

**SERO-EPIDEMIOLOGY OF BRUCELLOSIS IN SMALL
RUMINANTS IN PLATEAU STATE**

BY

BERTU, WILSON JAMES

DOCTOR OF VETERINARY MEDICINE (DVM,1995),

AHMADU BELLO UNIVERSITY, ZARIA

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PREVENTIVE MEDICINE, FACULTY OF VETERINARY
MEDICINE AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA**

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DECLARATION

I hereby declare that the work in this thesis titled '*Sero-epidemiology of Brucellosis in Small Ruminants in Plateau State*' was carried out by me in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria and Bacterial Research Division, National Veterinary Research Institute, Vom under the supervision of Professors I. Ajogi; J.O.O Bale and J .K. P Kwaga. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree or diploma at any university.

Bertu, Wilson James

Student

Signature

Date

CERTIFICATION

This thesis entitled “SERO-EPIDEMIOLOGY OF BRUCELLOSIS IN SMALL RUMINANTS IN PLATEAU STATE” by **Bertu, Wilson James** meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

PROFESSOR I. AJOGI (DVM (ABU), MPVM, Ph.D (IBA) Date

CHAIRMAN, SUPERVISORY COMMITTEE

PROFESSOR J.O.O BALE (B.SC (ABU), Date
M.Sc (ABU/READ), Ph.D (DUN.SCOTLAND)
MEMBER, SUPERVISORY COMMITTEE

PROFESSOR J.K.P KWAGA (DVM, M.Sc (ABU),
Ph.D (SASK.CANADA)
MEMBER, SUPERVISORY COMMITTEE

Date

DR D.H.M DUSAI (DVM, M.Sc, PGCVEE (ABU)
HEAD OF DEPARTMENT
VET.PUBLIC HEALTH AND PREVENTIVE MED.

Date

PROFESSOR S. A.NKOM
DEAN OF POST GRADUATE SCHOOL
AHMADU BELLO UNIVERSITY, ZARIA

Date

Dedication

This project is dedicated to my beloved wife, **Christiana** and children,

Naancin, Naandom and Naanret.

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ABSTRACT

A sero-epidemiological study of brucellosis in small ruminants (sheep and goats) was carried out in Plateau State to determine the status of the disease. A total of 1,347 serum samples from 851 goats and 496 sheep from nine Local Government Areas (LGAs) of the state were tested for brucella antibodies using Rose Bengal Plate test (RBPT) and Serum Agglutination test (SAT). The result revealed brucellosis prevalence of 46(9.2%) by RBPT and 26(5.2%) by SAT in sheep and 86(10.1%) and 51(6.0%) by RBPT and SAT respectively in goats. The overall prevalence of brucellosis in sheep and goats in Plateau State 14.5% and 16.0% respectively. The prevalence varied from one LGA to another and between Sheep and Goats in each LGA. In goats, the highest prevalence (11.5%) was recorded in Jos North LGA while the lowest (1.7%) was in Shendam LGA by SAT. In Sheep, Quanpan LGA had the highest prevalence of 17.6% while Mangu LGA had the least prevalence of 1.1% also by SAT. The difference in prevalence between sheep and goats was not statistically significant, ($P>0.05$). Age specific prevalence of brucellosis in both sheep and goats exist (17.3% in adults) and (21.4% in young) but was not statistically significant ($P>0.05$). There was no significant difference in prevalence between male (13.8%) and female (15.7%) sheep, ($P>0.05$). There was however a significant difference in prevalence between male (13.8%) and female (18.9%) goats, ($P<0.05$). There was no significant difference in prevalence between the samples obtained from slaughtered

animals (18.0%) and those from the field (17.0%), ($P>0.05$). The widespread brucellosis seroprevalence in Plateau state is of great economic and public health significance. There is the need to embark on control and eradication of small ruminant brucellosis in Plateau State. Culling of affected small ruminants and maintenance of good hygiene will be useful methods of control at this point in time. Isolation and characterization of *Brucella* species causing problems in small ruminants in Plateau State is suggested for further studies.

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CHAPTER ONE

INTRODUCTION

1.1 STATEMENT OF THE PROBLEM

Brucellosis is one of the most important reproductive diseases of domestic animals worldwide caused by a bacterial organism called *Brucella*. There are six species of *Brucella*, each infecting a specific animal host. They are *Brucella abortus* (Cattle), *Brucella melitensis* (Goats and Sheep), *Brucella ovis* (Sheep), *Brucella canis* (Dogs), *Brucella suis* (Pigs), *Brucella neotomae* (Desert wood rat). These organisms can also infect other domestic animals and man.

Brucellosis is endemic in Nigeria and has been reported in cattle, sheep, goats, dogs, pigs, horses, and camels ((Eze, 1978; Okoh, 1979; Bale and Kumi-Diaka, 1981; Bale, 1982; Adamu and Ajogi, 1999; Ocholi *et al.*, 2004). Wild animals are also susceptible to brucellosis and can serve as carriers to other animals as well as humans (Davis, 1990). The economic losses it causes in the livestock industry as well as the health risk it poses to human health cannot be overemphasized. Losses in livestock are in terms of abortion, infertility, low conception rate and low survival rate of neonates (Osori, 1976; Oyedipe *et al.*, 1981). Clinical signs and pathogenesis vary with the species of *Brucella* and the infected host: arthritis, epididymitis, infertility, abortion and fever are common (Elzer, 1998).

Although a lot of work has been done on bovine brucellosis in Nigeria, not much has been done on ovine and caprine brucellosis in Plateau State.

Nigeria's food animals' population has been estimated at 15.2 million cattle, 28 million goats, 23 million sheep (Anonymous, 2006). Sheep and goats are therefore important sources of protein supply to the populace; second only to cattle.

1.2 IMPORTANCE OF THE PROBLEM

Small ruminants make up the bulk of the population of food animals in Nigeria totalling 51 million. This is made up of 28 million goats and 23 million sheep, whereas there are 15.2 million cattle (FAO, 2006). Plateau State has a population of about 3 million sheep and goats put together. Their contribution to the protein needs of the country cannot therefore be overemphasised. Great proportion of families throughout the country and Plateau State in particular keep sheep and goats, which are major sources of income to these families. It is also a common practice for those who keep cattle especially the Fulani pastoralists as well as the native farmers to keep sheep and goats along side the cattle. This practice is found among the rural population of the middle belt and the northern parts of Nigeria. Sheep and goats are also often reared along with cattle in most private or Government-owned farms in the country, which are semi-intensive in nature (Ocholi, 2004).

The keeping of sheep and goats together with cattle provides an easy means of spread of *Brucella* infection from cattle to the small ruminants and vice versa. Ocholi *et al.* (2005a) reported the isolation of *Brucella abortus* as the cause of abortion in sheep that were reared together with cattle on a private farm. Previous work on isolation of *Brucella* in Nigerian livestock have indicated that only *Brucella abortus* has so far been isolated from cattle, (Eze, 1978; 1981; Falade 1981; Bale and Kumi-Diaka, 1981; Bale and Nuru, 1985), as well as from sheep and goats, in which cattle is the primary reservoir (Okoh, 1980; Ocholi *et al.*, 1993; Hale and Ajogi, 1997 and Ocholi *et al.*, 2005b). However, *Brucella melitensis* had been isolated from sheep and goat milk in Nigeria (Bale, 1981; Bale *et al.*,

2003). Since then, there has been no other report of *Brucella melitensis* isolation in Nigeria. All the *Brucella* isolated in recent time irrespective of the animal species from where they have been isolated have been *Brucella abortus* (Ocholi, 2005b).

The role of brucellosis in limiting livestock production and its economic impact on the livestock industry in Nigeria has been reported (Rikin, 1988; Ajogi and Akinwumi, 2001). These were only in cattle and do not include losses in productivity in other animals especially sheep and goats.

As a zoonosis of great importance, there is also a significant loss in infected human productivity due to Brucellosis (Madkour, 1981).

1.3 JUSTIFICATION

Brucellosis is a major cause of economic loss in the livestock industry and a disease of public health significance (WHO, 1986). It is endemic in Nigeria and almost all the states are affected including Plateau State. Most reported cases have been in cattle and not much in sheep and goats which are major sources of income and meat in Plateau state.

Plateau State is endowed with a large livestock population. Majority of which are sheep and goats with population of 964,188 and 1,865,805 respectively (Bourn *et al.*, 1992). Field reports of cases of abortion in small ruminants and cattle owners in the state have been on the increase. Although there are no published field studies to incriminate *Brucella* species as the cause, they could be suspected to be due to brucellosis on clinical grounds (Kabagambe, *et al.*, 2001) as it is the major cause of abortion and reproductive wastages in domestic animals.

In Plateau State, a wide scale investigation of brucellosis in sheep and goats has not been carried out.

Control measures in Nigeria are aimed at cattle vaccination with *Brucella abortus* Strain 19 (S. 19) vaccine while small ruminants are vaccinated with Rev.1 vaccine. However, Rev.1 vaccine has severe effect on the small ruminants (Falade, 1983a). As a result, vaccination of small ruminants against brucellosis has not been practised in Nigeria. This may give an opportunity for the disease to spread among the small ruminant population freely. They can in turn serve as sources of infection to cattle population as well as humans. The result is the persistence of the infection, making effective control and eradication of *Brucella* impossible. For the unrealized potentials of small ruminants to be fully derived, concerted efforts should be made to control brucellosis in these animal species. It is therefore pertinent to obtain the baseline data on the epidemiology of brucellosis in small ruminants in order to design a holistic control and eradication programme in Plateau State in particular and the country as a whole.

1.4 RESEARCH QUESTIONS

This study will attempt to answer the following questions.

- a) Is brucellosis prevalent in sheep and goats in Plateau state?
- b) Is the prevalence the same in sheep and goats?
- c) Is the prevalence of infection in sheep and goats similar throughout the state?

1.5 OBJECTIVES

The objectives of this work are to:

- i Determine the current prevalence of brucellosis in Sheep and Goats in Plateau State using Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT).
- ii Determine the prevalence in each zones and LGA of Plateau state.
- iii Determine the prevalence in relation to age, sex and in field and slaughtered animals

1.6 SIGNIFICANCE OF THE STUDY

- i The outcome of this serological survey would provide information that could be useful for understanding the pattern of brucellosis in sheep and goats in Plateau State.
- ii The survey will aid in identification of infected animals and locate pockets of infection.
- iii It could help in planning and execution of brucellosis control programme in the State.
- iv It will serve as a baseline data for small ruminant brucellosis in Plateau State and also as a reference point for other researchers.

CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND

Brucellosis is an infectious disease of many species of domestic animals and wild life worldwide. It occurs most commonly in cattle, sheep, goats, dogs, pigs. Occasionally, it also affects domestic animals like camels and horses and wild animals like bison, elk, and reindeer, caribou (Jensen *et al.*, 1999) also in marine animals like whales, dolphins, seals and porpoises.

The disease was first described by Hippocrates in 450 A.D. as a febrile disease. The causative agent was first isolated by Bruce in 1870 from the liver of British soldier who died of undulant fever (Malta fever) on the Mediterranean Island of Malta. Then it was called *Micrococcus melitensis*.

Ten years later, Bang and Stribolt (1880) isolated a similar organism named *B. abortus* from an aborted bovine fetus and fetal membranes in Denmark. The relationship of this disease in animals and man was not well understood until Zammit (1905) demonstrated the organism in the blood of goats and the isolation of the organism from apparently healthy goats by Horrecks in the same year. This was followed by the isolation of a similar organism named *B. suis* from an aborted pig by Traun (1914) in the USA. Evans, (1918) showed that these organisms were related and they were therefore included in the same genus *Brucella* by Meyer and Shaw (1920) in honour of David Bruce. After these three new species have been described, *B. ovis* from cases of epididymitis and abortions in sheep in New Zealand by Buddle and Boyes (1953). *B. neotomae* was isolated from a desert wood rat (*Neotoma lepida*) by Stoner and Lackman (1957) in Utah, USA and *B. canis* from cases of canine epizootic abortion and epididymitis in Beagle dogs by Carmichael

and Kenny in 1968 in the USA. There are six recognized species in the genus *Brucella* namely *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, *B. neotomae* and *B. ovis*.

In recent years, (Corbel and Brinley-Morgan, 1984) reported the isolation of *Brucella* species, which differ from the above mentioned from animals living in marine environment. Isolates were obtained from free-living Seals and Cetaceans around the coast of Scotland and captive Dolphins in the USA (Ewalt *et al.*, 1994). Their characterization showed that they could not be assigned to recognized species of *Brucella* but should be given a new nomen species called *Brucella maris* (Jahans *et al.*, 1997). However, based on molecular studies which indicate differences in the strains from cetaceans and seals, two new species, *B. cetaceae* and *B. pinipediae* have been proposed corresponding to cetaceans and seals respectively (Cloeckaert *et al.*, 2001).

2.2 DESCRIPTION OF THE CAUSATIVE AGENT

Brucellosis in sheep and goats is primarily caused by *B. melitensis* and rarely *B. abortus* (Luchsinger and Andarson, 1979; Garin-Bastuji *et al.*, 1998), or *B. suis* (Paolicchi *et al.*, 1993). *B. ovis* infects mainly sheep.

Morphologically, brucellae are coccobacilli or short rods 0.6 to 1.5um long and 0.5 - 0.7um in width. They are arranged singly and less frequently in pairs or small groups. The morphology of brucellae is frequently constant except in old cultures where pleomorphism, is evident. Brucellae are non-motile, non-spore forming, nonflagellate, nonencapsulated and have no pili.

They are Gram negative and usually do not show bipolar staining; they are not truly acid-fast but resist decolorization by weak acids. They therefore stain red by modified Ziehl-

Neelsen method that is sometimes used for the microscopic diagnosis of brucellosis from smears of solid or liquid specimens (Alton, *et al.*, 1988).

2.3 CULTURE AND GROWTH CHARACTERISTICS

Members are aerobic but some strains require atmosphere containing 5-10% CO₂ added for growth especially on primary isolation (Alton *et al.*, 1988). On repeated culture, isolates of *Brucella* can lose the requirement for added CO₂ for growth and may grow in air alone (Eze, 1981).

The optimum pH for growth is from 6.6-7.4 and culture media should be adequately buffered near pH 6.8 for optimum growth.

The optimum growth temperature is 36-38⁰C, but most strains can grow between 20⁰C and 40⁰C (European Commission, 2001; Anon, 2001). *Brucella* requires biotin, thiamin and nicotinamide and the growth are improved by serum or blood but haem (V-factor) and NAD are not required.

The growth of most *Brucella* strains is inhibited by media containing bile salts, tellurite or selenite. On suitable solid media, *Brucella* colonies are visible after 2 days incubation. After four days, *Brucella* colonies become round, 1-2mm in diameter, with smooth (S) margins, transparent and pale honey colour when plates are viewed in transmitted light and have a bluish translucent appearance in reflected light. When viewed from above, colonies appear convex and pearly white. Later, colonies become larger and slightly darker. Rough *Brucella* isolates produce similar colony size and shape but are more opaque off-white in colour with a rather granular surface. On blood agar, growth is slower than on serum dextrose agar (SDA) with the production of non-haemolytic, greyish- white glistening

colonies after 48-72 hours incubation. Little or slow growth is produced by many *Brucella* strains on MacConkey agar, even after five days at 37°C (Corbel *et al.*, 1979).

Growth on liquid media is usually poor unless culture is vigorously shaken. On semi-solid media, CO₂-independent *Brucella* strains produce uniform turbidity from surface down to a few millimetres depth while CO₂-dependent strains produce a disk of growth a few millimetres below the surface of the medium.

2.4 BIOCHEMICAL REACTIONS

Brucella metabolism is oxidative and cultures show no ability to acidify carbohydrate media in conventional tests. *Brucella* species are catalase positive and usually oxidase positive and they reduce nitrate to nitrite except *B. canis* strains.

The production of H₂S from sulphur containing amino acid varies. *B. melitensis* does not produce H₂S. Urease activity varies from fast to very slow. Indole and acetyl methyl carbinol are not produced from tryptophane and glucose respectively (Anon, 2001). Methyl red and Voges-Proskauer tests are negative and *Brucella* neither liquefy gelatine nor lyses red blood cells (Alton *et al.*, 1988).

2.5 ANTIGENIC CHARACTERISTICS

All smooth *Brucella* cross-react with one another in agglutination tests with unabsorbed polyclonal antisera, a cross-reaction which does not extend to non-smooth strains.

Lipopolysaccharide (LPS) comprises the major surface antigens of the corresponding colonial phase involved in agglutination. The (S-LPS) molecules carry the A and M antigens, which have different quantitative distribution among the smooth *Brucella* strains. This is of value in differentiating biovars of the major species using absorbed mono specific A and M antisera (Anon, 2001). Serological cross-reaction have been reported

between the smooth *Brucella* and various other Gram negative bacteria e.g. *E. coli* O: 116 and O: 157, *Salmonella* group N (O: 30) of Kaufmann-white and *Pseudomonas multophila*, *Vibrio cholerae* and especially *Yersinia enterocolitica* (O: 9). These organisms can induce significant antibodies, which cross-react with S-LPS of *Brucella* antigens in diagnostic tests (Anon, 2001; Corbel *et al.*, 1984).

2.6 SUSCEPTIBILITY TO PHAGES

There are about 40 phages which are lytic and specific to the genus *Brucella* and they are not known to be active against any other bacteria that have been tested. Therefore lysis by *Brucella* phages is a useful test to confirm the identity of *Brucella* species and for speciation within the genus (Anon, 2001).

Brucella phages currently in use for *Brucella* typing are Tbilisi (Tb), Weybridge (Wb), Izatnagar (Iz) and R/C. The first three are used for differentiation of smooth *Brucella* and R/C is used for rough *brucella* (*B. ovis*, *B. canis*) (Corbel *et al.*, 1987), Figure 2.1.

FIGURE 2.1: Differential characteristics of brucella phages to brucella species involved in small ruminant brucellosis

Species	Tb	IZ	R/C
<i>B. melitensis</i>	–	+	–
<i>B. ovis</i>	–	–	+
<i>B. abortus</i>	+	+	–

Key:

Tb = Tbilisi

Iz = Izatnagar

R/C = Rough culture

Source: Alton *et al.*, 1975

2.7 SUSCEPTIBILITY TO DYES

The effects of the dyes thionin and basic fuchsin on various *Brucella* species and biovars varies (Anon, 2001). Susceptibility to dyes at standard concentrations (20µg/ml) is used as routine typing test of *Brucella* species (Alton *et al.*, 1988). *B. melitensis* strains all grow in the presence of both dyes.

2.8 SUSCEPTIBILITY TO ANTIBIOTICS

Brucella species are sensitive to a wide range of antibiotics. Penicillin is used for the routine differentiation of the vaccinal strain *Brucella abortus* species biovar 1 strain 19, and streptomycin for *Brucella melitensis* biovar 1 strain Rev.1, the vaccine widely used for the immunization of cattle and small ruminants respectively, from the virulent field strains of their respective biovars by virtue of their different sensitivity to these antibiotics (Alton *et al.*, 1988). On primary isolation, brucellae are usually susceptible *in vitro* to gentamycin, tetracycline and rifampicin. Most strains are also susceptible to ampicillin, chloramphenicol, cotrimoxazole erythromycin, spectinomycin and streptomycin. Most strains are resistant to β-lactams, cephalosporins,

Polymixin B, bacitracin, cycloheximide, clindomycin, lincomycin, nystatin and vancomycin at therapeutic concentrations (Anon, 2001).

2.9 BRUCELLA SELECTIVE MEDIA

In an attempt to achieve success in the isolation of *Brucella* from contaminated clinical veterinary specimens as a result of heavy contamination of such specimens with various organisms, development of selective media became paramount. Further modifications have also been made to this medium by addition of antibiotics or dyes to the basal medium with the intention of eliminating other bacteria. These include Farrell medium for *B.abortus*

(Farrell, 1974; Ryan, 1967) and Thayer-Martin medium for *B.ovis* (Thayer and Martin, 1964).

