among herds reared under extensive husbandry system because chances of getting infection is possible most especially when herds share water points that are contaminated with uterine or vaginal discharges None of the herds sampled in this study practiced zero grazing.

The high prevalence of *Brucella* infection in the extensive management system may also be partly attributed to co-mingling in communal grazing areas particularly during the dry season. These findings concur with that of Musa *et al*. (1990) in Sudan who observed that

clinical manifestation of brucellosis often began during adverse weather conditions. During such times, animals become concentrated on scarce pastures and around watering points, which might become contaminated with aborted foetal materials or uterine discharges. The results also agreed with those of Ajogi (1987; 1988) who reported similar findings among pastoralist herds in Wase and Wawa-Zange Grazing reserves in Nigeria.

It is a tradition of the pastoralists to manage their livestock in an open kraal and the prevalence of *Brucella* infection was higher among herds kept in open Kraal (24.13%).
The high prevalence observed among herds that are kept in open kraal could be due to exposure when animals are kept in open and possibly an animal aborts. They may spread infection through uterine discharges, licking of one another or lying closely at night which could expose them to various risks other than brucellosis. These findings were similar to what was reported by Mai *et al*. (2012) in some pastoralist herds in Kano and Kaduna.

From this study, the highest prevalence rate was observed among large herds (30%), this was followed by Medium herds (20.14%) with no positive reactors detected among small herds. The high prevalence observed among large herds could be attributed to the stocking density. Animals concentrate around each other and contaminate the environment, thus encouraging spread among pen mates. These findings are in agreement with the reports by Oloffs (1996) in Uganda and Bertu, (2014) in Jos Plateau and Kachia Grazing Reserve. According to the epizootic rule (Akakpo and Bornarel, 1987), small herd, present low incidence compared to large herd size. Negative herds are more likely to be found among smaller herds than larger herds because the animals concentrated less around each and the herds are better managed hygienically hence less spread.

Majority of the herd owners were found to dispose placenta around herd premises. This could enhance spread within herds and to nearby herds. Spread of *Brucella* infection would be higher among herds that dispose contaminated placenta within its premises because of likely spread by dogs and other rodents that move within and between herds. In this study the prevalence was found to be higher in herds that disposed placenta either by burial or burning probably because of the ratio of animals infected to the number of animals in the herd. The finding in this study differ from that of Mai *et al*. (2012) who reported higher prevalence among some pastoralist herds in Kano and Adamawa States, Nigeria that disposed placenta indiscriminately.

Clearing of manure around herds was the factor that was used to evaluate hygiene practice among the herds under study. The prevalence was found to be higher among herds that do not clear cattle manure regularly (29.67%). *Brucella* was found to survive for several months in manure. As animals lie down in herds they could be contaminated and following licking by pen mates infection may ensue. An association between poor hygiene practices due to unhygienic herds with *Brucella* infection was observed in this study ($P < 0.05$).

Majority of the herd owners provided well water to their livestock, which is the common source of water. Herds that used well water presented higher infection rate possibly due to consumption of contaminated water. *Brucella* was reported to survive in water for several months under conditions of low temperature and high humidity (Alton *et al*., 1996), hence risk of infection from contaminated well water was possible.

Migration was a common phenomenon among the study herds most especially during the dry season and most of the herds rear multiple species of livestock. A statistical association was found between migration, rearing multiple species in a herd with *Brucella* infection ($P<0.05$). High seropositivity to *Brucella* infection was also observed among herds that share water and grazing points. When herds migrate, there is likelihood of acquiring infection at contaminated water and grazing points. Secondly keeping multiple species in herds could be a source of *Brucella* infection since infected animals could be introduced unknowingly. In some herds in Jigawa State, communal grazing is practiced which allows small ruminants from different herds to graze together during the dry season as a group. Such practice could serve as a source of introducing infection into herds when small ruminants are kept in association with cattle.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

An overall State wide seroprevalence survey of *Brucella* antibodies in cattle in Jigawa State demonstrated that the prevalence rates were 4.98%, 4.44% and 3.64% using RBPT SAT and CFT tests respectively. The prevalence of *Brucella* antibodies in milk was 0.5% by MRT and 0.72% using WAT test. Based on the CFT test an individual animal prevalence ranging from 0 to 18.5%, a within herd prevalence ranging from 0-57.14% and an overall herd prevalence of 20.48% were obtained.

The findings of *Brucella abortus* through culture and biochemical tests from two samples one each from vaginal swab of a Bunaji cow and placenta of a Friesian cow has demonstrated beyond doubt that *Brucella* species exists among cattle in Jigawa State.

