SERO-PREVALENCE OF BRUCELLOSIS IN SMALL RUMINANTS IN BAUCHI STATE, NIGERIA

BY

IBRAHIM YA'U

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE AWARD OF THE DEGREE OF MASTERS OF SCIENCE IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

SEPTEMBER, 2014

SERO-PREVALENCE OF BRUCELLOSIS IN SMALL RUMINANTS IN BAUCHI STATE, NIGERIA

BY

Ibrahim YA'U, DVM (UNIMAID), 2004

M.Sc./Vet-Med/08106/2008-2009

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE AWARD OF THE DEGREE MASTER OF SCIENCE IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

SEPTEMBER, 2014

DECLARATION

I declare that the work in this thesis entitled **''Sero-prevalence of brucellosis in small ruminants in Bauchi State, Nigeria,''** has been carried out by me in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Ibrahim YA'U	……	….……
Name of student	Signature	Date

CERTIFICATION

This thesis entitled **"SERO-PREVALENCE OF BRUCELLOSIS IN SMALL RUMINANTS IN BAUCHI STATE, NIGERIA"** by Ibrahim YA'U meets the regulations governing the award of the degree of Master of Science (M.Sc.) of the Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

Dr. M. Bello	_____	_____
Chairman Supervisory Committee. `	Signature	Date
Prof. J. K. P. Kwaga	_____	_____
Member, Supervisory Committee	Signature	Date
Dr. E. C. Okolocha		
Head, Department of	_____	_____
Veterinary Public Health and	Signature	Date
Preventive Medicine		
Professor A. Z. Hassan	_____	_____
Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

This thesis is dedicated to humanity, development of science, and my lovely family.

ACKNOWLEDGEMENTS

All thanks are to Allah who created the earth and the heaven and what are in between. I wish to express my profound gratitude and appreciation to my supervisors, Dr. M. Bello and Prof. J.K.P. Kwaga for their encouragement and support throughout the research period.

Similar appreciation goes to the Head of Department of Veterinary Public Health and Preventive Medicine Dr. E. C. Okolocha and all staff of the Department for their support and encouragement. Also my appreciation goes to Dr. Sulaiman Abubakar, for assisting me in buying the cELISA kit. My appreciation also goes to Bashir Usman, Ibrahim Muhammad Gamawa, and Victoria Ajiji for their assistance during sample collection. I want to specially acknowledge support and contributions of all the laboratory technologists of the Department, especially Mallam Yahuza Suleiman for their assistance in the laboratory procedures.

I want to acknowledge the support and assistance of my friends and colleagues especially the following: Drs. Yusha'u Baraya, Dauda Iliyasu, Peter Ehizibolo, Abdurrhman Mohammed, Bala Usman, Mannir Ari, Ma'aruf Lawal, and Muhammad Baba Saba. Similar appreciation to my lecturers especially Drs Muhammed Bello, Junaid Kabir, M. U. Kawu, S. T. Fadason, E. C. Okolocha, M. A. Raji, O. O. Okubanjo, and for their support and guidance on how to achieve academic excellence. Also my gratitude to the following Professors: J. O. O. Bale, I. Ajogi, Idris Abdulqadir, C. A. Kudi, S. A. Ojo, Idris A. Lawal, K. A. N. Esievo and H. M. Kazeem for their piece of advice and encouragement.

Permit me to appreciate my parents Alhaji Ya'u Adamu Sade and Hajiya Asma'u Muhammad Bello for their love, moral and financial support throughout the research period. May Allah reward them, amin. I also wish to thank my beloved wife (Malama Ruqayyatu Mahmoud Inuwa) and my lovely daughters Fatima and Aisha and son Mallam Ja'afar, for their support throughout the study period. Finally my gratitude goes to my brothers and sisters, Hajiya Asabe, Alhaji Hamsa Attahiru, Alhaji Samila (Talban Sade), Bello ,Alhaji Adamu, Abdulmalik, Sadiq, Hajiya Fatima, Usman, Salisu Ibrahim, Ahmad, Aisha, and Adamu. I thank you all for your prayers and support. May Allah in His infinite mercy reward you abundantly, Amen.