2.10 BRUCELLA EVOLUTION

By observing the evolutionary traits according to the ancestor-descendant rules of characters, it can be predicted that the ancestor of *Brucella* was an aerobic heterotroph free-living bacteria inhabiting the rhizosphere of plants (Moreno *et al.*, 2002). Complete chromosomal sequences of species belonging to these genera of alpha-proteobacteria have been released recently, which allow genetic comparison with *Brucella* (Moreno *et al.*, 2002; DelVecchio *et al.*, 2002). It is accepted that the *Brucella* ancestor was a bacterium that evolved into an animal intracellular parasite by losing structures, modifying existing ones and generating or acquiring new traits. These characteristics are reflected in the genome as well as in the phenotype and life cycle of the extant *Brucella* (Moreno *et al.*, 2002). Commensurate with these features are the genome size and the presence of two chromosomes with the same G+C content of *Brucella*, which is almost identical with its free-living plant and obligate intracellular alpha-proteobacteria relatives such as the free-living and opportunistic pathogen, *Ochrobacterium*.

Some of the orthologous characteristics identified as or predicted to be relevant to virulence did not probably undergo substantial modifications, as they are shared by the phylogenetic neighbours. Other modifications that is likely the result of the adaptive evolution to pathogenicity include several changes in the outer membrane features. These include changes in the lipopolysaccharide (LPS) lipid a structure in *Brucella* which was not substantially modified with respect to that of the phylogenetic neighbours (Velasco *et al.*, 2000). Similarly, the high conservation of the two-component BvrR/BvrS sensory regulatory system in *Brucella* examined and the striking difference in its periplasmic

sensing region with those of the phylogenetic neighbours and also supports the idea that this adaptation has preceded speciation. Also the type IV secretion system in plant endosymbionts functions in the establishment of the bacteroid condition, whereas in *Brucella* they may be devoted to transference of molecules inside phagosomes for controlling intracellular trafficking (Moreno *et al.*, 2002).

By comparing dendrogram and phylogenies it seems feasible to hypothesize that *B. abortus* and *B. melitensis* shared the same ancestor while *B. canis* and *B. suis* shared a common ancestor (Moreno and Moriyon, 2001). Based on nutritional requirement, *B. suis* is hypothesized the closest species to the *Brucella* ancestor (Plommet, 1991) and it is the most diverse of the classical brucellae in genomic structure and host preferences (Moreno and Moriyon, 2001). The strains of *B. suis* are the most closely related to *Ochrobacterium* and the most diverse among all the *Brucella* species that parasitize terrestrial mammals and the most closely related to the heterotroph ancestor (Moreno, 2002).

2.11 TAXONOMY OF BRUCELLA SPECIES AND BIOVARS

Brucellae have been proposed to be a monospecific genus in which all types should be regarded as biovars of *B. melitensis* (Verger *et al.*, 1985; 1987) in view of their high degree of DNA homology (>90% for all species). This proposal have not yet been agreed upon, therefore the old classification of the genus into six species, *B. melitensis*, *B. abortus*, *B. ovis*, *B. suis*, *B. neotomae*, *B. canis* (Corbel and Brinley Morgan, 1984) is still the classification used worldwide. Studies with a limited number of strains of classical species and biovars showed that they can be differentiated by restriction fragment length polymorphism (RFLP) analysis of whole DNA (Allardet-Servent, A *et al.*, 1988; Jensen *et al.*, 1999), the Bru-RS 2 elements (Halling and Bricker, 1994), the insertion sites of IS711 (Bricker and Halling 1994; Ouahrani-Betteche *et al.*, 1996; Clavareau *et al.*, 1998; Bricker,

1999), the REP and ERIC sequences (Mercier *et al.*, 1996; Tcherneva *et al.*, 2000) and DNA sites (Cloeckaert *et al.*, 1996). *Brucella melitensis*, *B. abortus*, *B. suis*, *B. neotomae* occur in the smooth form while *B. ovis* and *B. canis* are being encountered in the rough form.

For the species infecting small ruminants, *B. melitensis* have 3 biovars; *B. abortus* have seven biovars (1-6 and 9) and *B. ovis* has one (rough), Table 2.2.

- (i) Species differentiation is routinely based on lysis by phages and simple biochemical tests like oxidase and urease tests. Differentiation at biovar level between *B. melitensis*, *B. abortus*, and *B. suis* is currently performed by four main tests:
- (ii) CO₂ requirement
- (iii) Production of H₂S
- (iv) Sensitivity to dyes (thionin and basic fuchsin)
- (v) Agglutination with monospecific A and M antisera.

A recently developed agglutination test using latex beads coated with a pair of monoclonal antibodies directed against the rough lipopolysaccharide (R-LPS) and the 25kDa outer membrane protein (OMP25) respectively (Bowden *et al.*, 1997) makes it possible to accurately differentiate *B. ovis* from *B. canis*, and the occasional rough isolates of the smooth *Brucella* species.

FIGURE 2.2: Biovar differentiation of *Brucella* species involved in sheep and goat Brucellosis

Species	Biovar/ morphology	CO ₂ req	H ₂ S prodn	Dye sensitivity		Aggl. in Polyclonal antiserum		R-LPS	Latex mAb
				Thionin	Basic fuchsin	A	M		OmP 25
<i>B. melitensis</i>	1	-	-	+	+	-	+	-	-
	2	-	-	+	+	+	-	-	-
	3	-	-	+	+	+	+	-	-
	R	+	-	+	-	-	-	+	-
<i>B. ovis</i>	R	+	-	+	-	-	-	+	-
<i>B. abortus</i>	1	+	+	-	+	+	-	-	-
	2	+(2)	+	-	-	+	-	-	-
	3	+(2)	+	+	+	+	-	-	-
	4	+(2)	+	-	+	-	+	-	-
	5	-	-	+	+	-	+	-	-
	6	-	-	+	+	+	-	-	-
	9	±	+	+	+	-	+	-	-
	R	+or- (3)	± (3)	± (3)	± (3)	-	-	+	+

SOURCE: Alton *et al.*, 1975

2.12 EPIDEMIOLOGY OF BRUCELLOSIS

2.12.1 Geographical Distribution

Brucellosis is a disease of worldwide distribution occurring in domestic as well as wild animals. It has been reported wherever animals are raised all over the world (Seifert, 1996). Although some of the industrialized countries in Europe and America have achieved eradication of brucellosis through intensive control and eradication schemes, the disease is still a serious problem in developing countries (Ragan, 2002; Warner, 2001).

2.13 TRANSMISSION

In cattle, the excretion of *Brucella* may start 39 days after exposure (Phillipon *et al.*, 1970). Cow shed or pasture becomes infected after the infected animal aborts or has full term parturition and this may continue for 15 days. Although the infectious materials from the genital tract usually clears after 2-3 months, the cattle becomes carrier of *Brucella* and sheds it intermittently for many years (Herr *et al.*, 1990). In goats, excretion of the organism from the vagina is usually prolonged and copious (2 to 3 months). In sheep, excretion is generally less prolonged, usually ceasing within 3 weeks after abortion or full term parturition (Okoh, 1980). Shedding of *Brucella* is also common in udder secretions and semen, and may be isolated from various tissues such as lymph nodes, especially those associated with reproductive organs and from arthritic lesions (Alton *et al.*, 1988). The persistent infection of the mammary glands and supra-mammary lymph nodes leads to a constant or intermittent shedding of the organisms in milk in succeeding lactations. It provides an important source of infection for human and young animals (Anon, 2001).

Susceptible animals become infected by consumption of infected materials or by aerosols as animals tend to lick aborted fetuses or the genital discharges of an aborting animal (Bercovich, 1998). Exposure to *Brucella* infection also occurs *in-utero* or when animals

born to healthy dams are fed with colostrum or milk from infected dams (Catlin and Sheehan, 1986; Grillo, *et al.*, 1997; Bale and Nuru, 2001). Grazing animals on pastures where infected animals have grazed or coming in contact with infected premises or manure (Anon, 2001). Infected male shed the organisms in semen especially during the acute phase of the infection. Such males could transmit the infection to females through coitus. This is the reason why if infected semen is used for artificial insemination, it could introduce brucellosis to a free herd (Bale and Kumi-Diaka, 1981; Arthur, *et al.*, 1982; Blood and Radostits, 1989). Dogs have been found to be mechanical and biological vectors of brucellosis (WHO, 1986) as they are involved in transportation of infected material from one place to the other thereby introducing the infection to new areas and also infecting themselves.

Transmission in man is principally by contact with infected materials such as carcasses, aborted fetuses, placentas, vaginal discharges, manure and urine. Man to man infection is very rare (Currier, 1989). Horse riders also have high risk of infection because fistulus withers in an infected horse is a source of infection to the rider (Oladosu, 1986). Consumption of raw or unpasteurized milk and milk products is another important mode of transmission from animal to man (Ibrahim, 1974; Palanduz *et al.*, 1999). Other routes of infection in man include: inhalation of air droplet containing the organism (Currier, 1989), accidental self-inoculation with Strain 19 *Brucella abortus* vaccine (Weidmann, 1991).

2.14 RESERVOIRS OF *BRUCELLA*

Epidemiological evidence shows that *Brucella* species usually have definitive host preferences (Bercovich, 1998), although interspecies transmission does occur. *Brucella abortus* is recognized as the main cause of contagious abortion in cattle but can also cause abortion in sheep, goats, dogs, horses, camels, buffaloes as well as wild animals that have

contact with them (FAO, 2004). Serological and bacteriological studies show that cattle may acquire brucellosis due to other *Brucella* species from other animals (Nicoletti, 1980; Corbel, 1988; Forbes, 1990; Weidmann, 1991). *Brucella melitensis* is highly contagious to goats and sheep, but can as well infect cattle, dogs, camels, and pigs (Zowghi and Ebadi, 1988). *Brucella suis* commonly infects pigs but may also infect other animals like cattle, horses, dogs, and wild animals. *B. ovis* infects sheep while *B. canis* infects dogs primarily. Wild animals like buffaloes, wild pigs, deers, foxes, rodents, bison, elk, and antelopes are susceptible to *Brucella* infection and may serve as reservoir for domestic animals (Boer *et al.*, 1980; Tessaro *et al.*, 1986; Cook *et al.*, 1988; Davies, 1990). However, their role as reservoir is more in extensive farming especially in nomadic Fulani herd (Ocholi, 1990) than in intensive farming system.

2.15 RESISTANCE TO INFECTION

Resistance to brucellosis is influenced by age, sex, breed and stage of pregnancy of affected animal. Young animals tend to be less susceptible to infection than mature animals. Data also indicate that pregnant animals are more susceptible than non-pregnant animals and males because gravid uterus sustains the organism and thus influence the progression of the disease (Crawford *et al.*, 1990). It also appears that the younger the fetus is at the time of infection, the longer the incubation period. Data from naturally occurring and experimentally induced brucellosis suggest that natural resistance to brucellosis also influence the course and severity of the disease (Harmon *et al.*, 1989; Ho and Cheers, 1982).

2.16 THE DISEASE

Pathogenically, *B. melitensis* in sheep and goats is similar to *B. abortus* infection in cattle. Nevertheless, differences are significant and each species of *Brucella* causes a different

disease (OIE Manual, 1996). They are facultative intracellular parasites of the reticuloendothelial system. The virulence of *Brucella* varies considerably according to the species, strains and the size of infecting inoculum. Host susceptibility also varies and is associated with the reproductive status. Therefore in the field, all intermediate stages between typical acute infection and complete resistance may be observed and in addition, vaccinal immunity may modify the parasite-host relationship.

2.16.1 Phases of the Infection

The infection in females follows a very similar course to that of *B. abortus* in cattle. The major route of infection is through mucous membrane of the oropharynx and upper respiratory tract or conjunctiva. Another route is through the mucous membrane of the male and female genital tract. On entering into the body of the host, the organism encounters the cellular defences of the host but generally succeed in arriving via the lymph vessels at the nearest lymph node after escaping the cellular defenses (Ko and Splitter, 2003). The fate of the invading bacteria is mainly determined by cellular defences of the host chiefly macrophage and T-lymphocytes though specific antibody also plays a part. The outcome depends on the ruminant species infected, age, immune status of the host, pregnancy status, and the virulence and number of invading *Brucella* (Seifert, 1996).

When the bacteria prevail over the host's defenses a bacteraemia is generally established. The bacteraemia is always detected after 10 to 20 days and persists from 30 days to more than two months. If the animal is pregnant, bacteraemia often leads to the invasion of the uterus. At the same time, infection becomes established in various lymph nodes and organs, often in the udder and sometimes in the spleen.

The major clinical signs in the first stage of the disease are abortion but other signs due to localization of the organism may be observed e.g. orchitis, epididymitis, hygroma,

arthritis, metritis, subclinical mastitis, etc. However, numerous animals develop self-limiting infection or they may become asymptomatic latent carriers and potential excretors.

Infection does not normally occur if the female becomes infected at the end of the pregnancy. The second stage is characterized by either elimination of *Brucella* or more frequently, by persistent inflammation of mammary gland and supramammary and genital lymph nodes (Fenster Bank, 1987b) with constant or intermittent shedding of the organisms in milk and genital secretions. Animals generally abort once during the mid third of gestation but re-invasion of the uterus occurs in subsequent pregnancies with shedding in fluids and membranes. The pregnancy can also get to full term. The percentage of infected female lambing/kidding in a flock may reach 40 per cent. Females that are born into an infected area and get infected generally abort less than others. This explains the high level of abortions in newly infected flocks and their relatively low frequency in flocks where infection is enzootic. The udder is a very important predilection site for *Brucella* organisms. Infection in lactating, non-pregnant goats is likely to lead to colonization of the udder with excretion of *Brucella melitensis* in the milk (Renoux *et al.*, 1953). In goats, about two thirds of acute infections acquired naturally during pregnancy leads to infection of the udder and excretion of the bacteria in milk during subsequent lactation (Alton, 1985).

The main lesions which appear in the male animals are (orchitis and epididymitis) as well as inflammation of the joints and bursa. But abortion may also occur in the females presenting the typical yellowish sticky layers on the placenta. The consequences of brucellosis in small ruminants are infertility, high mortality rate in kids/lambs, mastitis and reduce milk production. The lesions on the placenta are more prominent with *B. melitensis*

infection than when *B. abortus* is the causal organism. After infection of *B. ovis* in small ruminants, epididymitis and inflammation of the vesicular glands are the most prominent symptoms in the male animals and this have a significant influence on the herd fertility.

2.16.2 *Brucella melitensis* Infection in Sheep and Goats

In endemic areas such as Mediterranean region, South West Asia, and parts of Latin America, it constitutes a serious zoonosis. Increased prevalence has been noted in areas with increased sheep farming.

Pathologically and microbiologically *B. melitensis* infection in sheep and goats is very similar to *B. abortus* infection in cattle. Most breeds of goats are fully susceptible to the infection but the susceptibility in sheep varies from one breed to the other e.g. the Maltese sheep are very resistant while the fat tailed sheep of South West Asia are very susceptible. These form a reservoir of infection that gives rise to widespread brucellosis outbreak in man.

In most countries around the Mediterranean and South West Asia, the brucellosis problem largely affects sheep, while in Latin America goats are chiefly involved (FAO/WHO, 1996). Although a variety of symptoms have been described in artificially infected goats, abortion in females and orchitis in males appear to be the main symptoms seen in natural cases of brucellosis in small ruminants.

2.17 PATHOGENESIS AND TRANSMISSION

The pathogenesis and transmission in sheep and goats are generally the same as in cattle. Materials excreted from the female genital tract form the supply of material for transmission to other animals. In goats, excretion of organisms from the vagina is

prolonged and copious. In both sheep and goats, the persistent excretion of *B. melitensis* in milk forms or provides an important source of human infection.

2.17.1 *Brucella ovis* Infection in Sheep

B. ovis has greater affinity for the reproductive tract of the male than in the female. Epididymitis and sterility are the most frequent and serious consequences of infection with *B. ovis*.

In rams, natural infection occurs over a long incubation period (6-17 weeks) during which time the animals are asymptomatic. The organism remains confined to the exposure sites and regional lymph nodes for 10-14 days before the infection advances to the bacteraemic stage with a more generalised infection that involves the spleen, kidneys and lymph nodes distant from the exposure sites.

During the later part of the bacteraemic stage the infection begins to localise in the genital organs. The localised lesions appear in the seminal vesicles, ampullae, testes, tail of epididymis and then less frequently the head of the epididymis. Some organisms may remain in the kidney and such animals become shedders of *B. ovis* in their urinary tract. These organisms can cause chronic interstitial nephritis in sheep.

In ewes under conditions stimulating natural infection, it was found that organisms disappear from the local site shortly after exposure, bacteraemia is prolonged and the organisms reappear in the genital tract at the third month of gestation. Abortion occurs when there is sufficient accumulation of necrosis of the placenta and separation from the caruncles. The slow progress of the infection probably accounts for the frequent finding of positive complement fixation test (CFT) titres but infrequent occurrence of abortion either by experimental or natural infection.

Foetal lambs can survive long periods *in-utero* in the presence of the infection and the primary consequences of the infection is not abortion but a placentitis that interferes with foetal nutrition and results in lambs with lowered birth weights.

Rams are more susceptible than ewes and are more actively responsible for the transmission of the organisms as well as serving as reservoir for the maintenance of the disease. Contaminated semen from infected rams is the source of the organism.

In breeding flocks the organisms are spread from infected rams to non-infected rams through passive venereal transmission. This occurs when the infected ram mates with a ewe followed by a non-infected ram within the same oestrous cycle. Ram to ram infection can occur by direct contact or by housing a non-infected ram in a barn or shed previously occupied by an infected ram. Sodomy is the major source of spread in young rams. Young rams are also susceptible to infection with *B. ovis* and in some flocks, up to 80 per cent of unmated young rams have been found to have clinical and serological evidence of the infection. Experimental transmission has been produced. Other means of infection is through the nasopharynx since rams often muzzle the genital organs of other rams. Ewes are more resistant compared to rams. In rare cases, direct contact during abortion between ewes can lead to infection. Although following abortion or parturition, an infected ewe may excrete *B. ovis* for almost 10 days; it does not maintain the infection from one breeding season to the other. The ewe may however transmit the infection if she comes into oestrus. *B. ovis* infection in rams has a deleterious effect on their semen quality, breeding efficiency and breeding capacity. In flocks where abortion has occurred, lamb yields have been known to drop from 100% to 25%. About 20% of the ewes may remain barren and up to 16% of lambs born alive die before six weeks of age. There is also a

prolongation of the lambing period as a result of interference with normal breeding operation.

2.17.2 *Brucella abortus* Infection in Sheep and Goats

Brucella abortus has predilection for uterus and placenta (Smith *et al.*, 1972; Blood and Radostits, 1989). This special predilection has been attributed to the presence of erythritol in the placenta, uterus, cotyledons and chorions which has been shown to stimulate the growth of *Brucella* both *in-vivo* and *in-vitro* (Smith *et al.* 1972; Corbel, 1988).

Erythritol is utilized by *Brucella abortus* as an energy source and trace amounts can stimulate its intracellular growth (Corbel, 1988). Erythritol is present in the normal and infected placenta of cattle, sheep, goats, and swine as well as the seminal vesicles and testes of the males of these species, but has not been found in the human placenta or in that of rabbit, guinea pig, and rats (Corbel, 1988). Erythritol favours the multiplication of *Brucella abortus* where it causes degeneration and necrosis of the cotyledons leading to abortion (Williams *et al.*, 1962; Smith *et al.*, 1972; Blood and Radostits, 1989). Following abortion, there is a further heavy invasion of the blood stream by the organism, which is followed by a rising titre. The stage in gestation when abortion occurs depends on the degree of infection, virulence of the *Brucella* and the stage of pregnancy when the infection occurs (Morgan, 1970). During pregnancy the disease is much more acute and tremendous multiplication occurs not only in the placenta but also in the lymph nodes leading to bacteraemia and vigorous antibody response (Corbel and Morgan, 1984).

The pathogenesis of the disease in the males is similar to that in the females except that the seminal vesicles and testes are involved; the reason for the predilection is similar to that in the females, the presence of erythritol (Smith *et al.*, 1972).

2.17.3 Clinical Manifestation

The incubation period of brucellosis varies from a few days to several weeks. According to Seifert (1990), the incubation period varies from 14 days to 120 days. The disease could run an acute or chronic course. When brucellosis is introduced into a clean herd of susceptible animals, it may run an acute course causing “abortion storm” in 50% or more of the pregnant animals. The incubation period is often tied up with the stage of pregnancy at the time of exposure (Seifert, 1996). In female animals, the major clinical signs are: abortion at third trimester of pregnancy, stillbirth, birth of weak offspring or dead full-term calves, retained placenta, infertility and sterility and decreased milk production (Corbel, 1988; Seifert, 1996; Anon, 2001). The aborted foetuses are covered by yellowish, slimy exudates. The placenta is oedematous and the cotyledons covered with sticky odourless brownish exudates. Hygroma swelling of the knee joint has been reported as a significant sign of brucellosis in the tropics especially among nomadic animals (Corbel, 1988; Blood and Radostits, 1989). This occurs in both sexes as a result of chronic brucellosis, Fensterbank, 1987).