From the results of the structured questionnaire survey it was found that mixing of animal species, large herd size, and disposal of manure near animal houses along with leaving animals in open spaces greatly enhances the spread of *Brucella* species. Furthermore natural breeding using diseased bull, poor hygiene practice, extensive husbandry management practices and migration facilitated the spread of *Brucella* infection among cattle herds.

In conclusion, evidence of the presence of *Brucella* infection using serology has been established in the study areas. *Brucella abortus* was isolated from cattle in the study areas. There were statistical associations between poor hygiene practice, migration, rearing of multiple species, extensive husbandry system, housing and breeding with prevalence of brucellosis in the study areas.

6.2 Recommendations

Based on the results of this work the following are hereby recommended:

1. Public awareness is advocated in order to boost the knowledge of farmers on brucellosis and other diseases in terms of general farm management, control and animal health care delivery system.
2. Farmers should be encouraged to test newly introduced animals for brucellosis and also to practice quarantine.
3. Farmers should improve sanitary measures in their herds through clearing of manure and proper waste disposal.
4. Herds where a positive reactor was reported should be monitored and herd owners should be advised to cull affected animals.
5. Pastoralists should avoid contact with herds at water and grazing areas, which will help in reducing spread of diseases like brucellosis.
6. Molecular characterization of the isolates obtained from this study should be carried out in order to gain more knowledge on the strains of *Brucella* in the study areas.

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APPENDIX I

STUDIES ON BRUCELLOSIS IN CATTLE IN JIGAWA STATE, NIGERIA

Department of Veterinary Medicine
Ahmadu Bello University Zaria

LGA: ----------

WARD: ----------

DATE: ----------

QESTIONNAIRE No: ----------

PART (A) Demography of herd owners

1. Name of owner (Optional): ----------
2. Sex: …….
3. Age: …….
4. Occupation: ……..……
5. Level of education:----------

PART (B) Herd Information

1. Types of Breeds: Exotic[ ] Local [ ] Cross [ ]
2. System of husbandry: Extensive [ ] Intensive[ ] Semi-intensive [ ]
3. Breeding programmes: Natural mating [ ] Artificial Insemination [ ]
4. Sources of animals: known farm [ ] Cattle market [ ]
5. Herd size : Small 1-30 [ ] Medium 30-50 [ ] Large 50-100 [ ]
6. Housing : Standard farm [ ] Fulani system [ ]
7. Categories of animals: Bulls [ ] Cows [ ] Heifers [ ] Calves [ ]
8. Animal age in month : 6-12 [ ] 13-24[ ] 25-36 [ ] 37-72[ ]

PART (C) Knowledge and Awareness of herd owners on Brucellosis

1. Have you heard of brucellosis? Yes [ ] No [ ]

2. Which name do you call it? Ciwon guiwa [ ] Bakkale [ ] other [ ]
3. Can you recognize it? Yes [ ] No [ ]
4. Base on clinical sign Yes [ ] No [ ]
5. Which of the signs

   Abortion? Yes [ ] No [ ]

   Retained placenta? Yes [ ] No [ ]

   Hygroma ? Yes [ ] No [ ]

   Weak calves? Yes [ ] No [ ]

Still birth? Yes [ ] No [ ]

6. Do you know how it is transmitted ? Yes [ ] No [ ]

   a. If yes, which of the ways

7. Ingestion: Yes [ ] No [ ], Water: Yes [ ] No [ ] Aerosol: Yes [ ] No [ ]

8. Do you know it can be transmitted by mating Yes [ ] No [ ]

9. Do you know it can reduce milk production Yes [ ] No [ ]

10. Do you know it can cause sterility Yes [ ] No [ ]

11. Do you know humans can be infected Yes [ ] No [ ]

12. Do you know how it is transmitted to humans Yes [ ] No [ ]

13. How do you handle the disease in your farm?

    Culling: Yes [ ] No [ ] Local treatment: Yes [ ] No [ ] Call a Vet: Yes [ ] No [ ]

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

15. Which type of vaccine?

    Strain19 ? Yes [ ] No [ ]

    CBPP? Yes [ ] No [ ]

    Others (specify) ? Yes [ ] No [ ]