ABSTRACT

This study was designed to determine the sero-epidemiological status of small ruminant brucellosis in Bauchi state, Nigeria. A total of 739 small ruminants comprising of 324 and 415 sheep and goats respectively from the three Senatorial Zones of the State were used for this study. Blood samples were collected from small ruminants slaughtered in the abattoirs and flocks of sheep and goats in the study area. Rose Bengal Plate test (RBPT) was used to screen all the sera obtained from the animals and Competitive Enzyme Linked Immunosorbent Assay (cELISA) was used to confirm the RBPT positive sera. Out of 324 sheep sampled, 114 (35.2%) and 77 (23.8%) were positive for *Brucella* infection by RBPT and cELISA, respectively. Similarly out of 415 goat sampled, 98 (23.6%) and 56 (13.5%) were positive by RBPT and cELISA respectively. In sheep, the highest prevalence of brucellosis using cELISA were found in Dass and Darazo LGAs with (37.2%) and (20.0%) being positive, respectively, while the lowest prevalence were found in Ningi and Gamawa LGAs with (16.0%) and (19.4%) being positive, respectively. However, in goats, the highest prevalence of brucellosis using cELISA test were recorded in Katagum LGA (25.5%) and Darazo LGA (22.6%) while the lowest prevalence were in Gamawa LGA (3.5%) and Ningi 4 samples (8.3%) respectively. There was no statistically significant difference ($P < 0.05$) between brucellosis and sex of the small ruminants. Questionnaires administered revealed low awareness of brucellosis in the study area especially among those age groups that are < 18 and those with Islamic or primary educational status, while the mode of transmission and how to protect against the disease were also not satisfactorily understood by the respondents. From this study, it was concluded that brucellosis among

small ruminants may be endemic and the level of awareness on its zoonotic importance and attitude of farmers towards management of animals in Bauchi state, Nigeria need to be improved. There is need to intensify campaigns on brucellosis especially on route of transmission and preventive measures among the farmers. The Government needs to outline strategies on how to employ the use of vaccine in prevention and culling of infected animals to ensure total eradication in the state, which can be achieved by adequate compensation to farmers by the government.

TABLE OF CONTENT

Title **Pages**

List of Figure

Figure **Page**

LIST OF TABLES

Table **Page**

LIST OF PLATES

Plate **Page**

LIST OF APPENDICES

Appendix **Page**

ABBREVIATIONS AND SYMBOLS

CDS	Center for Development Studies
CFT	Complement Fixation Test
EDTA	Ethylene diamino tetraacetic acid
cELISA	Competitive Enzyme Linked Immuno-sorbent Assay
EPR	Education, Practice and Research
FAO	Food and Agriculture Organisation
FDL	Federal Livestock Department
FAOSTAT	FAO Statistical Database
FDLPCS	Federal Department of Livestock and Pest Control Services.
G	Gram
Hrs	Hours
Kg	Kilogram
LGA	Local Government Area
L	Litre
ml	Millilitres
mg	Milligram
ng	Nanogram

nm	Nanometer
OD	Optical Density
OIE	Office International des Epizootics
OPD	Ophenylenediamine
RBC	Red Blood Cell
RIMS	Resource Inventory and Management Limited
SAT	Serum Agglutination Test
UK	United Kingdom
USA	United States of America
WAD	West African dwarf
WBC	White Blood Cell
WHO	World Health Organization
µl	Microliter

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Brucellosis is a worldwide zoonosis that is recognized as a major cause of significant economic losses in livestock due to its primary effect on the reproductive system in affected animals with concomitant reduction in production and also the serious threat to human health it poses (Young, 1991; Adams, 2002; Santellano-Estrada *et al*., 2004; Ehizibolo *et al*., 2011).