In the male animals, the major signs include, orchitis, epididymitis, inflammation of the seminal vesicles and swelling of the scrotum (Seifert, 1990).

In sheep and goats, the disease is characterized by high mortality rate in lambs and kids, mastitis, reduced milk production, abortion, epididymitis, orchitis and arthritis. Persistent infection of the supramammary lymph nodes is common in goats (Anon, 2001).

In horses, the disease is characterised by arthritis, chronic bursal enlargement of the neck, fistulus withers and poll evil, bursitis and causing lameness (Radostits *et al.*, 1997). In the mare, abortion and infertility may occur and the organisms have been isolated from mares which have aborted (Ray *et al.*, 1988). Some horses may present with general stiffness,

fluctuating temperature and lethargy (Macmillan, 1985). Brucellosis in dogs is characterized by abortion and infertility in bitches, epididymitis and scrotal dermatitis in male dogs (Okoh *et al.*, 1978; Eze, 1978; Corbel, 1988).

In man, the disease is characterized by intermittent or irregular fever, weakness, depression, night sweating, anorexia, constipation, myalgia and stiffness of the vertebral column (Falade, 1974; Thornes, 1977; Currier, 1989 and Weidmann, 1991).

2.18 SURVIVAL OF BRUCELLA IN THE ENVIRONMENT

Under appropriate conditions, *Brucella* can persist in the environment for very long periods (Corbel, 1988). The survival of *Brucella* organisms in the environment plays an important role in the epidemiology of the disease. Temperature, humidity and pH influence the organism's ability to survive (Bercovich, 1988).

The organisms resist drying, especially in organic substrates and can remain viable in the dust or soil for up to 10 weeks (Corbel, 1988). In damp soil, *Brucella* can survive for 60 days at 20⁰C and 144 days at 40% relative humidity (Bercovich, 1998). In tap water, *Brucella* can survive for several months at 4-8⁰C, 2½ years at 0⁰C and several years in frozen tissues or medium (Seifert, 1996). In butter, *Brucella* survives for up to 4 months, 6 weeks in milk and 2 weeks in cooled meat and up to 30 days in ice cream (Seifert, 1996). The organisms can survive 30 days in urine, at least 75 days in aborted fetuses and more than 60 days in uterine discharges (Corbel, 1988; Bercovich, 1998). They can also survive in cattle faeces for at least 1 year and in liquid manure and frozen soil for 2½ years (Seifert, 1996). Contaminated straw remains infectious for more than 1 month (Weidmann, 1991).

Brucella organisms are sensitive to direct sunlight, and under dry conditions, they survive only when embedded in proteins (Davis and Casey, 1973). They are sensitive to wide variety of disinfectants including strong acids and alkali, hypochlorites, iodophors, 1% Lysol and formaldehyde (Corbel, 1988; Seifert, 1996). However, the presence of organic matter exposure to low temperatures drastically reduces the efficacy of these chemical disinfectants (Corbel, 1988). Pasteurisation of milk at 68⁰C for 10-15 minutes can destroy the *Brucella* organisms (Merchant and Packer, 1975).

According to Corbel (1988), brucellae show high sensitivity to a wide range of antibiotics and chemotherapeutic agents including some penicillins, aminoglycosides, chloramphenicol, tetracyclines and sulphonamides. They are however resistant to vancomycin, bacitracin, polymyxins, metronidazole, nalidixic acid and antifungal agents.

2.19 PATHOLOGY

In the pregnant animal, the disease is principally characterized grossly by necrosis of the placenta and ulcerative endometritis. The placental cotyledons are covered by sticky exudates, swollen and necrosed tissues (Radostits, 1995). The intercotyledonary areas are frequently thickened and opaque with a leathery appearance (Corbel, 1988). Histologically, the disease is characterised by marked leukocytic infiltration especially with leucocytes and plasma cells, congestion and cellular proliferation leading to necrosis (Radostits *et al.*, 1995).

Infection in foetal lungs produces bronchopneumonia with congestion, fibrinous exudation and cellular infiltration. In males the lesions are confined to the genital organs. The testes, seminal vesicles and the epididymis are enlarged and there may be multiple granulomas with infiltration of lymphoid and plasma cells in the epididymis (Smith *et al.*, 1972).

2.20 IMMUNE RESPONSE

Infection with *Brucella* usually result in the induction of both humoral and cell-mediated immune responses, in which magnitude of response is affected by various factors including virulence of the strain, size of inoculum, pregnancy, sexual and immune status of the host (Joint FAO/WHO Expert Committee on Brucellosis, 1986).

Though the pattern of response has not been extensively studied in sheep and goats, information suggests that it is similar to that of cattle in which IgM is produced followed within 1-2 weeks by IgG. Both immunoglobulins fall to a low level in more chronic state of infection but IgG level higher.

B.melitensis Rev 1 vaccine strain when applied under standard conditions may induce a long lasting serological response to the agglutination test that seriously interferes with serological screening for infected animals (Alton, 1990; Macmillan, 1990; Elberg, 1996). As no difference have been found between the diagnostic antigens from field strains of *B. melitensis* and those from Rev 1 vaccine, serological test capable of distinguishing antibodies arising from infection and vaccination respectively have not been developed.

Cellular defences of the host normally determine the fate of the invading bacteria especially macrophages and T-lymphocytes. The most widely used correlates of cell mediated immunity (CMI) are: lymphocytes stimulation, macrophage inhibition, delayed-type hypersensitivity and Gamma interferon induction. The response can be expected as rapidly as few weeks just as in humoral immunity; but could be variable and may not be detected. Studies in experimental models have provided important insight into host defences that eventually control infection with *Brucella* organisms. Serum complement effectively lyses some rough strains (i.e. those that lack the O-polysaccharide side chain on their LPS) but little effect on smooth strains (those that have a long O-polysaccharide side

chain). Administration of antibody to mice prior to challenge with rough or smooth strain of *Brucella* reduces the number of organisms that appear in the liver and spleen. The effect is due mainly to antibodies directed against LPS with little or no contribution of antibodies directed against other cellular components (Montaraz *et al.*, 1986). Reduction in intensity of infection in mice can be transferred from immune to non-immune animals by both cluster of differentiation CD4⁺ and CD8⁺ T-cells (Araya *et al.*, 1989) or by immunoglobulins fractions of serum. Administration of antibodies to interferon gamma worsens experimental infection (Zhan and Cheers, 1993). More over, macrophages treated with interferon *in-vitro* inhibit intracellular bacterial replication. In ruminants, vaccination with killed vaccines provides some protection against challenge but live vaccines are much more effective.

2.20.1 The role of T-cells

The major role of T-cells in *Brucella* immunity is secretion of interferon Gamma for the activation of bactericidal function of macrophages and Cytotoxic T-lymphocytes activity as well as IgG_{2a} and IgG₃ isotype switching. The significance of CD4⁺ and CD8⁺ T-cells in *Brucella* immunity has been controversial.

In brucellosis, the different arms of the immune system namely antigen presenting cells, NK cells, CD4, CD8 cells and B-cells act together to provide a coordinate response (Golding *et al.* 2001). However, the primary mechanism of control involves cell mediated immunity, rather than antibodies although, some immunity against re-infection is provided by serum immunoglobulins (Young, 2000; Golding *et al.*, 2001; Cheers, 1984; Araya *et al.*, 1989). Large numbers of *Brucella* are present in the endothelial cells and chorions. Histologically, the disease is characterized by a marked leukocytic infiltration, with

lymphocytes and plasma cells, congestion and cellular proliferation leading to necrosis (Laing, 1979).

2.20.2 Evaluating Cellular Immune Response

T-cell counting is the standard method for evaluating the cellular immune response against intracellular pathogens such as *Brucella*, (Celik and Akbutut, 2005). Previous studies on murine models have shown that cytokine secreting CD4⁺T cells are the fundamental components of immunity against *Brucella*. Nevertheless, it is known that CD8⁺ cells are very important in protective immunity (Cheers, 1984). More recently, optimal protection against intracellular *Brucella* has been regarded as a coordinated interaction between different T- cell subsets (Araya *et al.*, 1989). Earlier murine brucellosis studies indicated the importance of T- cells subset in the resolution of infection but produced conflicting data suggesting that either CD4 and or CD8 T-cells were responsible for protection (Araya *et al.*, 1989; Meilke, 1991; Pavlov *et al.*, 1982). In murine experiment, depletion of CD8 T-cells prevented the elimination of *Brucella* as did treatment with anti Thy 1.2 monoclonal antibodies, suggesting that CD8 T-cells are critical for eliminating intracellular infection (Mielke, 1991).

The paradigm of disease resolution was founded on adoptive T-cells transfer or *in-vivo* subpopulation depletion using monoclonal antibodies both with potential pitfalls caused by contamination by unwanted cell population or the unknown elimination of certain cells. To clarify the controversy over T-cell subsets responsible for *Brucella abortus* disease resolution, gene knock out mice have been used. As expected in an immune response to a complex bacterium, CD4 as well as CD8 T-lymphocytes recognize and proliferate to numerous antigens of *Brucella*.

2.21 DIAGNOSIS

Diagnosis depends on a combination of clinical examination, bacteriological examination and serology.

2.21.1 Clinical Signs

Brucellosis could be suspected in any herd with history of abortion during the third trimester of pregnancy, infertility, orchitis, epididymitis, stillbirths, neonatal mortality and hygroma. These signs are however not pathognomonic to brucellosis (Bercovich, 1998).

2.21.2 Direct Smear

The cultural and microscopical methods described for cattle are also applicable to the diagnosis of *B. melitensis* and *B. ovis* in sheep and goats. The only unequivocal method for the diagnosis of brucellosis in small ruminants is based on the isolation of *Brucella* organisms (Alton *et al.*, 1988). This bacteriological diagnosis of *B. melitensis* and *B. ovis* can be made by means of microscopic examination of smears from vaginal swabs, placentas, or aborted fetuses stained with the Stamp (1950) modification of the Ziehl-Neelsen method. However morphologically related microorganisms such as *Chlamydia psittaci* or *Coxiella burnetii* can mislead the diagnosis (Radostits *et al.*, 1997).

2.21.3 Cultural Isolation and Biotyping

Isolation of *B. abortus*, *B. melitensis* and *B. ovis* requires 50-100ml of serum per litre of medium and an added 5-10% CO₂ in the atmosphere for growth and that the organism occurs only in the rough phase.

The materials that can be cultured are semen from rams, and vaginal discharge, milk and aborted fetus and fetal membranes from ewes that have aborted or delivered lambs prematurely.

Vaginal swabs and milk from aborted ewes or goats are the best materials/samples for the isolation of *B. melitensis*, while spleen and lymph nodes (iliac, mammary and prefemoral) are the most reliable samples for isolation purposes in necropsied animals (Marin *et al.*, 1996a).

The Farrell's selective medium developed for the isolation of *B. abortus* from milk (Farrell, 1974) is also recommended for the isolation of *B. melitensis* (Alton *et al.*, 1988).

However, Nalidixic acid and Bacitracin concentrations in this medium may have inhibitory effects on some *B. melitensis* strains (Marin *et al.*, 1996a). Thus its sensitivity for the isolation of *B. melitensis* from naturally infected sheep is sometimes lower than that obtained with the less selective Thayer – Martin's modified medium (Marin *et al.*, 1996a). The sensitivity of bacteriological diagnosis is significantly increased by the simultaneous use of these two media (Marin *et al.*, 1996b).

The identification of *Brucella* involves Gram's reaction, colonial and cellular morphology and routine biochemical tests (Corbel, 1987). Species are distinguished on the basis of lysis by bacteriophage and oxidative reactions on amino acids and carbohydrate substrates. Typing of isolates into biovars are based on CO₂ requirement, sensitivity to thionin and basic Fuchsin dyes, H₂S and urease production (Alton *et al.*, 1988).

2.21.4 Animal Inoculation

Laboratory animals can be used for the diagnosis of animals suspected to have brucellosis. Guinea pigs are reported to be the most susceptible to brucellosis than any other laboratory animal. However, mice and rabbits are also susceptible and may be used. Two guinea pigs per sample are recommended for inoculation (Morgan *et al.*, 1978).

A suspension of suspected material (foetal lungs, abomasal content, placenta, blood, uterine discharges, lymph nodes and milk) is injected intraperitoneally or intramuscularly into guinea pigs, killed and necropsied 3-6 weeks after inoculation (Morgan *et al.*, 1978). If positive, *Brucella* organisms produce septicaemia disease in the guinea pigs (Smith *et al.*, 1972). The recovery of *Brucella* from the spleen or lymph nodes cultured on appropriate media and or a positive SAT at 1/10 or above is evidence of infection (Morgan *et al.*, 1978; Alton *et al.*, 1988).

2.22 SEROLOGICAL DIAGNOSIS

The current serological tests used for the diagnosis of *Brucella melitensis* and *Brucella ovis* in sheep and goats were initially developed for the diagnosis of *Brucella abortus* in cattle. Although not formally validated for use in sheep and goats, these tests especially Rose Bengal Plate test, Complement fixation test and more recently ELISA have been used for the serological diagnosis of brucellosis in sheep and goats (Farina, 1985; Macmillan, 1990). The outer membrane of the bacteria contains the main antigens involved in *Brucella* antibody detection. The resulting reaction is an agglutination where antibodies to *Brucella* are present and this gives a positive result.

Where *Brucella* antibodies are absent, there is no agglutination and the test is said to be negative. The ELISA test has been found to be highly sensitive and specific (Ocholi, 1990) and plate and capillary agglutination test have been used for the diagnosis of ovine and caprine brucellosis. Several factors influence the outcome of serological diagnosis of brucellosis such as long and variable incubation period (Smith *et al.*, 1972; Blood and Radostits, 1989; Morgan, 1967) vaccination status (Morgan, 1969) and type of exposure (Hornitzky and Searson, 1989).

2.22.1 Rose Bengal Plate Test (RBPT)

This test was developed by Rose and Roekpe (1957) for the diagnosis of bovine brucellosis to differentiate specific *Brucella* agglutinins from non-specific factors. When the antigen was buffered at pH 4.0 they observed that agglutination of *Brucella abortus* cells by non-specific agglutinins of bovine serum was inhibited whereas the activity of specific *Brucella* antibodies was not affected. Despite the scanty and sometimes conflicting information available (Trap and Gaumout, 1975), this test is internationally recommended for the screening of brucellosis in small ruminants (Joint FAO/WHO Expert Committee on Brucellosis, 1986). However, the standardization of the antigen suitable for *Brucella abortus* in cattle reduces the sensitivity of the test for the diagnosis of *Brucella melitensis* infection in sheep. If the antigen is standardized differently, to give a higher analytical sensitivity, the diagnostic sensitivity to *B. melitensis* infection will be improved. The Rose Bengal test is based on the detection of specific antibodies of the IgM and IgG types but more effective in detecting antibodies of the IgG₁ type than the IgG₂ and IgM types (Levieux, 1974). Although the low pH (+3.6) of the antigen enhances the specificity of the test, the temperature of the antigen and the ambient temperature at which the reaction takes place may influence sensitivity and specificity (Macmillan, 1990).

2.22.2 Serum Agglutination Test (SAT)

The SAT has been the most widely used serological test for the diagnosis of brucellosis in animal and man. It has been used for the control and eradication of brucellosis in most countries of the world such as Britain, United States of America, Canada, Israel, Germany, etc. (Nicoletti, 1969; Morgan, 1977; Stemshorn *et al.*, 1985; Dahoo *et al.*, 1986). The original form of the test was established by Huddleson and Abell in 1928 and then Welch and Mickle in 1933 who prepared the antigens. The SAT contributed to the international harmonization of brucellosis control and eradication programme, although it is not the

most sensitive. The test measures agglutinating antibodies of the IgM, IgG₁, IgG₂, IgA types (Levieux, 1974) but the IgM types are more reactive (Bercovich, 1998). Since SAT may yield both false positive and false negative results, it effectively detects brucellosis only on a herd basis (Corbel *et al.*, 1984; Nielsen *et al.*, 1988). The SAT has been widely used for the serological tests in Nigeria (Falade, 1974; Nuru and Dennis, 1975; Adekolu-John, 1989; Atsanda and Agbede, 1996; Ajogi *et al.*, 1996a; Olayinka and Ogundipe, 2001).

2.22.3 Milk Ring Test

Milk Ring Test (MRT) was first developed by Fleischner in 1937 for detecting the presence of *Brucella* antibodies in milk using stained standard *Brucella* antigen (Morgan, 1967). It is a valuable screening test both in the periodic testing of brucellosis-free dairy herds and in locating potentially infected herds (Corbel, 1988). The diagnostic significance of MRT is based on the presence of lacteal anti-*Brucella* IgM and IgA bound to milk fat globules, which rises to the cream layer of the milk. The *Brucella* antigen is stained blue with haematoxylin. When the stained antigen is added to the milk, a stained antigen-antibody complex is formed (if antibody is present) which adheres to the fat globules and rise to form a blue coloured cream layer seen as ring at the top of the tube. If there are no antibodies in the milk, the stained *Brucella* antigen remains in suspension in the milk column (Morgan *et al.*, 1978).

2.22.4 Complement Fixation Test (CFT)

As in cattle brucellosis, this test despite the complexity and the heterogeneity of the techniques used in different countries, there is agreement that this test is effective for the serological diagnosis of brucellosis in sheep and goats (Farina, 1985; Macmillan, 1990; Alton, 1990). The CFT is based on the detection of specific antibodies of the IgM and

IgG₁ that fix complement (Levieux, 1974). It is highly specific but laborious and requires highly trained personnel as well as suitable laboratory facilities. Its specificity is very important for the control and eradication of brucellosis but may test negative when antibodies of the IgG₂ type hinder complement fixation (Macmillan, 1990).

2.22.5 Antiglobulin (Coombs) Test

Coombs test detects incomplete *Brucella* antibodies of the IgG₂ type and it is used to confirm SAT results. Although the test is laborious, it is particularly important when the SAT and CFT results are negative or inconclusive (Kiss, 1971). The main limitation of this test is that the test results are indicative for infection only when its titres are at least two times the titre of the SAT.

2.22.6 Skin Hypersensitivity Test

Data from countries that have eradicated brucellosis indicate that only testing with several serological tests identifies infected herds (Stemshorn, 1984; Dahoo *et al.*, 1986; FAO/WHO, 1986; Weidmann, 1991). However since the reliability of serological tests depend on the presence of antibodies; infected animals with low antibody will not be detected. To improve on brucellosis diagnosis, researchers have used various brucellins to illicit an allergic response in infected animals (Fensterbank, 1978; Cunningham *et al.*, 1980; Chukwu, 1985b).

The test involves injecting the allergin into the flank or intra-palpebrally and measuring the thickness of the skin (Weidmann, 1991). The test is of great importance in animals with latent infection of *Brucella* in areas with low prevalence and areas known to be free from brucellosis (Bercovich, 1998).

2.22.7 2-Mercaptoethanol Test (2-MET)

The test is based on the observation that the activity of IgM is destroyed after the serum has been treated with 2-mercaptoethanol while the activity of IgG is not affected (Morgan *et al.*, 1967). It is done by adding equal volumes of 0.2M 2-mercaptoethanol to serum diluted 1:5 and conducting a serial dilution tube test (Morgan *et al.*, 1978). Seifert (1996) reported that the test is simple modification of the tube agglutination test (TAT) in which the non-specific IgM antibodies are eliminated; the test detects chronic infections with fewer false positives. The 2-mercaptoethanol and the Rivanol tests detect specific IgG (Rossi and Cantini, 1969) and are usually used to differentiate between infected and vaccinated cattle.

2.22.8 Enzyme Link Immunosorbent Assay (ELISA)

The most widely used enzyme immunoassay in detecting brucellosis is indirect ELISA (iELISA). The method involves binding of the antigen on a solid phase usually a polystyrene microtitre plate so that antibody if present in a sample, binds to the immobilized antigen-enzyme conjugate which in combination with a chromogenic substrate gives a coloured reaction indicative of the presence of antibody in the sample. It is this method that is now familiar to most researchers.