PART (D) Brucellosis Exposure factors

1. New Animal Introduction? Yearly: [ ] monthly : [ ] Weekly [ ] At will: [ ]
2. Do you quarantine new introduction? Yes [ ] No [ ]
3. Do you screen against brucellosis? Yes [ ] No [ ]
4. Do you do any form of screening (specify) Yes [ ] No [ ]
5. Where is your ssource of water? Tap [ ] Well [ ] River [ ] Stream [ ]
6. Herd Hygiene ; Do you clear cattle dung Yes [ ] No [ ] Don't remove cattle dung Yes [ ] No [ ]

7. Do you encounter the following signs in your herd? Yes [ ] No [ ]

Abortion ? Yes [ ] No [ ]

Retained placenta ? Yes [ ] No [ ]

Hygroma ? Yes [ ] No [ ]

Weak calves? Yes [ ] No [ ]

History of still birth? Yes [ ] No [ ]

8. Do you mix your herd with other peoples herd ? Yes [ ] No [ ]

9 Do you share drinking points ? Yes [ ] No [ ]

10 Do you use communal pastures for grazing? Yes [ ] No [ ]

11 Do you raise different species of animals ? Yes [ ] No [ ]

12. How do you dispose placenta? within premises [ ] burning [ ]

13. Do you migrate? Yes [ ] No [ ]

APPENDIX II

CFT Conversion Table

Conversion of Brucellosis Complement Fixation Test Titre to International Complement Fixation Test Units (I.C.F.T.U.)

Dilution of serum	Degree of Complement Fixation			
	1+	**2+**	**3+**	**4+**
½	8	10	12	13
¼	17	20	23	27
1/8	33	40	46	53

1/16	66	80	93	106
1/32	133	160	186	213
1/64	266	320	371	426
1/128	531	640	742	851
1/256	1062	1280	1485	1702
1/512	2125	2560	2970	3405
1/1024	4250	5120	5939	6810

APPENDIX III

Brucellosis in Europe

	Bovine	**Caprine**	**Porcine**	**Ovine**
Country	**(*B. abortus*)**	**(*B. melitensis*)**	**(*B. suis*)**	**(*B. ovis*)**
Albania	-	+	+	+
Belgium	+	-	-	-
Bulgaria	-	-	+	+
Croatia	-	-	+	+
Czech Republic	-	-	?	-
France	+	++	?	+
Germany	+	-	?	+
Greece	+	++	ND	ND
Ireland	+	-	-	-
Italy	+	+	-	ND
Latvia	-	-	+	-

Lithuania	-	-	-	?
Macedonia	+	+	-	-
Malta	+	+	-	-
Poland	+	+	?	-
Portugal	+	+	-	+
Romania	-	-	+	-
Russia	++	++	+	+
Slovakia	-	-	ND	-
Slovenia	-	-	-	+
Spain	+	+	-	+
Ukraine	ND	ND	ND	ND
Yugoslavia	+	+	+	-

- Not present
+ Low sporadic incidence
++ High incidence,
? Presence uncertain
ND No data None of the four types of brucellosis exist in Austria, Denmark, Estonia, Finland, Hungary, Iceland, Luxembourg, Moldavia, Netherlands, Sweden, Switzerland, and the United Kingdom

Source: FAO-WHO-OIE Animal Health Yearbooks, 1994, 1995

APPENDIX 1V

Brucellosis in Asia

	Bovine	**Caprine**	**Porcine**	**Ovine**
Country	**(*B. abortus*)**	**(*B. melitensis*)**	**(*B. suis*)**	**(*B. ovis*)**
Afghanistan	+	+	ND	ND
Bangladesh	+	+	ND	ND
Bhutan	+	-	-	ND
China	+	+	+	+
Hong Kong	ND	ND	?	ND
India	+	+	?+	-
Indonesia	+	ND	+	+
Iran	+	+	-	-
Israel	-	+	-	-
Iraq	+	+	ND	ND

Jordan	-	++	-	-
Korea (S)	++	-	?+	-
Kuwait	++	++	-	-
Malaysia	+	-	?-	-
Mongolia	++	+	-	+
Myanmar	+	ND	+	ND
Oman	++	ND	ND	ND
Qatar	ND	ND	ND	ND
Sri Lanka	++	+	-	+
Syria	+	ND	ND	ND
Thailand	+	-	+	-
Turkey	++	++	-	ND
UAE	-	+	-	+
Yemen	+	+	-	-

- Not present
+ Low sporadic incidence
++ High incidence,
? Presence uncertain
ND No data
None of the four types of brucellosis is present in Bahrain, Cyprus, Japan, Malaysia (Sabah), Philippines, or Singapore
No data for countries of the former Soviet Union or Qatar