The indirect ELISA, using more or less purified S-LPS of *B. melitensis* as antigen and polyclonal conjugate (anti-IgG H+L) has been reported to be sensitive enough for the diagnosis of infection in sheep and goats (Jimenezde Bagues *et al.*, 1995; Diaz-Aparicio *et al.*, 1994; Blasco *et al.*, 1994b; Delgado *et al.*, 1995). While the use of ELISA for diagnosis of bovine brucellosis is well developed, more extensive field trials are required to validate the test for use in sheep and goats.

As mentioned before, the use of a purified periplasmic protein (26kDa) in iELISA or c-ELISA protocols could be useful for differentiating *B. melitensis* infections from Rev 1 vaccinated sheep (Debbarh *et al.*, 1996).

2.22.9 Other Serological Tests

Other serological tests for the diagnosis of brucellosis worthy of mention are:

Radioimmunoassays (RIA)

Radioimmunodiffusion (RID)

Agar gel immunodiffusion (AGID)

Indirect haemolysis test (IHLT)

Heat inactivation test (HIT)

2.23 MOLECULAR DIAGNOSIS

2.23.1 Polymerase Chain Reaction (PCR)

Although culturing is a specific method and is confirmatory, its sensitivity depends on the viability and numbers of *Brucella* within the sample, the nature of sample (foetal organs, foetal membranes, lymph nodes, etc.) and the number of specimens tested from the same animals (Hornitzky and Saerson, 1986). The time required for culturing field specimens can be long and tissues or fluids that are only contaminated with low number of *Brucella* may not be detected. Therefore in the case of fluids or tissues contaminated with non viable or a low number of *Brucella*, PCR could be a potentially useful method for the diagnosis of brucellosis. Several authors reported a good sensitivity of PCR for detection of *Brucella* DNA from pure cultures (Baily *et al.*, 1992; Romeo *et al.*, 1995; Da Costa *et al.*, 1996). Others showed that PCR could be a potentially useful tool when used alone or

in combination with labelled probes to differentiate some *Brucella* species and biovars (Mercier *et al.*, 1996; Ouahrani-Betteche *et al.*, 1996).

The possibility of PCR techniques to detect the DNA of dead bacteria, or in paucibacillary samples and even in samples highly contaminated with other microorganisms, could increase the rate of detecting animals infected with *Brucella*.

2.23.2 Other molecular methods.

- i. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE).
- ii. Pulse-field gel Electrophoresis (PFGE).

2.24 PREVENTION AND CONTROL

2.24.1 Immunization

2.24.1a *B. melitensis* Rev.1 vaccine

In most countries where *B. melitensis* infection is endemic, immunization will be the most appropriate first step, either as a preliminary to eradication or as sole measure. The live attenuated *B. melitensis* Rev.1 strain is presently recognized as the best available vaccine for the prophylaxis against brucellosis in sheep and goats. The value of the vaccine for this purpose has been confirmed through various independent field and controlled experiments (Alton, 1990; Blasco, 1997). The ability of Rev.1 to produce a high level of immunity against both artificial and natural challenge has been convincingly demonstrated both for sheep and goats (Alton, 1990).

The duration of immunity conferred by vaccinating with Rev.1 was investigated by vaccination of Maltese goats at 4 to 12 months of age and challenged them at about 21/2 years later and other at 41/2 years after vaccination. The resistance of the animals

challenged (at 21/2 years) and those at 41/2 were found to be the same, thus the immunity was considered to be life long.

More recent work has demonstrated the efficacy of Rev.1 in sheep either vaccinated as lambs conjunctivally or subcutaneously and challenged 9-10 months (Fensterbank, 1985) or 7.5-15.5 months later, respectively (Verger *et al.*, 1995) or vaccinated as adult conjunctivally and challenged 21/2 years after. Likewise, good results of protection were obtained when young goats were vaccinated at 4 months of age conjunctivally and subcutaneously and challenged 8.5-12.5 months later (Fensterbank *et al.*, 1987). To avoid stimulating serological reactions that would interfere with subsequent diagnosis, immunization is usually restricted to lambs and kids aged 3-8 months.

Similarly a reduced dose (10^3 - 10^6 CFU) has been used subcutaneously in field trials and reported as an effective method to control brucellosis in small ruminants and being relatively safe in pregnant sheep and goats (Kolar, 1984; Gasca *et al.*, 1985; Al Khalaf *et al.*, 1992; Sales-Henriques *et al.*, 1992; Al Shamkh, 1995; Uysal, 1995; Delgado *et al.*, 1995).

2.24.1b *B. abortus* Strain 19 Vaccine

This is the most commonly used vaccine for the prevention of bovine brucellosis (Nicoletti, 1990). This strain was isolated from the milk of a Jersey cow in 1923. It was left at room temperature in the laboratory for one year and was found to have become attenuated. The strain was found to be capable of inducing protective immunity in cattle though effectiveness may vary depending on age of vaccination, dose, route of administration and prevalence of brucellosis in vaccinated herd (Nicoletti, 1990). *B. abortus* Strain 19 is an attenuated organism of smooth morphology which normally does not grow in the presence of erythritol. The strain is of low virulence such that vaccination

with $40-120 \times 10^9$ CFU (classical dose) of living *B.abortus* organisms gives a good protection. However, it has disadvantages such as induction of abortion in pregnant cows, induction of persistent antibody response due to the presence of LPS with an O-chain on S.19 that cannot be serologically differentiated from that due to natural infection and its excretion in milk which can serve as a source of infection to man (Plommet, 1991). This vaccine possesses LPS with O-chain that makes the production and persistence of antibodies possible in serum following the administration of the vaccine. The presence of these antibodies makes the continued use of the vaccine incompatible with simultaneous application of test and slaughter procedures in the control of brucellosis.

This undesirable effect can be eliminated by adopting the following procedures.

1. Calves are vaccinated with a reduced dose of $3-10 \times 10^9$ CFU between 4 and 8 months of age and then the same dose is repeated when they are adults.
2. Calves are vaccinated conjunctivally with 2 drops of the vaccine containing $4-10 \times 10^9$ CFU at the age of 4-10 months and a second conjunctival vaccination with the same dose six months later (Nicoletti, 1990).

2.24.2 PREVENTION

The first and the most important means of keeping a herd free of brucellosis is to prevent its entry into the farm. This could be achieved through the following procedures:

2.24.2.1 Vaccination

The S.19 vaccine for *Brucella abortus* provides increased resistance against field strain infection following natural infection. Properly vaccinated animals are less likely to be infected and therefore are not a source of field strain of the organism. The RB51 vaccine is another effective vaccine for vaccinating cattle and gives a life long immunity. The optimum age of vaccination is between 4 and 8 months. It is also permitted and efficacious

for adult cattle to be vaccinated using a reduced dose of the vaccine, such as one-tenth and one-twentieth (Alton, 1980; Nicoletti, 1990).

2.24.2.2 Restriction of Movement

There should be restriction of movement of the animals to prevent contact with animals from other farms. This will ensure that no infected animals come in contact with the herd at any point in time to introduce infection into the farm. Only certified brucellosis-free animals should be allowed entry. This also applies to transport of animals and certain animal products within countries and between countries, following the general principles and procedures as specified in the International Zoo-Sanitary Code of the OIE. This also describes the essential testing of animals and quarantine measures.

2.24.2.3 Quarantine of Replacement Animals

All replacement animals intended to be introduced into the herd must be subjected to strict quarantine until they are certified brucellosis free before they are finally introduced into the farm. The period of quarantine should be sufficient enough to detect those incubating brucellosis. The time range is between 4 months to 12 months or until the breeding animal has completed a gestation without a laboratory evidence of infection (Radostits *et al.* 1997).

2.24.2.4 Avoid Herding of Cattle with Sheep and Goats

The common practice of rearing cattle along with sheep and goats must be discouraged. This is because such husbandry practices favour the spread and maintenance of brucellosis in the herd. As vaccination is being done to the cattle without attending to the sheep and goats, they become sources of infection to the cattle once they acquire the infection. Abortion due to *Brucella abortus* which is attributable to this husbandry system has been reported (Ocholi *et al.*, 2005).

2.24.2.5 Periodic Testing of the Animals

There should be constant sero-monitoring of the herd in order to keep track with brucellosis status of the herd. Any reactor is a suspect and must be handled with care and segregated for further investigation. Confirmed positive animal should be culled immediately.

2.24.2.6 Maintain Good Hygiene on the Farm

This is an important measure in a herd that desire to be free from brucellosis. A high level sanitation will prevent the accumulation of the organism in the environment thereby reducing the risk of other animals becoming infected.

2.24.3 Control of infection

When brucellosis has entered a farm or a herd, efforts must be geared towards controlling its spread, minimize losses and risk of human infection. The aim is to eventually eliminate the disease on the farm. The following are therefore the major steps to be taken.

2.24.3.1 Testing and Culling of Reservoir of Infection

Artificial insemination breeding cattle in the herd must be tested and positive ones culled immediately. This removes infected animals from the herd and therefore reduces exposure and transmission of the disease within the herd. Healthy animals are vaccinated to ensure protection.

2.24.3.2 Mass Education of farmers and Workers

All personnel on the farm must be adequately educated about brucellosis, its signs and symptoms such as abortion, retained placenta and infertility. They must report such signs promptly and take adequate precautionary measures when attending to such animals. Animals manifesting such signs and symptoms must be isolated and tested for brucellosis.

2.24.3.3 Isolation of Animals at Parturition

All animals approaching delivery should be considered potential dangers to other animals. They should be isolated and restricted at a maternity pen. After parturition, they are tested and all non-living products of parturition are adequately disposed of and the environment properly cleaned to ensure no traces of contamination is left in the pen. The animals must be certified free from brucellosis before they leave the pen.

2.24.3.4 Abortion Cases

All abortion products must be burnt and the contaminated area well disinfected in order to destroy any *Brucella* organisms that may be present. The animals that aborted must be quarantined and tested before it is released into the herd.

2.24.4 Eradication by Test and Slaughter

It is usually accepted that elimination by test and slaughter policy is justified on economic grounds only when the prevalence of infected animals in the area is two per cent or less (Nicoletti, 1993).

On a national scale, eradication can be done by identifying infected herds and slaughter all affected animals in the herd. However, this can be possible only when there is an adequate arrangement to compensate the affected livestock owners.

This could take place when the animals are under strict surveillance and restricted movement. On a smaller scale, the animal should be isolated at parturition since all parturitions should be considered potential sources of infection. All the non-living products of parturition should be burnt or buried deeply in a pit. Contaminated areas should properly be disinfected and cleaned.

All livestock owners should properly be educated on the aims and objectives of the control programme so that there is a total co-operation from them at various levels of the programme. They should be informed of the hazards of this disease to their families and themselves and the economic benefits of the control programme, etc. and goats in the designated areas and reactors are removed until all animals tested negative to the disease in the long run.

2.25 PROBLEMS MILITATING AGAINST CONTROLLING BRUCELLOSIS IN NIGERIA

Problems militating against successful brucellosis control programme in Nigeria include:

1. The herding of different animals species together e.g. cattle, sheep, goats. This is a common practice in Nigeria both with the private farms and the peasant and nomadic farmers. Such husbandry favours the spread of brucellosis from one species to the others. This makes it very difficult to achieve control and eradication compared to a single species situation. Herds containing a mixture of breeds have been found to be at greater risk of infection. This is due to the random introduction of different breeds into the farm making control difficult.
2. The indiscriminate introduction of imported purchased animals of high production without the necessary advice from a veterinary expert and in the absence of the proper veterinary infrastructure can lead to abortion storm (Ocholi *et al.*, 2005). In Nigeria, farmers do not care to know the brucellosis status of their own animals let alone that of the new animals they are introducing into their farms. These practices can render control programmes ineffective. notes that the development of National dairy or private dairy herd through the introduction of large exotic dairy animals has led to increased problem with brucellosis (Nicoletti, 1980). Large numbers of susceptible cows make control of brucellosis difficult in these herds.

3. There are no laws restricting animals' movement from one part of the country to another. This makes contact between infected and non-infected animals possible thereby increasing the spread of *Brucellosis* for as long as the final destination of the movement. Infected materials left along the route also serve as source of infection for many other animals. In dairy sector, the extent of contact between herds and between individuals may also be an important factor influencing the rate of spread of the infection.
4. The porous nature of Nigerian borders. Nigeria is bordered by about four countries: Cameroon, Chad, Niger and Benin Republic. Movement of humans and animals occurs through these borders freely. Such animals are brought in without any consideration to their brucellosis status. These will continue to introduce brucellosis into the country even if Nigeria had succeeded in controlling the disease giving rise to resurgence of infection.
5. Test and slaughter policy will hardly be accepted especially by Fulani herdsmen even with compensation. This is because their prestige is in the number of animals they have and would not want to exchange it for money. Since brucellosis does not kill adult infected animals in epidemic proportion, they will hardly be convinced that their animals are infected.

They could rather sell the affected animal to another farmer or migrate away from the location to escape the policy.
6. Involvement of wildlife such as buffaloes also compounds control problem. These animals serve as reservoir hosts and continue to transmit the infection to nomadic herds in the bush or around wildlife parks. Even if it is possible to eradicate the disease in domestic animals, these wild animals will continue to serve as source of infection to the domestic animals.

7. Control programme will require considerable resources and organisation as well as the cooperation of herders and farmers. Inadequate laboratory facilities, lack of manpower and financial restrictions are serious constraints to the establishment of a brucellosis control programme in Nigeria.

2.26 PUBLIC HEALTH IMPORTANCE

Brucellosis is a zoonotic disease of major public health significance. It is widely distributed all over the world with regions of endemicity being Mediterranean, Middle East, Latin America and parts of Asia.

Humans are accidentally infected by *Brucella* organisms and are normally dead-end hosts. The disease primarily is an occupational risk in exposed professionals such as veterinarians, farmers, laboratory workers, abattoir workers and others who work with animals and their products. The maximum danger being the lambing, calving or kidding period. Others get infected through dairy products consumption without necessarily having contact with the animals. Humans are susceptible to such species like *B. melitensis*, *B. suis*, *B. abortus* and *B. canis*. Infection due to *B. melitensis* and *B. suis* often give rise to a severe and prolonged form of the disease.

Brucellosis in humans is a multisystemic, acute to chronic disease characterized by fever, headache, joint pains, musculo-skeletal pains, night sweating, malaise and wasting condition (Alausa, 1979; Adekolu-John, 1989; Currier, 1989; Falade, 2002). Symptoms of acute brucellosis are intermittent fever and flu-like conditions (McDermott and Arimi, 2002). The economic losses due to human brucellosis although difficult to estimate, result from physical and psychological suffering due to infection, hospitalization, cost of drugs and the loss of work or income due to illness, physical incapacity and reduction in productivity (WHO,1986).

2.27 ECONOMIC IMPORTANCE

Brucellosis causes economic losses through abortion, temporary infertility due to metritis, decreased milk production in dairy cows, deaths of young animals due to stillbirths, weak offspring and sterility, increased cost of animal replacement and lowered sale value of infected animals (Rikin, 1988; Radostits, et al., 1995; Ajogi *et al.*, 1996; FAO, 2004).

There are also indirect economic losses due to enforcement of control and eradication measures. These include cost of vaccines and vaccination, personnel cost and logistics. There are also losses due to compensation paid for slaughtered infected animal (FAO, 2004). The economic loss due to brucellosis in Nigerian livestock was assessed to be 224 million U.S Dollars by Esuruoso (1979). Ajogi and Akinwumi (2001) estimated annual losses in Wase and Wawa-Zange cattle reserves at N9.38 Million and N3.38 Million respectively. Others were by Chukwu (1985b) and Rikin (1988). Indirect losses especially those that require brucellosis-free status to access regional or international livestock markets, have not been estimated in sub-Saharan Africa but could be a constraint to future trade (McDermott and Arimi, 2002).

2.28 OCCURRENCE IN NIGERIA

2.28.1 Bovine brucellosis

Brucellosis is an endemic disease in Nigeria having been reported sporadically all over the country as far back as 1927. More of the reports come from established farms than from the nomadic cattle rearers who control about 90% of the animal population. Evidence of the disease has been demonstrated in small ruminants as well as cattle, dogs, pigs, horses and camels. Due to poor disease reporting system in Nigeria, the distribution of the disease in accurate proportion is not well documented. Similarly, surveillance has not been very efficient leading to poor determination of the incidence and prevalence of brucellosis in

Nigeria. The earliest reference to the occurrence of the disease in Nigeria is contained in the annual report of the Veterinary Department for the year 1927 in which 10 cases of contagious abortions were recorded (Anon, 1927). This was followed by Earshaw and O'Brien (1928) who reported cases of knee-joint hygroma in indigenous cattle. Hall (1934) diagnosed contagious bovine abortion in government ranch where sero-agglutination revealed a positive reactor rate of about 15 per cent.

The annual reports of the veterinary department in Nigeria for the year 1946 indicated increase in reactor rates among government owned cattle herds (Mettam, 1946, 1947, 1948). Various serological and cultural studies over the years have proved that brucellosis is widespread in Nigerian (Esuruoso, 1965; Adams and McKay, 1966; Banerjee and Bhatt, 1970; Esuruoso and Hill, 1971; Esuruoso and Van Blake, 1972; Esuruoso, 1974; Nuru, 1974; Nuru and Dennis, 1975; Eze, 1978; Falade, 1980; Bale and Kumi-Diaka, 1981; Eze, 1981; Agunloye *et al.*, 1988; Ocholi, 1990; Ngulukun, 2002). There were also reports from slaughter houses (Nuru, 1975; Chukwu, 1987; Cadmus *et al.*, 2006) corroborating with the above. Prevalence rates vary between 20-60%).

2.28.2 Caprine Brucellosis

The first evidence of brucellosis infection in goats was presented by Adams and McKay (1966) and later by Kramer *et al.* (1967). Since then little information was available until the first comprehensive reports of brucellosis among goats in Nigeria was presented by Falade *et al.* (1974).

Other reports on caprine brucellosis presented from various areas of Nigeria were, (Falade, 1978; Bale, 1980; Falade, 1980; Falade, 1981; Bale *et al.*, 1982) all revealing that prevalence rates varies between 2.1-14.5%. A recent study by Cadmus *et al.* (2006) in Ibadan, Oyo State revealed a prevalence of 0.86%.

2.28.3 Ovine Brucellosis

Early efforts at ascertaining causes of abortion in sheep failed until Okoh (1980a) confirmed the isolation of *B.abortus* from sheep in an investigation farm in Kano State of Nigeria. Subsequent serological (Okoh, 1980a; Falade and Shonekan 1982; Okewole *et al.*, 1988) and cultural studies (Bale *et al.*, 1982; Ocholi *et al.*, 2005a) have confirmed ovine brucellosis in Nigeria.

2.28.4 Brucellosis in Horses

Horses have recently gained popularity in Nigeria due to increasing interest in polo games and horse racing. There are very few reports available on the presence of the disease in horses. Bale and Kwanashie (1984) demonstrated *Brucella* antibodies in horses in Northern Nigeria where 14 (8%) out of 166 were reactors. The isolation of *B. abortus* from an aborted equine foetus (Oladosu *et al.*, 1984) confirmed the presence of the disease in horses in the country. Recently, *B. abortus* was also isolated from horses on a government-owned farm (Ocholi *et al.*, 2004). This is a further confirmation of the existence of equine brucellosis in Nigeria.

2.28.5 Brucellosis in Pigs

Because of differences in cultural and religious belief, Pig production in Nigeria is limited to only a few states from the middle belt to the southern parts. The production is in the hands of rural subsistent farmers mostly, with only a few of them attaining semi-commercial or commercial levels. The first report of swine brucellosis in Nigeria is contained in reports by Mettam (1947) and Adams and McKay (1966). Since then, there has been no sufficient information to indicate the distribution and prevalence of the disease in Nigeria. It is however certain that the disease is present in the country as a serological

study from 6 piggeries in Northern states of Nigeria showed that 12 (2.7%) out of 443 pigs sampled had *Brucella* antibodies, (Bale and Nuru, 1985).

2.28.6 Brucellosis in Dogs

In the past there were doubts as to the presence of canine brucellosis in Nigeria when serological survey by Falade (1977) found no antibodies to *Brucella* in dogs. However *B. canis* had been detected through serology where a prevalence rate of 28.6% was obtained (Adesiyun *et al.*, 1986) and through cultural recovery from a case of abortion in an imported Boxer (Okoh *et al.*, 1978). With the current upsurge in dog breeding using imported dog species and the attendant random movement of dogs through trade, the spread of brucellosis among dogs in the country is most likely to be increased. This is because puppies or adult dogs from infected kennels may be sold to non-infected kennels.

2.28.7 Brucellosis in Camels

Brucellosis in camels in Nigeria was first diagnosed based on serological survey by Okoh (1979) where 1.0% prevalence of *Brucella* antibodies was obtained. Subsequent serological test by Adamu *et al.* (1997) and Adamu and Ajogi (1999) showed increasing rates of 19.1% in three surveys and 12.5% in one abattoir surveys respectively.

2.28.8 Human Brucellosis

The distribution of human brucellosis is directly related to the distribution of animal brucellosis. Brucellosis is a widespread disease with millions of individuals at risk worldwide, especially in developing countries where a significant animal reservoir exists (Clarke, 2005). Therefore the risk in humans is a function of the risk in livestock and the livestock- human infection rate. In these countries, milk pasteurization is not routine and the consumption of raw milk and milk products is common.

The first case of brucellosis in a human being reported in Nigeria was in 1962 (Collard, 1962) when *Brucella* antibodies was demonstrated in the sera obtained from healthy persons in various parts of Nigeria. Between 1962 and 1967 records obtained from university college hospital, Ibadan indicated 9 cases of human brucellosis and *Brucella* species were isolated from 4 blood and one bone marrow culture (Falade, 1981). Most cases of human brucellosis were essentially an occupational hazard, occurring amongst workers in the livestock industry. Epidemiological studies revealed significantly higher prevalence of infections among occupationally exposed persons including herdsmen, abattoir workers, laboratory workers and veterinarians than occupationally non-exposed population such as blood donors and pregnant women (Falade, 1974; Alausa and Osoba, 1975; Alausa and Awoseyi, 1976; Alausa, 1979). Brucellosis in humans presents as a febrile flu-like illness with intermittent fever (Berman, 1981; Chukwu, 1987; Seifert, 1986).

2.29 TREATMENT IN HUMANS

Despite extensive studies for many years, the optimum antibiotic therapy for brucellosis is still in dispute. WHO recommends 600 to 900 mg of rifampicin and 200 mg of doxycycline daily for a minimum period of six weeks for adults. Some still claim that the long established combination of intramuscular streptomycin with an oral tetracycline gives fewer relapses. Infections with complications such as meningoencephalitis and endocarditic require combination therapy with rifampicin, tetracycline, and aminoglycosides. A combination of rifampicin and cotrimoxazole is the best treatment for uncomplicated cases in children. However relapses may not be completely ruled out.

2.30 SAFETY PRECAUTIONS IN BRUCELLA WORK

Brucellosis is one of the most easily acquired laboratory infections. World Health Organization (WHO) classified it in risk group III. There is therefore a great need for strict safety measures in working with Brucella infected animals as well as the environment where work on Brucella is carried out. Adequate accommodation, hygienic facilities together with good bacteriological techniques are necessary for safety.

3. When working on a potentially dangerous material, the operator should wear impermeable clothing, eye-shield, disposable gloves, mask, etc.
4. Mouth pipetting should be prohibited emphatically since it is a major cause of laboratory infection by the bacteria.
5. Prevention of aerosols is very important. Processes that cause frothing must be avoided. Screw capped bottles and tubes should be used.

Virulent suspensions should not be blown out of pipettes. After use, all pipettes, swabs sticks, bottles, Petri dishes should be immersed in a metal container that has lid and are autoclaved before handled.

Working with Biohazard hood class I should be under well protected wears such as hand gloves (long ones), mask, etc. The cabinet should be emptied and properly disinfected between each operation.

2.31 FIELD MATERIALS

All field materials such as aborted material, uterine discharges, milk and milk products are potentially dangerous materials to exposed personnel. Veterinarians, laboratory workers, abattoirs workers, animals attendants should take precautions. Such products should be handled using protective clothing and should be disposed off properly and all

contaminated areas disinfected properly after use. Personnel should never eat, drink or smoke while working in contaminated or infected environment as these serve as means of infection.

2.32 PACKAGING AND TRANSPORT OF SPECIMENS

Specimens should be collected in leak proof containers and should be well protected as mentioned above. The materials used for sample collection should be disinfected and sterilized after use.

CHAPTER THREE

MATERIALS AND METHOD

3.1 STUDY AREA AND SITES

The study was carried out in Plateau State which is located in the middle belt (North Central Geopolitical zone) of Nigeria. It lies between latitude 8.50° and 10.46° North and longitude 8.20° and 10.36° East. The state shares common boundaries with Kaduna state in the North and West, Bauchi state in the East, Nassarawa in the South and Taraba state in the South East. The State has a tropical climate with a near temperate temperature in the Northern part. It lies within the Guinea savannah vegetation zone of Nigeria with an average annual rainfall of 1200mm. The weather and rocky terrain are very conducive for livestock and arable crop production. The state is made up of 17 Local Government Areas which are divided into three geo-political zones namely: Plateau North, Central and South. The Northern zone is made up of 6 Local Government Areas, 5 in the Central zone and 6 in the Southern zone. Samples were collected from three Local Government Areas in each zone having the highest population of sheep and goats. The following LGAs were selected:

Plateau North:	Jos North, Jos South, Bassa LGAs
Plateau central:	Mangu, Bokkos, Pankshin LGAs
Plateau South:	Langtang North, Shendam and Quanpan LGAs

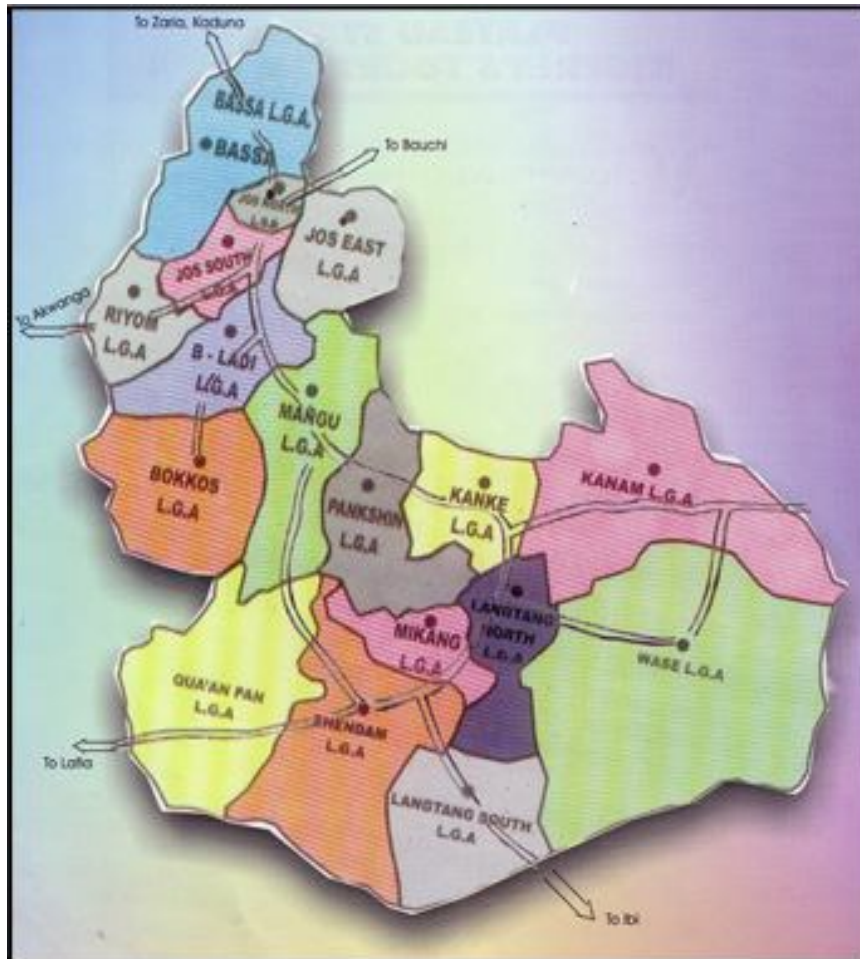
3.2 CRITERIA FOR LOCAL GOVERNMENT AREAS SELECTION

The criteria that were used for the selection of these Local Government Areas were: presence of large population of small ruminants, presence of a major market and presence of modern slaughter slabs or abattoir. The State ministry of Agriculture and Local government veterinary offices and extension officers were contacted to determine LGAs

that fit these criteria. Informations on the location of markets, farms and slaughter slabs or abattoir in each Local Government Area were obtained from Veterinary Extension officers.

3.3 DESCRIPTION OF STUDY SITES

PLATE 3.1: Map of Plateau State showing the 17 LGAs where the study was conducted.



Jos North, Jos South and Bassa LGAs are located at the extreme northern part of Plateau State. Jos North shares boundary with Bauchi State while Jos South and Bassa both share boundaries with Kaduna State.

Mangu, Bokkos and Pankshin LGAs are located in the centre of the State while Bokkos LGA shares boundary with Nassarawa State on its West, Mangu and Pankshin LGAs are bordered on the East by Bauchi State.

Langtang North, Quanpan and Shendam are located on the southern end and the lower part of the State. Quanpan is bordered on the South by Nassarawa, West by Kaduna State while Shendam is bordered on the East by Taraba State and on the South by Benue and Nassarawa States. Langtang North is bordered in the North by Kanke and Kanam LGAs, in the East by Wase, in the South by Langtang South and in the West by Shendam and Mikang LGAs respectively. The Southern zone is the hottest (30-40⁰c) zone of Plateau State and lies below the Plateau highlands. It is about 200-250km from the State Capital.

3.4 ANIMAL BREEDS

The most predominant breed of sheep in Plateau State is the Yankasa, which are distributed throughout the State. The Uda and Balami breeds are few and are found only in the State Capital and some few Local Government headquarters.

The predominant goat population is West African dwarf and Sokoto red. The West African dwarf goat makes the greater populations in the villages while the Sokoto red goats are found mostly in the state capital and Bukuru metropolis and a few in the other selected Local Government Areas .

3.5 HUSBANDRY SYSTEMS

The common animal husbandry system practised in the State is the extensive, seasonal confinement system. During the dry season, the animals are allowed to roam freely and fend for themselves within the community. They come home during the day to drink water and eat any available household wastes. In the evening, they return to their various households to rest for the night.

During the rainy season when crops are planted, the animals are usually taken out for grazing in the morning and brought back home in the late evening by a member of the household. They could also be tethered outside or within the compound and are provided grass throughout the wet season. Most households keep between 10 to 50 sheep and goats together in the same dwelling compound.

3.6 SAMPLE SIZE

The sample size was obtained based on the prevalence of the disease in Nigeria as reported by Bale (1982) which was 14.1% in sheep and 16.1% in Goats. This is because there is no available record on the prevalence of Brucellosis in small ruminant in Plateau State. There is also no current information on the population of sheep and goats in the state. Therefore based on the prevalence of 14.1% and 16.1% for sheep and goats respectively, the sample sizes were calculated to be 185 for sheep and 206 for goats (appendix I). However, 851 goats and 496 sheep were sampled from 9 Local Government Areas of Plateau State giving a total of 1,347 samples. The sample size was increased to allow for adequate coverage.

FIGURE 3.1: Distribution of Sheep and Goats Sampled from Nine LGA of Plateau State

LGA	Sheep	Goat	Total
Bassa	16	71	87
Bokkos	91	81	172
Jos-North	98	157	255
Jos-South	91	180	271
L – North	50	50	100
Mangu	94	147	241
Pankshin	9	64	73
Q/Pan	17	43	60
Shendam	30	58	88
Total	496	851	1,347

3.7 SAMPLING PROCEDURE/COLLECTION OF BLOOD

Villages with high number of small ruminants based on informations obtained from the Agric Department of every selected LGA were given numbers. Selection of a village to be sampled was based on a simple random sampling. Ten villages were sampled in each LGA.

Clutch sampling procedure (Thrusfield, 1997) was used to select the animals for bleeding. A systematic random sampling was used and every third animal in a flock or at slaughter was sampled throughout the sample collection period. Samples from slaughter slabs and abattoir were collected first in each LGA before field sampling started. This was done to avoid the possibility of sampling an animal twice.

An assistant was used to restrain live animals properly and then five (5) ml of venous blood was collected from the jugular vein into sterile vacutainers on which the species, breed, age, sex, date and LGA of the animals were well labelled. The same amount of blood was also collected at the abattoirs or slaughter slabs directly from the jugular vein of a slaughtered animal into a similarly well-labelled bottle.

3.8 HANDLING OF SERUM SAMPLES

The blood samples were allowed to clot by laying them down in a slanting position, then transported to the laboratory in a leak-proof container with ice packs and centrifuged at 1000rpm for 5 minutes. Serum was then decanted into 5ml plastic tubes and stored in the refrigerator at -20°C until required for testing.

3.9 PROCUREMENT OF ANTIGENS AND CONTROL SERA

3.9.1 Rose Bengal Plate Test (RBPT) *Brucella abortus* antigen

This was obtained from Veterinary Laboratories Agency, New Haw, Weybridge, England and Onderstepoort Veterinary Institute, South Africa.

Brucella abortus antigen has been recommended for use in tests involving all the smooth brucella species (*B.abortus*, *B .melitensis*, *B. suis*) because they have no antigenic variations. The use of species specific antigens for serological tests is only limited to *B .canis* and *B. ovis* which are rough brucella species.

3.9.2 Serum Agglutination Test (SAT) *Brucella abortus* antigen

This antigen was obtained from Onderstepoort Veterinary Institute, South Africa.

3.9.3 Positive and Negative Control Sera

These were supplied by Central Veterinary Laboratory, New Haw, Weybridge, England through National Veterinary Research Institute, (NVRI) Vom, Plateau State, Nigeria.

3.10 PREPARATION OF PHENOL SALINE

Phenol saline was prepared as described by Morgan *et al.* (1978) by dissolving 85g of sodium chloride (NaCl) and 50g of phenol crystals in 10 litres of distilled water and mixed thoroughly.

3.11 SEROLOGICAL TESTS

Serum samples were tested for *Brucella* antibodies by Rose Bengal plate test (RBPT) and Serum Agglutination Test (SAT) as described by Alton *et al.* (1988).

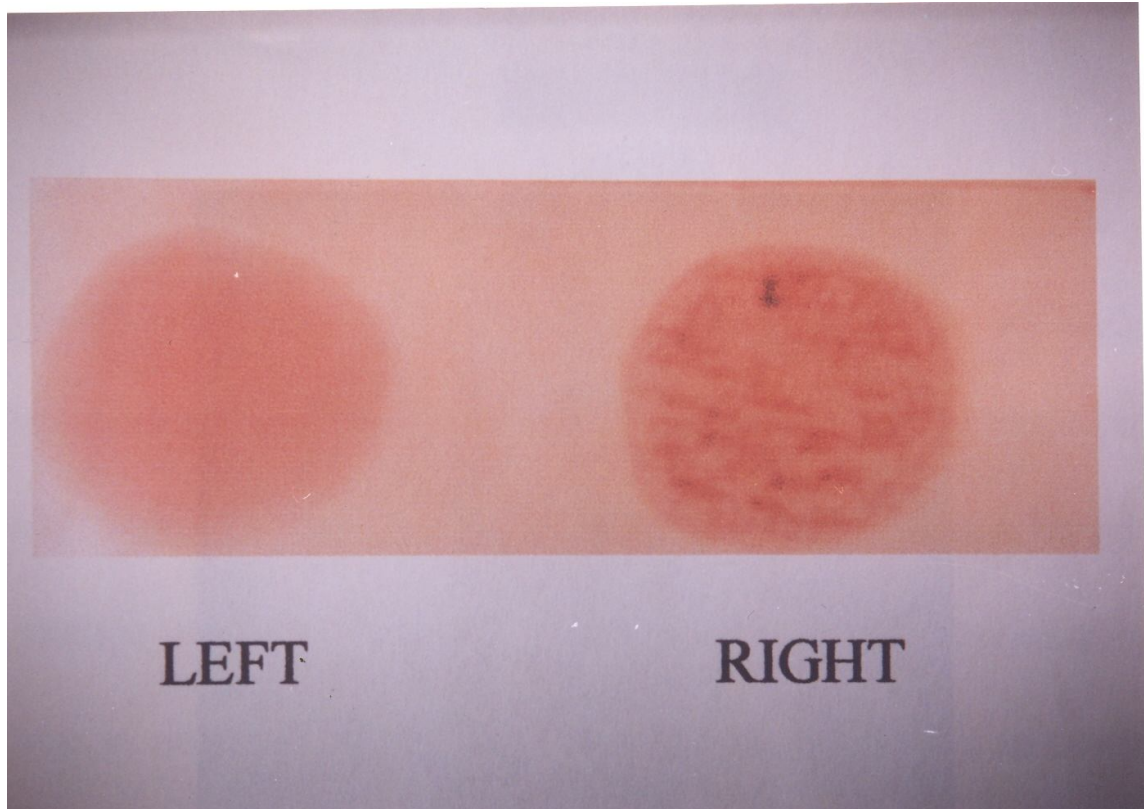
3.11.1 Rose Bengal Plate Test (RBPT)

This test was carried out using Rose Bengal plate test antigen obtained from Onderstepoort Veterinary Institute (OVI) South Africa, according to the method described by Alton *et al.* (1988).

Thirty (30) microlitre of antigen was placed on a white ceramic tile and the same volume of 30 microlitre test serum was placed beside the antigen. The two were mixed thoroughly using sterile applicator stick and rocked gently for 4 minutes after which they were observed for agglutination.

The formation of distinct pink granules (agglutination) was recorded as positive while the absence of granule formation was recorded as negative.

Plate 3. 7: Rose Bengal plate test (RBPT)results



Left- Negative (no agglutination)

Right- positive (shows agglutination)

2.11.2 Serum Agglutination Test (SAT)

This was carried out using the agglutination method as described by Alton *et al.* (1988). The SAT antigen was obtained from Onderstepoort Veterinary Institute (OVI), South Africa.

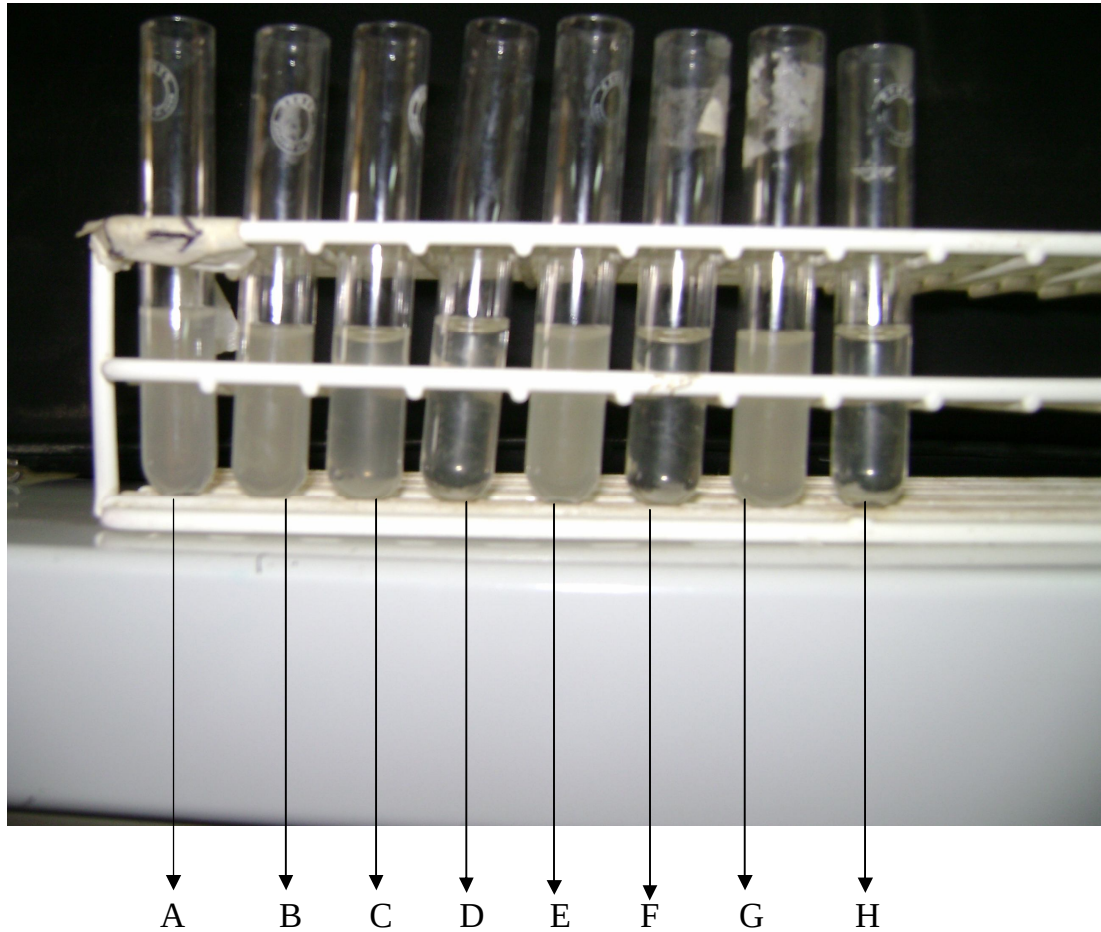
The British method in which five test tubes were required per sample was used. For the 1st tube, 0.8ml of phenol saline was dispensed while 0.5ml was applied to the 2nd, 3rd 4th and 5th tubes using microtitre pipette fitted with corresponding tips. Similarly, 0.2ml of the test serum was added to the 1st tube and mixed properly. Serial dilution was then carried out by pipetting 0.5ml of mixture in the 1st tube to 2nd, then to the 3rd, then to the 4th and then the 5th tubes. The final 0.5 ml from the 5th tube was discarded.