Source**:** FAO-WHO-OIE Animal Health Yearbooks, 1994, 1995

APPENDIX V

Brucellosis in the Americas

	Bovine	**Caprine**	**Porcine**	**Ovine**
Country	**(*B. abortus*)**	**(*B. melitensis*)**	**(*B. suis*)**	**(*B. ovis*)**
Antigua/ Barbuda	?	-	-	-
Argentina	++	-	+	
Belize	-	-	-	ND
Bolivia	++	+	+	ND
Brazil	++	-	+	-
Canada	-	-	-	+
Chile	++	-	-	+
Colombia	+	-	-	-
Cuba	?	-	++	-

Dominican Republic	++	-	+	-
Ecuador	++	ND	ND	ND
El Salvador	++	ND	+	ND
Guatemala	+	-	+	-
Haiti	+	-	-	-
Honduras	?	-	++	-
Jamaica	?+	-	-	-
Mexico	+	+	ND	-
Nicaragua	++	ND	ND	ND
Peru	++	ND	ND	++
Paraguay	+	ND	-	+
Uruguay	+	-	-	+
United States	+	-	(+)	+
Venezuela	++	-	++	?

- Not present
+ Low sporadic incidence
++ High incidence
? Presence uncertain
ND No data

None of the four types of brucellosis is present in Barbados, Falkland Islands, Surinam, or St. Kitts/Nevis

Source: FAO-WHO-OIE Animal Health Yearbooks, 1994, 1995

APPENDIX V1

Brucellosis in Africa

	Bovine	**Caprine**	**Porcine**	**Ovine**
Country	**(*B. abortus*)**	**(*B. melitensis*)**	**(*B. suis*)**	**(*B. ovis*)**
Algeria	+	?	ND	+
Angola	?	?	?	?
Botswana	+	ND	-	ND
Cape Verde	?	?	?	+
Central African Republic	++	ND	+	ND
Chad	++	?	?	ND
Congo	+	-	-	-
Côte d'Ivoire	+	-	-	+
Egypt	+	+	ND	-

Eritrea	+	?	ND	+
Ghana	+	-	-	-
Guinea	+	ND	-	ND
Kenya	+	+	ND	ND
Libya	+	+	-	-
Mauritius	-	-	-	-
Morocco	+	?	-	-
Mozambique	++	+	++	+
Namibia	+	-	-	?
Niger	+	+	ND	+
Nigeria	++	+	+	ND
Seychelles	+	-	-	-
South Africa	++	+	-	+
Sudan	++	+	-	-
Tanzania	+	ND	ND	ND
Tunisia	+	++	-	-
Zaire	+	ND	+	ND
Zimbabwe	+	+	-	+

- Not present
+ Low sporadic incidence
++ High incidence
? Presence uncertain
ND No data

No data on any of the four types of brucellosis are available for Gambia, Mali, and Mauritania
Source: FAO, WHO-OIE Animal Health Yearbooks 1994, 1995

APPENDIX VII

Table 4.5: Within Herd Prevalence (WHP) based on RBPT, SAT and CFT in various Herds of North Central Senatorial Zone of Jigawa State Nigeria

Herds	Number of animals tested	RBPT Positive	Within herd Prevalence (%)	SAT Positive	Within herd prevalence(%)	CFT Positive	Within herd Prevalence(%)
Dutse	**96**	**16**	**16.6**	**14**	**14.5**	**14**	**14.5**
DT1	21	15	71.4	12	57.14	12	57.14
DT2	10	1	6.6	1	6.6	1	6.6
DT3	12	0	0	0	0	0	0
DT4	10	0	0	0	0	0	0
DT5	12	0	0	0	0	0	0
DT6	12	0	0	0	0	0	0
DT7	10	0	0	0	0	0	0
Kiyawa	**82**	**0**	**0**	**0**	**0**	**0**	**0**

B/Kudu	**90**	**14**	**15.5**	**12**	**13.3**	**9**	**10**
BK1	14	3	21.4	3	21.4	2	14.2
BK2	10	0	0	0	0	0	0
BK3	25	10	40	9	36	7	28
BK4	11	1	9	1	9	1	9
BK5	10	0	0	0	0	0	0
BK6	10	0	0	0	0	0	0
BK7	10	0	0	0	0	0	0
Gwaram	**91**	**9**	**9.8**	**7**	**7.6**	**7**	**7.6**
GW1	23	4	17.3	4	17.3	4	17.3
GW2	11	1	9	1	9	1	9
GW3	10	1	10	1	10	1	10
GW4	15	0	0	0	0	0	0
GW5	12	3	25	1	8.3	1	8.3
GW6	10	0	0	0	0	0	0
GW7	10	0	0	0	0	0	0
Buji	**89**	**8**	**8.9**	**7**	**7.8**	**7**	**7.8**
BUJ1	10	0	0	0	0	0	0
BUJ2	17	5	29.4	5	29.4	5	29.4
BUI3	15	1	6.6	0	0	0	0
BUJ4	16	2	12.5	2	12.5	2	12.5
BUJ5	10	0	0	0	0	0	0
BUJ6	10	0	0	0	0	0	0
BUJ7	11	0	0	0	0	0	0
Miga	**87**	**0**	0	**0**	**0**	**0**	**0**