0.5ml of antigen (diluted 1:10 with phenol saline) was added to all the tubes. The tubes were covered, shaken and incubated at 37⁰C for 20 hours.

The antibody titre of a serum sample was the last dilution giving a definite 1+ agglutination and above. In this SAT, reactions at dilutions of 1/10, 1/20, 1/40, 1/80, and 1/160 represent agglutination activity of >12.5, >25, >50, >100, >200 IU/ml respectively (Morgan *et al.*, 1978; Ogundipe *et al.*, 1993). Titres of 1:40 (50 IU/ml) and above were taken as diagnostic for *Brucella abortus* (Morgan *et al.*, 1967; Ogundipe *et al.*, 1994).

Known positive and negative control sera were set up along with the test sera.

Plate 3.9: Serum agglutination test (SAT) results after 20 hours of incubation.



KEY

A B E G = Negative SAT results

C D F H = Positive SAT results

POSITIVE Showing very clear tubes with distinct agglutination floculi at the bottom.

NEGATIVE Showing homogeneously cloudy tubes

3.11.3 Degree of Clearance Reading

++++	100% agglutination (background very clear)
+++	75% agglutination (background clear)
++	50% agglutination (background slightly cloudy)
+	25% agglutination (background moderately cloudy)
-	No agglutination (remain homogenously cloudy)

X/Y: X= degree of agglutination, Y= dilution (denominator)

The results were recorded to show the degree of agglutination (clearance) in the numerator and the dilution (denominator). Example: 3/40 = 3+ agglutination at a dilution of 1/40 (third tube).

3.12 CONSIDERATION OF THE SEROLOGICAL TESTS

The results of the two tests (RBPT and SAT) were considered in parallel. This means that a sample was accepted as positive if it tested positive to either one or both of tests.

3.13 METHOD OF DATA PRESENTATION AND ANALYSIS

3.13.1 Data presentation

Data generated from this study are presented in Tables and Figures. The prevalence of brucellosis in sheep and goats was calculated as percentage of animals that were positive to the serological tests in relation to the total number of animals tested. The prevalence rates in sheep and goats, males and females were calculated. Prevalence in different geopolitical zones of the state and based on age and between field samples and slaughtered animals were also calculated.

3.13.2 Statistical Analysis

Data obtained were subjected to statistical analysis using Chi-square (X^2) (Snedecor and Cochran, 1980; Uzoagulu, 1998) to see the association of brucellosis with sex, age, zones and between field samples and slaughtered reactors. The test was carried out at 5% level of significance.

The Kappa statistic (Noordhuizen, *et al.*, 1997) was used to determine the level of agreement due to chance and provides a more realistic evaluation of the actual level of agreement for the pair of tests.

CHAPTER FOUR

RESULTS

4.1 PREVALENCE OF BRUCELLOSIS IN SMALL RUMINANTS IN PLATEAU STATE

Out of a total of 496 sheep sampled, 46 (9.3%) and 26 (5.2%) were positive for brucellosis by RBPT and SAT respectively. Also out of 851 goats sampled, 86 (10.1%) and 51 (6.0%) were positive by RBPT and SAT respectively (Table 4.1). The overall prevalence is 14.5% and 16.0% for sheep and goats, respectively.

4.2 PREVALENCE OF BRUCELLOSIS IN EACH OF THE LGAs

In Bassa LGA where 16 sheep and 71 goats were sampled, there was no positive reaction for sheep by the RBPT while 1(6.3%) was positive by SAT. Out of 71 goats, 4(5.6%) were positive by RBPT while 6(8.5%) were positive by SAT. One (1.2%) sample was positive by both tests (Table 4.2 and Appendix vi).

Similarly, out of a total of 91 sheep and 81 goats screened from Bokkos LGA, 10(11.0%) and 5(5.5%) sheep were positive for *Brucella* antibodies by RBPT and SAT respectively and 6(7.4%) and 5(6.2%) goats were positive by RBPT and SAT respectively. Two (1.2%) samples were positive by both tests (Table 4.2 and Appendix vi).

Out of a total of 98 sheep and 157 goats in Jos North LGA, 7 (7.1%) and 11 (11.2%) sheep and 19(12.1%) and 18(11.5%) goats were positive for brucellosis by the Rose Bengal Plate test (RBPT) and Serum agglutination test (SAT) respectively. Thirteen (5.1%) samples were positive by both tests (Table 4.2 and Appendix vi).

A total of comprises 91 sheep and 180 goats were sampled from Jos South LGA. 13(14.3%) and 3(3.3%) sheep were positive by RBPT and SAT respectively while

15(8.3%) and 10(5.6%) goats were positive by RBPT and SAT respectively. Three (1.1%) samples tested positive by both tests (Table 4.2 and Appendix vi).

Out of a total of 50 sheep and 50 goats screened from Langtang North LGA, 5 (10.0%) and 2 (4.0%) Sheep were positive for *Brucella* antibodies by RBPT and SAT respectively while 5(10.0%) and 3(6.0%) goats were positive by RBPT and SAT respectively. Two (2%) tested positive for both tests (Table 4.2 and Appendix vi).

Out of 96 sheep screened from Mangu LGA, 6(6.3%) and 1(1.0%) were positive by RBPT and SAT respectively while 23(15.6%) and 6 (4.1%) out of a total of 147 goats were positive by RBPT and SAT respectively. One (1.1%) sample was positive by both tests (Table 4.2 and Appendix vi).

Out of a total of 9 sheep screened from Pankshin LGA 1 (11.1%) was positive for *Brucella* antibody by RBPT, 1 (11.1%) was positive for *Brucella* antibodies by SAT respectively while 9 (14.1%) and 3 (4.7%) goats were positive for *Brucella* antibodies by RBPT and SAT respectively out of 64. Two (2.7%) samples were positive by both tests (Table 4.2 and Appendix vi).

Out of a total of 17 sheep screened from Quan/pan LGA, 1(5.9%) and 2 (11.6%) were positive for *Brucella* antibodies by RBPT and SAT respectively while 7(16.3%) and 1(2.3%) out of 43 goats were positive by RBPT and SAT respectively. One (1.7%) sample was positive by both tests (Table 4.2 and Appendix vi).

Similarly, from a total of 30 Sheep and 58 Goats from Shendam LGA, 4(13.3%) and 2(6.7%) sheep were positive by RBPT and SAT respectively while 5(8.6%) and 1(1.7%) goats were positive by RBPT and SAT in the same order. One (1.1%) sample was positive by both tests (Table 4.2 and Appendix vi).

Table 4.1: Overall seroprevalence of Brucellosis in Sheep and Goats in Plateau State.

Small ruminant	Total	RBPT +ve	SAT +ve	RBPT/SAT+ve	Overall % +ve
	sample				
Sheep	496	46(9.3%)	26(5.2%)	72	14.5%
Goats	851	86(10.1%)	51(6.0%)	137	16.0%

TABLE 4.2: Distribution of brucellosis Seroprevalence in the 9 LGAs of Plateau state

LGA	SHEEP			GOATS		
	No Tested	RBPT+(%)	SAT+ (%)	No Tested	RBPT +(%)	SAT +ve%
Bassa	16	0 (0.0)	1 (6.3)	71	4 (5.6)	6 (7.0)
Bokkos	91	10 (11.0)	5 (5.5)	81	6 (7.4)	5 (6.2)
Jos-North	98	7 (7.1)	8 (8.2)	157	19 (12.1)	18 (11.5)
Jos-South	91	12 (13.2)	3 (3.3)	180	9 (5.0)	10 (5.6)
Langtang North	50	5 (10.0)	2 (4.0)	50	4 (8.0)	3 (6.0)
Mangu	94	6 (6.4)	1 (1.1)	147	23 (15.6)	6 (4.1)
Pankshin	9	1 (11.1)	1 (11.1)	64	9 (14.1)	2 (3.1)
Q/Pan	17	1 (5.9)	3 (17.6)	43	7 (16.3)	1 (2.3)
Shendam	30	4 (13.3)	3 (10.0)	58	5 (8.6)	1 (1.7)
Total	496	46 (9.3)	26 (5.2)	851	86 (10.1)	51 (6.0)

4.3 SEROPREVALENCE OF BRUCELLOSIS IN THE THREE ZONES OF PLATEAU STATE

Table 4.3 presents Sero-prevalence of brucellosis in the three zones of Plateau state.

In Plateau North where a total of 205 sheep and 408 goats screened, 19 (9.26%) and 12(5.9%) Sheep were positive by RBPT and SAT respectively while 32(7.8%) and 33(8.1%) goats were positive by RBPT and SAT, respectively.

Out of a total of 196 sheep and 292 goats screened from the Central zone of Plateau State, 17(8.7%) and 7(3.8%) Sheep were positive by RBPT and SAT respectively while 38(13.0%) and 13 (4.5%) goats were positive by RBPT and SAT, respectively.

From a total of 97 sheep and 151 goats screened from the southern zone of Plateau State, 10 (10.3%) and 7(7.2%) Sheep were positive by RBPT and SAT respectively while 16(10.6%) and 5(3.3%) goats were positive by RBPT and SAT, respectively.

The highest prevalence in Goats (13.6%) was recorded in the Central zone of Plateau State. 10.6% was recorded in Southern zone of Plateau while the lowest (7.8%) was in the Northern zone of Plateau State. For sheep, the highest prevalence (10.3%) was from the Southern zone of Plateau State, Northern zone of Plateau (9.26%) while the lowest (8.7%) was in the Central zone of Plateau. Variations in management practises, dynamics of animal movement and habit of borrowing or lending animals for breeding by local farmers are responsible for these differences.

TABLE 4.3: Prevalence of brucellosis in the three Zones of Plateau State

Zone	Sheep			Goats		
	No tested	RBPT +(%)	SAT + (%)	No tested	RBPT+ (%)	SAT + (%)
North	205	19 (9.3)	12 (5.9)	408	32 (7.8)	33 (8.1)
Central	194	17 (8.7)	7 (3.6)	292	38 (13.0)	13 (4.5)
South	97	10 (10.3)	7 (7.2)	151	16 (10.6)	5 (3.3)

Plateau north / Plateau Central ($X^2 = 0.0175$), ($P > 0.050$)

Plateau North / Plateau South ($X^2 = 2.4907$), ($P > 0.05$)

4.4: SEROPREVALENCE OF BRUCELLOSIS IN SHEEP AND GOATS IN PLATEAU STATE BASED ON BREEDS.

Out of a total of 496 sheep sampled, 450 were Yankasa breed while 46 were Uda and Balami together. Out of the 450 Yankasa breed, 38(8.4%) were positive by RBPT while 22(4.9%) were positive by SAT.

From the 24 Uda breed, 5(20.8) and 2(8.3%) were positive by RBPT and SAT respectively. Out of the 22 Balami breed, 3(13.6%) were positive by RBPT while 2(9.1%) were positive by SAT. The prevalence in Yankasa breed was lower compared to that of the Uda and Balami (Table 4.4a).

From a total of 851 goats sampled, 750 were West African Dwarf (WAD) goats while 101 were Red Sokoto goats (RSG). Out of the 750 WAD goats 70(9.3%) were positive by RBPT while 43(5.7%) were positive by SAT.

Similarly, out of 101 Sokoto Red goats, 16(15.8%) were positive by RBPT while 8(7.9%) were positive by SAT. The prevalence of brucellosis was higher in the Red Sokoto goats compared the West African Dwarf goats (Table 4.4b).

4.4a: Seroprevalence of brucellosis in Sheep based on Breeds

Breeds	No Tested	RBPT +ve	% +ve	SAT +ve	% +ve
Yankasa	450	38	8.4	22	4.9%
Uda	24	5	20.8%	2	8.3%
Balami	22	3	13.6	2	9.1%

($X^2= 3.9076$), ($P<0.05$)

Table 4.4b: Seroprevalence of brucellosis in Goats based on Breeds

Breeds	No Tested	RBPT +ve	%	SAT +ve	%
WAD*	750	70	9.3	43	5.7
RSG*	101	16	15.8	8	7.9

* WAD=West African dwarf goats

RSG= Red Sokoto goats

($X^2=4.5286$), ($P< 0.05$)

4.5 SEROPREVALENCE OF BRUCELLOSIS IN GOATS AND SHEEP IN PLATEAU STATE BASED ON SEX

From a total of 496 sheep tested, 159 were males while 337 were females. Out of the 159 males, 22 (13.8%) were positive. Similarly, 53 (15.7%) out of 337 females were positive for Brucella antibodies (Table 4.5).

A total of 851 goats were tested out of which 333 were males while 518 were females. From the 333 males, 46 (13.8%) were positive for Brucella antibodies while 98 (18.9%) of the 518 female goats were positive (Table 4.5).

The prevalence of 17.7% in female Sheep and Goats combined was higher compared to that of male sheep and goats combined which was (13.8%) (Table 4.5).

Table 4.5: Prevalence of brucellosis in goats and sheep in Plateau State based on sex.

Animal	Males			Females		
	No tested	No +ve	% +ve	No Tested	No +ve	% +ve
Goats	333	46	13.8	518	98	18.9
Sheep	159	22	13.8	337	53	15.7
Total	492	68	13.8	855	151	17.7

Goats $(X^2 = 3.998), (P < 0.05)$

Sheep $(X^2 = 0.275), (P > 0.05)$

Total $(X^2 = 3.572), (P > 0.05)$

4.6 SERO-PREVALENCE OF BRUCELLOSIS IN SHEEP AND GOATS IN PLATEAU STATE BASED ON AGE

Out of a total of 496 sheep, 458 were adult (6 months and above) while 38 were young (less than 6 months). Similarly, out of 851 goats, 793 were adult while 58 were young. This shows that there were more adult sheep and goats screened compared to the young ones. This is because the young animals were sampled only from field animals. All the samples collected from slaughtered animals were all from adults.

Out of the 458 adult sheep, 81(17.7%) were positive to the two tests combined while 8 (21.0%) of the young were also positive by the two tests ((Table 4.6).

Out of the 793 adult goats, 135(17.0%) were positive to both tests while 13 (22.4%) of the 58 young were positive by the two tests (Table 4.6)..

The overall result however shows a lower prevalence in the adults (17.3%) compared to the young (21.9%) (Table 4.6).

Table 4.6: Prevalence of brucellosis in Sheep and Goats in Plateau State based on age

Animal	Adults			Young		
	No tested	No +ve	% +ve	No tested	No +ve	% +ve
Sheep	458	81	17.7	38	8	21.0
Goats	793	135	17.0	58	13	22.4
Total	1251	216	17.3	96	21	21.9

Sheep: ($X^2 = 0.2754$), (P>0.05)

Goats: ($X^2 = 1.0928$), (P>0.05)

4.7 SERO-PREVALENCE OF BRUCELLOSIS FROM FIELD SAMPLES AND SLAUGHTER ANIMALS

The seroprevalence of brucellosis among field animals and slaughtered animals for both sheep and goats is here presented (Table 4.7 and 4.8).

Out of a total of 496 sheep sampled, 287 were field samples while 209 were slaughter slabs/ abattoir samples. From the 287 field samples, 42(14.6%) were positive while 34(16.3%) out of the 209 samples obtained from slaughter slabs and abattoir were positive, (Table 4.7). There were more sheep sampled from the field compared to those from the slaughter slabs/abattoir.

Out of a total of 851 goats sampled, 294 were field samples while 557 were slaughter slabs and abattoir samples. Out of the 294 field samples, 57(19.4%) were positive (Table 4.8). Similarly, out of the 557 samples from slaughter slabs and abattoir, 104(18.7%) were positive.

A total of 581 samples comprising of 287 sheep and 294 goats were collected from the field out of which 99(17.0%) were positive.

A total of 766 samples comprising of 209 sheep and 557 goats were collected from slaughter slabs and abattoir out of which 138(18.0%) were positive. There were more samples from slaughtered animals compared to field animals so there was more likelihood of more positive animals from the slaughter houses compared to those from the field.

Table 4.7: Sero-prevalence of brucellosis in sheep and goats from field samples in Plateau State

Animal species	No tested	No +ve	%+ve
Sheep	287	42	14.6
Goats	294	57	19.4
Total	581	99	17.0

Sheep ($X^2 = 0.2494$), ($P > 0.05$)

Goats ($X^2 = 0.064$), ($P > 0.05$)

Table 4.8: Sero-prevalence of brucellosis in sheep and goats from abattoir/ slaughter slabs samples in Plateau State

Animal	No tested	No +ve	% +ve
Sheep	209	34	16.3
Goat	557	104	18.7
Total	766	138	18.0

$$X^2=(3.393), (P>0.05)$$

4.9 BRUCELLA ANTIBODY TITRE PROFILE IN SHEEP IN THE VARIOUS LGAs

An overall antibody titre obtained in sheep in all the LGAs by Serum Agglutination test (SAT) is here presented (Appendix IV). Thirty five samples were positive on screening but on titration, 9 had titres below 1:40 (>50 iu/ml) and were considered negative. 26 had titres between 1: 40 (>50 iu/ml to 1: 160(>200 iu/ml) and were confirmed positive. Jos North LGA had the highest SAT positive samples (8) and the highest titre (1: 160) while Bassa, Mangu and Pankshin had the least positive samples of one each, all at titres of 1:40. Bokkos had 5 while Jos South and Quanpan had 3 positive samples each and all had titres between 1: 40 to 1:80. Langtang North and Shendam had 2 positive samples each and both had titres of 1: 40.

4.10 ANTIBODY TITRE PROFILE FOR BRUCELLOSIS IN GOATS IN THE NINE LGAS.

Antibody titre profile as determined by SAT for goats in all the selected LGA is as presented (Appendix V). 70 samples were positive on screening but on titration, 19 had titres of below 1: 40 (<50 iu/ml) and were considered negative. 51 had titres between 1:40 (>50 iu/ml and 1:160 (>200 iu/ml) and were confirmed positive. The highest positive (18) was recorded in Jos North LGA which also had the highest titre of 1: 160 (>200 iu/ml). The least positive (1) was in Quanpan and Shendam LGAs. The second highest was Jos South having 10 positive. Mangu had 6 while Bassa and Bokkos had 5 positive each. Langtang North had 3 while Pankshin had 2.

4.11 AGREEMENT OF POSITIVE SERA BETWEEN RBPT AND SAT

Out a total of 1397 sera screened in this work, 189 were positive either by RBPT, SAT or both. 27(14.3%) were positive to both RBPT and SAT, 114(60.3%) were positive by RBPT only, while 48 (25.4%) were positive by SAT only (Appendix III).

CHAPTER FIVE

5.0

DISCUSSION

This study reveals brucellosis prevalence of 9.2% by RBPT and 5.2% by SAT in sheep and 10.2% by RBPT and 6.0% by SAT in goats and an overall prevalence of 14.5% in sheep and 16.0% in goats indicating a high prevalence rate. To the best of my knowledge, this appears to be the first comprehensive serological study of small ruminant brucellosis in Plateau State. There is no information on any previous work of this nature that has been conducted in small ruminant brucellosis in the State.

These prevalences agree with those by Okoh (1980) who reported a prevalence of 14.5% in sheep in Kano and Bale *et al.* (1982) who conducted a study in Northern Nigeria and reported a prevalence of 14.1% in sheep and 16.1% in goats. The findings in this study are however higher compared to those by Okewole *et al.* (1988) who reported 12.05% brucellosis prevalence in sheep and 9.4% in goats in Northern Nigeria. Other reported prevalences which are lower compared to the findings of this work include those by Brisibe *et al.* (1993) who reported 2.5% to 8.8% in sheep and 2.8% in goats in Maiduguri Metropolis and Shehu *et al.* (1999) who reported 6.6% in sheep and 4.75% in goats in a seroprevalence study of brucellosis in ruminants in Bauchi state.

Falade and Shonekan (1982) reported 2.56% in sheep and 9.0% in goats and Ogundipe *et al.* (1993) reported 5.88% all in Oyo State which are also lower compared to these finding.

In a recent survey of Brucellosis in seven government farms in Northern Nigeria, Bale, *et al* (2003) reported a prevalence of 15.9% in sheep and 34.8% in goats which are higher compared to the findings in this study. Another higher report is that by Ojo *et al.* (2007) in a goat farm in Abeokuta which revealed 45.7% prevalence.

Based on the findings of this study, brucellosis is endemic in Plateau State and the prevalence is high among sheep and goats in the State. The prevalence appears to be widespread in all the Local Government Areas surveyed. The high prevalence and the wide distribution are not surprising since small ruminants are not being vaccinated against brucellosis in Plateau State and in Nigeria. So far there has been no effort made for the prevention and control of brucellosis in small ruminants in the state and Nigeria as a whole. This leaves a gap in the effort to control brucellosis in our domestic animals in either Plateau state or Nigeria in general. This is because as long as small ruminants continue to remain unvaccinated, they will become infected and serve as reservoirs of the organism thereby spreading it to cattle and humans.

Secondly, the result is not surprising given that small ruminants from different households are allowed to roam about in communal grazing areas where they get mixed up with others from neighbouring areas which may be brucella infected animals. Further more, most small ruminant farmers (70%) in Plateau state borrow or lend breeding males to other farmers or even take females to be served at a neighbour's farm. All these tend to serve as easy means of transmission of brucellosis between farms, neighbouring villages and Local Government Areas.

Although the prevalence is widespread, differences do occur from one LGA to the other and between sheep and goats in each of the LGA. This may be due to differences in management system practised in each LGA, for instance, seasonal confinement system is practised mostly in LGAs in the Southern and Central zones of Plateau while semi-intensive and zero grazing are mostly practised in LGAs that make up the State Capital. The rate of introduction of (infected) replacement animals into the different Local

Government Areas may also contribute the spread of infection from their previous locality to their new destinations.

Also there is usually lack of effective control posts to check the health status of small ruminant as they are transported across the states. The absence of strict sanitary measures put in place to reduce contamination of the environment after an abortion by infected animals also facilitates the spread of infection within a flock (Anon, 2001).

Although the prevalence in sheep is lower than that in goats, there is no significant difference between the prevalences ($X^2 = 0.5986$, $P > 0.05$). The reason for higher seropositivity among goats compared to sheep, although not statistically significant, may be as a result of differences in the sample size of goats compared to sheep. Another reason could be the difference in the feeding habit of sheep and goats. Goats being browsers may be able to pick more brucella infected materials than sheep as they browse on pasture and contaminated materials on or close to the ground surface (Kabagambe, *et al.*, 2001). Sheep however are more selective and tend to pick pasture from the tips higher above the ground thereby avoiding contaminants from the ground. It is therefore possible to have a higher infection rate in goats than sheep as revealed in this study.

The higher prevalence of brucellosis in goats (16.0%) compared to sheep (14.1%) in this study is similar to that reported by Bale *et al.* (1982) which was 16.1% in goats and 14.1% in sheep. The results also agreed with the report by Falade *et al.* (1981) who reported 9.0% in goats compared to 4.8% in sheep. Brisibe *et al.* (1993) in their study reported higher prevalence of 2.8-11.0% in goats compared to 2.5-8.8 in sheep also agree with the findings of this study. Recent studies Bale *et al.* (2003) in seven government farms in Northern Nigeria and Ojo *et al.* (2007) in Abeokuta revealed high prevalences of 34.8% and 45.75% respectively in goats.

There is however a contrary finding by Marrison *et al.* (1975) and Okewole *et al.* (1988) who reported higher prevalence rates in sheep (1.8-14.1%) compared to goats (1.5%) and 12.05% in sheep compared to 9.4% in goats, respectively .

Although Krammer *et al.* (1967) carried out their study only in sheep alone in Eastern Nigeria and only in goats alone in Northern Nigeria; they reported a higher prevalence of 1.0% in goats compared to 0.87% in sheep. Okoh (1980) worked on only sheep in Kano and reported a prevalence of 14.5% while Falade and Shonekan (1982) reported 2.56% prevalence in sheep in Ibadan. The study carried out by Falade and Sellers (1974) in goats in 10 states of Nigeria revealed a prevalence of 4.27% while Adams and McKay (1966) reported zero prevalence in both sheep and goats in government farms in Eastern Nigeria.

Just as prevalence of brucellosis is widespread in Nigeria and varies from one state to the other, the prevalence of brucellosis also varies from one LGA to another in Plateau state. There are areas of low and high prevalences in different parts of Plateau state. This may not be unconnected to differences in husbandry practices in the different LGAs as well as level endemicity of the disease in the different LGAs. The dynamics of animal trade and movement in and out of the various LGAs could also be responsible for the variation in prevalence.

Although there were variations in the prevalence of brucellosis in sheep and goats from Plateau North, Plateau Central and Plateau South, the differences were however not statistically significant ($P>0.05$).

The rate of introduction of non indigenous sheep (Uda and Balami) and Sokoto Red goats from outside the state is more in the Northern zone of Plateau State because of the location of the State Capital in this zone as well as being the gateway from the Northern States of

Nigeria from where these non indigenous small ruminants are brought. Some of these animals may be carriers of brucella infection as there was significantly higher prevalence in these compared to the indigenous Yankasa sheep and the West African dwarf goats. Secondly, more of the animals in this zone are kept in confinement (intensive management) than other zones, being largely urban dominated. This may be responsible for the high prevalence in Plateau North as infection is easily transmitted within the entire herd under intensive management system particularly in zero-grazing (Kabagambe *et al.*, 2001) commonly practised in the State Capital.

Plateau Central and Southern zones being largely rural settings, practise free range management system. The use of all the rocky terrains as communal grazing areas for both cattle and small ruminants in Plateau central could favour the spread of brucellosis from cattle to small ruminants and from one small ruminant herd to the other. Susceptible animals are likely to graze on pastures contaminated by infected cattle or infected small ruminant herds also using the same grazing area and thus become infected. This scenario has been reported by Kabagambe *et al.* (2001). However, as long as animals remain on free range, the rate of infection will be low as animals do not spend time on contaminated areas. Plateau South seems to have a higher prevalence compared to Plateau central even though it is the zone with the lowest population of small ruminants. There was a crisis which devastated both human and animal population in this zone. The farmers are restocking their farms with animals from neighbouring areas. The indiscriminate introduction of these replacement animals from other areas may be responsible for the relatively high prevalence in this zone. This is because either the animals were brought in with the infection or the area is highly infected causing immediate infection of introduced animals since no consideration was given to the brucellosis status of the introduced animals or that of the area. Another reason is the high humidity (75% to 80%) in the

Southern zone of Plateau State. At 40% relative humidity, brucella can survive in the soil for 144 days (Bercovich, 1998). This implies that brucella can survive longer in this zone being highly humid compared to the other zones of the State making infection and re-infection of susceptible animals possible.

There are very few breeds of sheep and goats in Plateau state. The Yankasa sheep and the West African dwarf goats are the indigenous and predominant breeds in the state. These are mostly reared on free range and do not experience strict confinement. This makes it almost impossible for them to remain in a contaminated area beyond a few seconds and therefore unlikely to get infection from such an area. This is possibly the reason why the prevalence of brucellosis in these breeds is low in this study.

The Balami and the Uda breeds of sheep and the Sokoto red goats are mostly brought in from the far north by traders and private individuals who can afford them. Secondly, they are mostly kept in strict confinement and fattened under zero grazing. They are usually found only in the urban and semi-urban areas of the state. Strict confinement and or in association with poor hygienic practices encourage the spread of brucellosis from one infected animal to the entire herd under confinement. It is therefore not surprising that the prevalence of brucellosis in these breeds is higher compared to that of the Yankasa and the West African dwarf breeds of sheep and goats respectively. The difference in prevalence between the Yankasa and Uda and Balami and between West African dwarf goats and Sokoto red goats are statistically significant ($P < 0.05$).

This study considered sex as an important factor in the epidemiology of brucellosis because of the great affinity of brucella to reproductive organs especially in pregnant animals. We therefore tried to compare the prevalence between male and female animals

sampled. The result shows a higher prevalence (15.8%) in female sheep than male sheep (13.8%). The difference was however not statistically significant ($P > 0.05$).

Similarly, a higher prevalence (18.9%) was recorded among female goats compared to the males (13.8%). There was a significant difference between the prevalence in male and female goats ($P < 0.05$).

This shows that sex plays a significant role in seroprevalence of brucellosis, though both males and females are susceptible. The higher prevalence in females compared to males in both species in this study may be due to higher sample size of females compared to males. There is also the factor of a pregnancy hormone called erythritol which enables brucella to multiply rapidly in the uterus during pregnancy. This may be responsible for higher prevalence rates in the females compared to the males (Smith *et al.*, 1972; Corbel, 1988).

This study investigated the prevalence of brucellosis in adult and young animals sampled to establish its relationship with age. The prevalence was higher in the young (21.9%) than in the adult (17.3%) even though there were more samples tested from the adult than the young animals. This was however not statistically significant ($P > 0.05$).

This result is contrary to the fundamental fact that brucellosis is more of a disease of reproductively matured animals than the young. The high prevalence in the young in this study may suggest that some of the young animals sampled might have acquired the infection through suckling of milk from infected dams or through transplacental transmission from the dam to the fetus in-utero. It could also be due to the presence of maternal antibodies in some of the young animals rather than due to infection. Calves which become infected through ingestion of infected milk could develop antibodies and also shed brucella organisms in their faeces thereby contaminating the grazing grounds

(Banerjee and Bhatta, 1970; Arthur *et al.*, 1982), thus serving as source of infection to other animals. The prevalence of brucellosis in the old animals is not surprising. There is no any form of control of brucellosis in small ruminants therefore infected adult animals become carriers and continue to be seropositive.

The result of this study shows that brucellosis is prevalent in both field sampled animals and those that were slaughtered at the abattoir/ slaughter slabs. The prevalence is higher in slaughtered animals sampled at the abattoirs and slaughter slabs (18.0%) compared to field sampled animals (17.0%). There was however no significant difference between the prevalence of field and slaughtered animals ($P > 0.05$).

The reason for the high prevalence in slaughtered animals is possibly because a lot of sheep and goats, males or females sold for slaughter at the abattoir/ slaughter slabs might be carriers of brucellosis. There are also cases where sick animals in a flock are sold for slaughter to avoid losing them through death. All families in the study area rear both sheep and goats for production and economic benefits to meet family needs. Older animals are sold in preference to younger ones because they are no longer productive. Such are likely to be chronic carriers of brucellosis and hence more seropositive. The prevalence in field sampled animals shows that infected animals still present in various sheep and goat flocks.

Several serological tests have been developed and used for the diagnosis of brucellosis world wide ranging from simple cheap and rapid tests to complex and expensive ones. The conventional tests commonly used include Rose Bengal plate test, (RBPT), Serum Agglutination test (SAT), Complement Fixation test (CFT) and Enzyme-Linked Immunosorbent Assay (ELISA). However, each of these tests has limitations of not detecting all the relevant antibodies at different stages of the infection. The antibody response in brucellosis consists of an early IgM isotype response. This is followed by

production of IgG₁ antibody and later by small amounts of IgG₂ and IgA (Levieux, 1978b). Since it is clear that no individual test is perfect, all serological diagnosis of brucellosis will require the use of two or more tests to ensure the sensitivity and specificity of the serological diagnosis (Nicoletti, 1990). According to Stemshorn *et al.*, (1985), a screening test should be economical, rapid and highly sensitive but need not be specific. The primary objective of a screening test in the control and eradication of brucellosis is to detect animals that are infected and may spread the disease.

The RBPT and SAT used in this study fit the above observation and their combination is useful as each of them detects antibodies that the other fails to detect as a single test. The SAT is very effective and even superior to the CFT in the diagnosis of earlier phases of brucellosis because it has the capacity to detect antibodies of the immunoglobulin M isotype which predominate in the beginning of the infection (Chappel, 1989; Joint FAO/WHO, 1986). Several laboratories still prefer to use this test because of its simplicity compared to the CFT technique despite the controversy about its limitations. Secondly, it can be standardized according to international units, making its results comparable in all countries (Herr *et al.*, 1982).

The RBPT on the other hand is also standardized, simple to perform and inexpensive. It is considered suitable for screening individual animals. The antigen is used at pH of 3.65. The low pH prevents some agglutination by IgM and encourages agglutination by IgG₁ thereby reducing non-specific reactions (Corbel, 1984 Allan *et al.*, 1976). The RBPT has been reported to be good in detecting IgG₁ and IgG₂ which are antibodies produced in late *Brucella* infections. In a study conducted by Alton *et al.* (1985), 78 out of 79 culture positive cattle were positive by RBPT. Similarly, it has been reported that all animals that were culture positive in a study gave positive reactions to the RBPT. This implies that the

RBPT is very sensitive and possibly serve as a confirmatory test. Office of International Epizootics (1996) recommended the RBPT and ELISA as screening tests and SAT and CFT as supplemental tests. Muma *et al.* (2007) evaluated three serological tests, Rose Bengal plate test (RBPT), competitive ELISA and Fluorescent Polarization Assay (FPA) for brucellosis in naturally infected cattle in Zambia and found out that the RBPT performed well in both Sensitivity and Specificity. The c-ELISA had the highest sensitivity but low in specificity while the FPA had the highest specificity but low in sensitivity.

The limitations to SAT are due to its inability to detect chronic infections (Corbel, 1988, Nicoletti, 1990; Radostits *et al.*, 1995), differentiating antibodies resulting from vaccination and those due to infection (Weidmann, 1991; Seifert, 1996) and reacting with non-specific antibodies and cross-reacting with antibodies of other organisms such as *Yersinia enterocolitica*, *Vibrio cholerae*, *E. coli*, and *Salmonella sp* (Corbel *et al.*, 1984) which may result in diagnostic problems. These limitations were however taken care of in this study as follows:

- a) There is no routine vaccination in sheep and goats in Nigeria and in the study area in particular; therefore there is no ground for antibodies due to vaccination interfering with the SAT result in this study.
- b) The possibility of cross-reactivity with antibodies of other organisms was not determined as it is beyond the objectives of this study. However, low SAT titres (1:10 and 1:20) which are most likely the results of cross-reacting antibodies were considered negative. This therefore eliminates the interfering effect of cross-reacting antibodies. Only titres of 1:40 and above were considered positive for brucella infection.

Twenty seven (14.3%) sera were positive for both RBPT and SAT, 114 (60.3%) were positive for RBPT alone while 48 (25.4%) were positive for SAT only (Appendix III) out of a total of 189 positive sera. This is a reflection of test variations and different stages of infection. The samples that were positive for both RBPT and SAT show that both IgM and IgG immunoglobulin isotypes were prevalent in those animals indicating a point of transition from early to chronic infection. The proportion of such reactors was low showing that the frequency of such occurrence is usually rare. The fact that a high proportion of the sera (60.3%) were positive to RBPT only and negative to SAT shows that the dominant immunoglobulin in such sera is the IgG1 and IgG2 isotype which predominate in chronic infections.

The high percentage (60.3%) of RBPT positive sera in this study is not surprising as it reflects carrier status usually attained by brucella infected animals. Brucella infected animals usually become carriers and may continue to shed the organisms for a long period of time. The 48 sera positive to SAT only and negative for RBPT in this study indicates early infection in which case the predominant immunoglobulin is the IgM. This low prevalence (25.4%) compared to that of RBPT (60.3%) indicates that there are more chronic cases than those in acute or early stage of infection. The finding in this study agrees with that of others that while some samples may be positive to the SAT, they may be negative to RBPT and vice versa. A number of samples may be positive by both SAT and RBPT at the same time. In a study by Cunningham (1980) in cattle, out of 4867 serum samples tested, 835 were positive to the RBPT and 596 to the SAT. Of the 596 sera that were positive to SAT, 564 were simultaneously positive to both SAT and RBPT while 32 were SAT positive but negative to RBPT. This goes to confirm that no single test is particularly sensitive and specific or a gold standard for the diagnosis of brucellosis.

In determining the agreement between the two tests, all chance proportion agreement was 88% (Appendix III). However, many of the samples that contributed to this high level of agreement were negative on both tests.

There was no agreement between RBPT and SAT, ($Kappa = 0.03$), (Appendix III) suggesting that the two tests may be detecting different antibody isotype. This is so because RBPT detects predominantly IgG₁ and IgG₂ which are produced in later stage of infection while the SAT detects predominantly the IgM antibody isotype which are produced in acute infection and fails to detect IgG₁, (Nielsen *et al.*, 1988).

Brucellosis is an economically important disease. The prevalence of 9.3% in sheep and 10.1% in goats in this study has far reaching economic implications. The primary economic losses are abortion and neonatal mortality. Other associated losses include decreased milk production, death, culling and condemnation of animals due to infertility, loss in animal export of the country, cost of medication in terms of treatment of sick animals, cost of replacement animals. Esuruoso (1979) put the annual financial loss due to brucellosis in Nigeria at 224M Dollars, ₦148.8M by Rikin, (1988) Ajogi and Akinwumi, (2001) also determined the cost-benefit analysis of cattle herds with and without brucellosis per animal and confirmed that the disease causes great economic losses to the farmer. When these losses are considered at present monetary value with small ruminant inclusive, the losses could be very enormous.

The fact that brucellosis is endemic in the study area and prevalent among sheep and goats poses a great risk to human health. This means that the risk to human is also widespread especially in occupationally exposed individuals (Alausa, 1979). The prevalence of brucellosis among field animals is a great risk to the farmers and their families. This is because they live together with the animals in the compounds and have close contact with

them to the extent that the animals will eat left over food in the plates of the farmers. The farmers also come in direct contact with infected materials such as aborted fetuses, placentas, vaginal discharges and contaminated environment.

Infection due to direct contact with infected animal has been reported. The habit of removing aborted fetuses and retained placentas with bare hands by farmers exposes them to brucella infection. Other sources of exposure of farmers to brucella infection are: contaminated environment, consumption of fresh or raw milk and improperly cooked meat from an infected animal.

The prevalence of brucellosis in sheep and goats in the abattoir and slaughter slabs is also significant because the butchers, meat inspectors, veterinarians, meat sellers and other meat handlers are at high risk of infection through direct contact with infected animals and meat at slaughter. Meat consumers in the state are also at risk of infection with brucellosis as they handle and process meat with bare hands and if improperly cooked meat is eaten. Since the presence of brucellosis in animals is a determinant of its possible presence in humans, the widespread prevalence in Plateau state poses a great danger to human health in the state.

6.0 CONCLUSIONS AND RECOMMENDATIONS

- 1) The result of this study revealed *Brucella* antibodies prevalence of 9.3% by RBPT and 5.2% by SAT in sheep and 10.1% by RBPT and 6.0% by SAT in goats in Plateau State and an overall prevalence of 14.5% in sheep and 16.0% goats. The study shows that brucellosis is prevalent among sheep and goats in Plateau State.
- 2) It also shows that brucellosis is widespread across the state and the prevalence varies from one Local Government Area to another.
- 3) There was no significant difference in the prevalence between goats and sheep.