APPENDIX VIII

4.6: Within Herd Prevalence (WHP) based on RBPT SAT and CFT in the various herds of North East Senatorial Zone of Jigawa State

Herds	Number of animals tested	RBPT Positive	Within herd prevalence (%)	SAT Positive	Within herd Prevalence	CFT Positive	Within herd Prevalence
Hadejia	**97**	**21**	21.6	**18**	**19.58**	**16**	**18.55**
HJ1	20	**7**	35	6	30	6	30
HJ2	14	0	0	0	0	0	0
HJ3	15	6	40	4	26.6	3	20
HJ4	12	1	8.3	1	8.3	1	8.3
HJ5	10	0	0	0	0	0	0
HJ6	14	4	28.5	4	28.5	3	21.4

HJ7	12	3	25	3	25	3	25
Guri	**86**	**0**	**0**	**0**	**0**	**0**	**0**
Kirikasamma	**88**	**0**	**0**	**0**	**0**	**0**	**0**
Birniwa	**90**	**0**	**0**	**0**	**0**	**0**	**0**
Auyo	**92**	**19**	**20.65**	**18**	**19.56**	**15**	**18.47**
AU1	24	9	37**.5**	8	33.3	**7**	29.1
AU2	12	3	25	3	25	3	25
AU3	10	4	40	4	40	3	30
AU4	12	0	0	0	0	0	0
AU5	10	0	0	0	0	0	0
AU6	13	3	23	3	23	2	15.3
AU7	11	0	0	0	0	0	0
Kaugama	**83**	**0**	**0**	**0**	**0**	**0**	**0**
Mallammaduri	**86**	**0**	**0**	**0**	**0**	**0**	**0**
Total	**622**	**40**		**36**		**31**	

APPENDIX IX

Table 4.7: Within Herd Prevalence (WHP) based on RBPT, SAT and CFT in various herds of the North West Senatorial Zone of Jigawa State, Nigeria

Herds	Number of animals tested	RBPT Positive	Within herd prevalence (%)	SAT Positive	Within herd prevalence	CFT Positive	Within herd prevalence
Yankwashi	**92**	**13**	**14.1**	**13**	**14.1**	**10**	**10.86**
YK1	12	0	0	0	0	0	0
YK2	13	2	15.38	2	15.38	2	15.3
YK3	15	1	6.6	1	6.6	1	6.6
YK4	20	7	35	7	35	5	35
YK5	11	3	27.2	3	27.2	2	27.2
YK6	10	0	0	0	0	0	0
YK7	10	0	0	0	0	0	0

Kazaure	**90**	**12**	**13.3**	**11**	**12.2**	**11**	**12.2**
KZ1	14	4	28.5	**4**	28.5	4	28.5
KZ2	**12**	0	0	0	0	0	0
KZ3	**20**	6	30	5	25	6	25
KZ4	**13**	0	0	0	0	0	0
KZ5	**10**	1	10	1	10	1	10
KZ6	**11**	1	9	1	9	1	9
KZ7	**10**	0	0	0	0	0	0
Maigatari	**87**	**9**	**10.3**	**7**	**8.04**	**7**	**8.04**
MG1	13	1	7.6	0	0	**0**	0
MG2	12	2	16.6	2	16.6	**2**	16.6
MG3	10	0	0	0	0	**0**	0
MG4	15	2	13.3	2	13.3	2	13.3
MG5	14	4	28.5	3	21.4	3	21.4
MG6	13	0	0	0	0	0	0
MG7	10	0	0	0	0	0	0
Garki	**87**	**0**	**0**	**0**	**0**	**0**	**0**
Ringim	**89**	**0**	**0**	**0**	**0**	**0**	**0**
Taura	**87**	**0**	**0**	**0**	**0**	**0**	**0**
Gumel	**90**	**0**	**0**	**0**	**0**	**0**	**0**
Total	**622**	**34**		**31**		**28**	