- 4) There was no significant difference in prevalence between the adult and the young sheep and goats.
- 5) There was no significant difference between sheep and goats sampled from the field and those sampled at slaughter in the abattoir and slaughter slabs.
- 6) There was no significant difference in prevalence between males and females in sheep.
- 7) There was a significant difference in prevalence between male and female goats.
- 9) Based on the wide –scale seroprevalence of the disease in Plateau State, the economic and public health consequences are also widespread.
- 10) The prevalence of brucellosis in slaughter slabs and abattoir also shows that the butchers, veterinarians and livestock dealers are exposed to infection with *Brucella*. This is because they hardly put on any protective wears in the course of handling either the live or slaughtered animals or the meat. This was witnessed during sample collection for this study in the abattoir and in all slaughter slabs visited.

Based on the findings of this work and the economic and public health significance of brucellosis, it is recommended that:

- a) Plateau state Government should as a matter of urgency embark on the control and eradication of Brucellosis in small animals.
- b) The Federal government should make available *Brucella* vaccine to the state for the regular vaccination of sheep and goats as being done in cattle.
- c) Our rural farmers and the general public should be educated using all available media on the need to maintain good hygienic practices on their animal houses and ensure adequate disposal of aborted and parturition materials and manure.

- d) The rearing of sheep and goats together with cattle should be discouraged as cattle can serve as source of *Brucella* infection to them and vice versa.
- e) Brucellosis surveillance should be put in place in Plateau state with all the Local Government Areas fully involved.
- f) Using the data obtained from surveillance, all sheep and goats that are reactors should be culled immediately.
- g) As preventive measures, veterinarians, livestock farmers, laboratory workers, butchers and meat sellers should wear protective materials in the course of their duties.

For further studies, isolation and characterization of brucella species causing problems in small ruminants in Plateau state is suggested.

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APPENDIX I
POPULATION SIZE CALCULATION.

$$N = Z^2 PQ / L^2$$

Where

$$Z = 1.96$$

P = Prevalence

Q = 1 - Prevalence

L = Level of allowable error (0.05)

For Sheep, P = 14%, therefore,

$$N = (1.96)^2 \times 0.14(1-0.14) / (0.05)^2 = 185$$

For Goats, P = 16.1%, therefore,

$$N = (1.96)^2 \times 0.161(1-0.161) / (0.005)^2 = 206$$

APPENDIX II

DETAILED CHI-SQUARE CALCULATIONS

a) Level of significance between the prevalence of Brucellosis in sheep and goats.

Hypothesis:

Ho: There is no significant difference between the prevalence of Brucellosis in sheep and goats in Plateau State.

Ha: There is a significant difference between the prevalence of Brucellosis in sheep and goats in Plateau State.

$$X^2 = \frac{\{(observed - Expected)\}^2}{Expected}$$

	+	-	Total
Sheep	72	424	496
Goats	137	714	851
Total	209	1138	1347

Observed frequency (O)		
Sheep / +	=	72
Sheep / -	=	424
Goats / +	=	137
Goats / -	=	714

Expected frequency (E)	=	E	$X^2 = \frac{(O-E)^2}{E}$
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Sheep/ + =	$496 / 1347 \times 209 / 1 = 76.96$	$= (72 - 76.96)^2 / 76.96 =$	0.3195
Sheep/ - =	$496 / 1347 \times 1138 / 1 = 419.04$	$= (424 - 419.04)^2 / 419.04 =$	0.0587
Goats/ + =	$851 / 1347 \times 209 / 1 = 132.04$	$= (137 - 132.04)^2 / 132.04 =$	0.1862
Goats/ - =	$851 / 1347 \times 1138 / 1 = 718.96$	$= (714 - 718.96)^2 / 718.96 =$	0.0342
		$X^2 = \text{SUM total} =$	0.5986

Calculated X^2 (0.5986) is less than table X^2 (3.841)

Accept Ho: There is no significant difference between the prevalence of Brucellosis in Sheep and Goats in Plateau State.

b) Level of significant difference in brucellosis prevalence based on zones of the state.

Hypothesis:

Ho: here is no significance difference in prevalence between North and central zones of Plateau state.

Ha: There is significant difference in prevalence between North and Central zones of Plateau state.

$$X^2 = \frac{\{(Observed - Expected)^2\}}{Expected}$$

	+	-	Total
North	96	517	613
Central	75	413	488
Total	171	930	1101

Observed frequency (O)

North/ += 96

North/ -= 517

Central/ += 75

Central/ -= 413

Expected frequency (E)

$$\frac{(Observed-Expected)^2}{Expected}$$

$$N/ + = 613/ 1101 \times 171 \times 1 = 95.21$$

$$\frac{(96-95.21)^2}{95.21} = 0.0066$$

$$N/ -= 613/ 1101 \times 930 \times 1 = 517.79$$

$$\frac{(517-517.79)^2}{517.79} = 0.0012$$

$$C/ + = 488/ 1101 \times 171 \times 1 = 75.79$$

$$\frac{(75-75.79)^2}{75.79} = 0.0082$$

$$C/ + = 488/ 1101 \times 930 \times 1 = 412.21$$

$$\frac{(413-412.21)^2}{412.21} = 0.0015$$

$$X^2 = \text{Sum Total} = 0.0175$$

Calculated X^2 (0.0175) is less than Table X^2 (3.841).

Accept Ho: There is no significant difference in prevalence between Northern and Central zones of the State.

Hypothesis:

Ho: There is no significant difference in prevalence between North and Southern zones of Plateau state.

Ha: There is significant difference in prevalence between North and Southern zone of plateau state.

$$X^2 = \frac{\{(Observed - Expected)\}}{Expected}$$

	+	-	Total
North	96	517	613
South	38	210	248
Total	134	727	816

Observed frequency (O)

N/ + = 96

N/ - = 517

S/ + = 38

S/ - = 210

Expected frequency (E)	$X^2 = \frac{(\text{Observed}-\text{Expected})^2}{\text{Expected}}$	
N/ + = 613/ 816x 134x1= 100.66	$\frac{(96-100.66)^2}{100.66} =$	0.2102
N/ - = 613/ 816 727x 1= 546.14	$\frac{(517-546.14)^2}{546.14} =$	1.5548
S/ + = 248/ 816x 134x 1=40.73	$\frac{(38-40.73)^2}{40.73} =$	0.1830
S/ - = 248/ 816 x 727x 1=220.95	$\frac{(210-220.95)^2}{220.95} =$	0.5427
	Sum Total =	2.4907

Calculated X^2 (2.4907) is less than Table X^2 (3.841).

Accept H_0 : There is no significant difference in prevalence between Northern and Southern zones of the State.

c) Level of significant difference in prevalence of brucellosis between breeds of sheep.

Hypothesis:

H_0 : There is no significant difference between Yankasa and other breeds of sheep (Uda and Balami).

H_a : There is significant difference in prevalence between Yankasa and other breeds of sheep.

	+	-	TOTAL
Yankasa	38	412	450
Others	8	38	46
Total	46	450	496

Observed frequency (O)

Y/ + =38

Y/- = 412
 O/+ = 8
 O/- = 38

Expected frequency (E)	$X^2 = \frac{(O - E)^2}{E}$
Y/+ = $\frac{450}{496}$ X 46 = 41.7	$\frac{(38-41.7)^2}{41.7} = 0.3283$
Y/- = $\frac{450}{496}$ X 450 = 417.3	$\frac{(412-417.3)^2}{417.3} = 0.0673$
O/+ = $\frac{46}{496}$ x 46 = 4.3	$\frac{(8-4.3)^2}{4.3} = 3.1837$
O/- = $\frac{46}{496}$ x 450 = 41.7	$\frac{(38-41.7)^2}{41.7} = 0.3283$
$X^2 = \text{SUM}$	$\text{Total} = 3.9076$

Calculated X^2 (3.9076) is greater than table X^2 (3.841)
 Accept H_a : There is significant difference in prevalence between Yankasa and others (Balami and Uda) breeds of sheep.

d) Level of significant difference in prevalence between West African dwarf goats and Sokoto red goats.

	+	-	Total
WAD	70	680	750
SRG	16	85	101
Total	86	765	851

Observed Frequency (O)
 WAD/ + = 70
 WAD/ - = 680
 SRG/ + = 16
 SRG/ - = 85

Expected Frequency (E)	$X^2 = \frac{(O - E)^2}{E}$
$\frac{750 \times 86}{851} = 75.79$	$\frac{(70-75.79)^2}{75.79} = 0.0499$
$\frac{750 \times 765}{851} = 674.2$	$\frac{(680-674.2)^2}{674.2} = 0.0499$

$$\frac{101 \times 86}{851} = 10.2 \quad \frac{(16-10.2)^2}{10.2} = 3.298$$

$$\frac{101 \times 765}{851} = 90.79 \quad \frac{(85-90.79)^2}{90.79} = 0.3692$$

$$\text{Total} = 4.1594$$

Calculated X^2 (4.1594) is greater than table X^2 (3.481).

Accept H_a : There is a significant difference in prevalence between WAD and SRG.

e) Level of significant difference of brucellosis prevalence between Male and Female Goats

Hypothesis:

H_0 : There is no significant difference in prevalence between male and female goats.

H_a : There is significant difference in prevalence between male and female goats.

$$X^2 = \frac{\{(\text{Observed} - \text{Expected})^2\}}{\text{Expected}}$$

Observed frequency

Male/+ = 46

Male/- = 189

Female/+ = 98

Female/- = 418

$$\text{Expected frequency (E)} \quad X^2 = \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

$$\text{Male/+} = 335/851 \times 144/1 = 56.69 \quad \frac{(46-56.69)^2}{56.69} = 2.014$$

$$\text{Male/-} = 335/851 \times 707/1 = 278.31 \quad \frac{(189-278.31)^2}{278.31} = 0.410$$

$$\text{Female/+} = 516/851 \times 144/1 = 87.31 \quad \frac{(98-87.31)^2}{87.31} = 1.308$$

$$\text{Female/-} = 516/851 \times 707/1 = 428.69 \quad \frac{(418-428.69)^2}{428.69} = 0.266$$

$$\text{Sum total} = 3.998$$

Calculated X^2 (3.998) is greater than Table X^2 (3.841).

Reject Ho: There is a significant difference in prevalence between male and female goats.

- f) Level of significant difference in Brucellosis prevalence between male and female Sheep.

Hypothesis:

Ho: There is no significant difference in prevalence between male and female sheep.

Ha: There is significant difference in prevalence between male and female sheep.

$$X^2 = \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

Observed frequency (O)

M/+ = 22

M/- = 137

F/+ = 53

F/- = 284

Expected frequency (E)

$$X^2 = \frac{(\text{Obs} - \text{Exp})^2}{\text{Exp}}$$

$$\text{M/+} = 159/496 \times 75/1 = 24.04 \quad \frac{(22-24.04)^2}{24.04} = 0.1734$$

$$\text{M/-} = 159/496 \times 421/1 = 134.96 \quad \frac{(137-134.96)^2}{134.96} = 0.031$$

$$\text{F/+} = 337/496 \times 75/1 = 50.96 \quad \frac{(53-50.96)^2}{50.96} = 0.082$$

$$\text{F/-} = 337/496 \times 421/1 = 286.04 \quad \frac{(284-286.04)^2}{286.04} = 0.015$$

$$X^2 = \text{Sum total} = 0.3014$$

Calculated X^2 (0.3014) is less than Table X^2 (3.841)

Accept Ho: There is no significant difference in prevalence between Male and Female sheep.

- g) Level of significance of prevalence between the young and the adult Sheep.

Hypothesis

Ho: There is no significant difference in prevalence between the young and adult sheep.

Ha: There is a significant difference in prevalence between the young and adult sheep.

$$X^2 = \frac{\{(\text{Observed} - \text{Expected})^2\}}{\text{Expected}}$$

	+	-	Total
A	81	377	458
Y	8	30	38
Total	89	407	496

Observed frequency (o)

A/ + = 81

A/ - = 377

Y/ + = 8

Y/ - = 30

Expected frequency (E)

$$X^2 = \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

$$A/ + = 458 / 496 \times 89 / 1 = 82.36 \quad \frac{(81 - 82.36)^2}{82.36} = 0.0225$$

$$A/ - = 458 / 496 \times 407 / 1 = 375.82 \quad \frac{(377 - 375.82)^2}{375.82} = 0.0037$$

$$Y/ + = 38 / 496 \times 89 / 1 = 6.82 \quad \frac{(8 - 6.82)^2}{6.82} = 0.2045$$

$$Y/ - = 38 / 496 \times 407 / 1 = 31.18 \quad \frac{(30 - 31.18)^2}{31.18} = 0.0447$$

$$X^2 = \text{Sum total} = 0.2754$$

Calculated X^2 (0.2754) is less than Table X^2 (3.841)

Accept H_0 : There is no significant difference between the prevalence of brucellosis in the young and adult Sheep.

f) Level of significant difference in prevalence of Brucellosis between the adult and young goats.

Hypothesis

H_0 : There is no significant difference in prevalence between the young and adult goats.

H_a : There is significant difference in prevalence between the young and adult goats.

$$X^2 = \frac{\{(\text{Observed} - \text{Expected})^2\}}{\text{Expected}}$$

	+	-	Total
A	135	658	793
Y	13	45	58

Total	148	703	851
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Observed frequency

A/ + =135

A/ - =658

Y/ + =13

Y/ - =45

Expected frequency (E) = , $X^2 = \frac{(\text{Observed}-\text{Expected})^2}{\text{Expected}}$

A/ + = $793/851 \times 148/1 = 137.91 = \frac{(135-137.91)^2}{137.91} = 0.0615$

A/ - = $793/851 \times 703/1 = 655.09 = \frac{(658-655.09)^2}{655.09} = 0.0130$

Y/ + = $58/851 \times 148/1 = 10.09 = \frac{(13-10.09)^2}{10.09} = 0.8412$

Y/ - = $58/851 \times 703/1 = 47.91 = \frac{(45-47.91)^2}{47.91} = 0.1771$

$X^2 = 1.0928$

Calculated X^2 (1.0928) is less than Table X^2 (3.841)

Accept H_0 : There is no significant difference in prevalence of brucellosis between the Adult and Young goats.

h) Level of significance of prevalence of brucellosis between sheep sampled in the field and those slaughtered in abattoirs.

Hypothesis:

H_0 : There is no significant difference in prevalence between sheep in the field and those slaughtered in abattoirs.

H_a : There is significant difference in prevalence between sheep in the field and those slaughtered in abattoirs.

$X^2 = \frac{(\text{Observed}-\text{Expected})^2}{\text{Expected}}$

	+	-	Total
Field	42	245	287
Slaughter	34	175	209
Total	76	420	496

Observed frequency (O)

Field/+ = 42

Field/ - = 245
 Slaughter/+ = 34
 Slaughter/- = 175

Expected frequency (E) $X^2 = \frac{(\text{Observed}-\text{Expected})^2}{\text{Expected}}$

F/ += 287/496x76/1= 43.98 $\frac{(42-43.98)^2}{43.98} = 0.089$

F/ -= 287/496x420/1 = 243.02 $\frac{(245-243.02)^2}{243.02} = 0.016$

S/ + = 209/496x76/1 = 32.02 $\frac{(34-32.02)^2}{32.02} = 0.122$

S/ - = 209/496x420/1 = 176.98 $\frac{(175-176.98)^2}{176.98} = 0.022$

$X^2 = 0.2494$

Note: F= Field
 S= slaughtered

Calculated X^2 (0.2494) is less than Table X^2 (3.841)
 Accept H_0 : There is no significant difference in prevalence between field and Slaughtered Sheep.

i) Level of significant difference in prevalence of Brucellosis between goats sampled in the field and those slaughtered in the abattoirs.

Hypothesis:

H_0 : There is no significant difference in prevalence between goats sampled in the field and those from abattoir.

H_a : There is significant difference in prevalence between goats sampled from the field and those from abattoir.

$X^2 = \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$

	+	-	Total
Field	57	237	294
Slaughtered	104	453	557
Total	161	690	851

Observed frequency (O)

Field/+ = 57

Field/ -= 237

Slaughtered/ + = 104

Slaughtered/ - = 453

Expected frequency (E)

$$X^2 = \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

$$F/ + = 294/851 \times 161/1 = 55.62 \quad \frac{(57-55.62)^2}{55.62} = 0.034$$

$$F/ - = 294/851 \times 690/1 = 238.38 \quad \frac{(237-238.38)^2}{238.38} = 0.008$$

$$S/ + = 557/851 \times 161/1 = 105.38 \quad \frac{(104-105.38)^2}{105.38} = 0.018$$

$$S/ - = 557/851 \times 690/1 = 451.62 \quad \frac{(453-451.62)^2}{451.62} = 0.004$$

$$X^2 = 0.064$$

Calculated X^2 (0.064) is less than Table X^2 (3.841)

Accept H_0 : There is no significant difference in prevalence of brucellosis between Field and Slaughtered goats.

APPENDIX III

TEST OF AGREEMENT BETWEEN SAT AND RBPT

KAPPA STATISTIC

		SAT		
		+	-	Total
	+	27a	114b	141a+b
RBPT	-	48c	1158d	1206c+d
	Total	75a+c	1272b+d	1347n

Proportion of agreement (chance + genuine agreement)

$$\frac{a + d}{n} = \frac{27 + 1158}{1347} = 0.88$$

Agreement due to chance alone = $\frac{Ea + Ed}{N}$

$$Ea = \frac{(a + b)(a + c)}{n} = \frac{141 \times 75}{1347} = 7.85$$

$$Ed = \frac{(c + d)(b + d)}{N} = \frac{1206 \times 1272}{1347} = \frac{1534032}{1347} = 1138.85$$

$$\frac{Ea + Ed}{N} = \frac{7.85 + 1138.85}{1347} = \frac{1146.7}{1347} = 0.85$$

Genuine Agreement = Proportion of Agreement – chance

$$0.88 - 0.85 = 0.03$$

INTERPRETATION

Kappa = 0, There is no agreement

Kappa = 1, there is perfect agreement

Kappa = 0.4-0.5: there is moderate agreement

Kappa = 0.5-0.6: there is acceptable agreement

Kappa = 0.6-0.8: there is good agreement

APPENDIX IV

DISTRIBUTION OF SAT TITRE IN SHEEP IN THE 9 LGAS OF PLATEAU STATE

LGA	No. tested	No + on. screenin g	>12.5 (iu/ml)	>25 (iu/ml)	>50 (iu/ml)	>100 (iu/ml)	>200 (iu/ml)	TOTAL
			1:10	1:20	1:40	1:80	1:160	
Bassa	16	3	1	1	1	0	0	1
Bokkos	91	7	1	1	3	2	0	5
Jos North	98	11	1	2	2	5	1	8
Jos south	91	4	1	0	2	1	0	3
Langtang N	50	2	0	0	2	0	0	2
Mangu	94	1	0	0	1	0	0	1
Pankshin	9	1	0	0	1	0	0	1
Quanpan	17	3	0	0	2	1	0	3
Shendam	30	3	1	0	2	0	0	2
Total	496	35	5	4	16	9	1	26(5.2%)

APPENDIX V

DISTRIBUTION OF SAT TITRE IN GOATS IN THE 9 LGAS OF PLATEAU STATE

LGA	No. tested	No+ screened	12,5iu/ ml	25iu/ ml	50iu/ ml	100iu/ ml	200iu/ ml	Total
			1:10	1:20	1:40	1:80	1:160	
Bassa	71	6	1	0	3	2	0	5
Bokkos	81	5	0	0	3	2	0	5
Jos North	157	33	15	0	11	5	2	18
Jos South	180	11	1	0	9	1	0	10
Langtang North	50	3	0	0	1	2	0	3
Mangu	147	8	2	0	4	2	0	6
Pankshin	64	2	0	0	1	1	0	2
Quan- Pan	43	1	0	0	1	0	0	1
Shendam	58	1	0	0	0	1	0	1
Total	851	70	19	0	33	16	2	51(6.0%)

APPENDIX VI
DISTRIBUTION OF AGREEMENT BETWEEN SAT POSITIVE AND RBPT
POSITIVE SERA.

LGA	RBPT/SAT +ve	RBPT +ve	SAT +ve
Jos North	13	13	16
Jos South	3	25	10
Bassa	1	3	6
Mangu	2	27	5
Pankshin	2	10	1
Bokkos	2	14	3
Langtang North	2	8	3
Shendam	1	8	2
Quan-Pan	1	6	2
Total	27(14.3%)	114(60.3%)	48(25.4%)