Brucellosis is caused by fastidious, intracellular, non-spore forming, non-motile, non-encapsulated, gram negative coccobacillus (or short rod) bacterium of the genus *Brucella* which contains six well defined species distinguished according to their preferential host, biochemical tests and cell surface characteristics (FAO, 2005; Zygmunt *et al.*, 2009). They include *Brucella abortus* (in cattle), *B. melitensis* (in goats and sheep), *B. suis* (in pigs) *B. ovis* (causing epididymitis in sheep and goat), *B. canis* (in dogs), and *B. neotomae* (affecting desert wood rats in the USA). *B. inopinata* (isolated from a human patient).

Four more species were recently proposed, *B. ceti* and *B. pennipedialis* isolated from marine animals, *B. inopinata* (isolated from a human patient) and *B. microti* isolated from the common vole (OIE, 2009). However, cross infection does occur among the species with ingestion, inhalation and direct contact as the major routes of infection in both animals and human (Alton *et al*., 1988; Winchell *et al*., 2010).

In Nigeria, the disease has been reported in cattle (Esuruoso and Van Blake, 1972; Esuruoso, 1974; Eze, 1978; Bale and Kumi-Diaka, 1981; Falade and Shonekan, 1981), dogs (Osinubi *et al.,* 2004), sheep and goats (Okoh, 1980; Okewole *et al.,* 1988; Hale and Ajogi, 1997; Ocholi *et al*., 2005; Ojo *et al.,* 2007; Bertu *et al.,* 2010) and in horses (Bale and Kwanashi, 1985; Ehizibolo *et al.,* 2011).

The epidemiology of brucellosis reveals some important factors which contribute to the prevalence and spread of the disease in livestock including farming system and practices, farm sanitation, livestock movement, mixing animals and sharing of grazing sites (Kadohiri *et al.,* 1997; Kabagambe *et al.,* 2001; Omer *et al.,* 2007). McDermott and Arimi (2002) in their epidemiological review of brucellosis in sub-Saharan Africa reported that the disease is very common in cattle, but less well studied in small ruminants. High prevalence rates have been reported in developing countries because of traditional food consumption and close contacts with infected animals (Nicoletti, 1984).

1.2 Statement of Research Problem

Small ruminants make up the bulk of the population of food animals in Nigeria, totalling up to 51 million, consisting of 28 million goats and 23 million sheep (FAO, 2006). Brucellosis is an endemic disease in Nigeria with serological studies in various parts of the country indicating the existence of the disease virtually in all domestic animals and humans (Ajogi, 1997). Economic losses associated with the disease include loss of milk, infertility and sterility, cost of vaccines and lowered value of animal culled due to disease (Bale, 1991). Despite the endemic nature of the disease, the incidence and prevalence of the disease is difficult to ascertain due to uncoordinated reporting and

undedicated government effort at consistent epidemiological investigation of the disease in Nigerian livestock population (Bawa *et al.,* 1987; Ocholi *et al.,* 1993).

Small ruminants contribute immensely to the protein needs of most countries. In Nigeria and Bauchi State in particular, many families keep sheep and goats as a source of complimentary income. The estimated population of sheep and goats by 2010 in Bauchi was put to be 3,396,376 and 5,086,267 respectively (FDL, 2011). It is a common practice among Fulani pastoralists as well as farmers to keep sheep and goats alongside with cattle. This situation usually provides an opportunity for the spread of brucellosis from infected cattle to small ruminants (Ocholi *et al.,* 2005). The role of brucellosis in limiting livestock production and its economic impact on the livestock industry in Nigeria is widely recognised (Rikin, 1988; Ajogi and Akinwumi, 2001). This does not include losses in productivity in small ruminants.

Livestock rearing is one of the oldest human occupations in Bauchi State, Nigeria. The majority of the people in the State are Hausa and Fulani that live in close association with their livestock, in poor sanitary conditions.

1.3 Justification

Small ruminants are a major source of meat supply in Bauchi State. It is evident that brucellosis is endemic and represents a major constraint to livestock production in Nigeria (Ate *et al.,* 2007). Studies suggest increasing trend in the prevalence of the Brucellosis (Ocholi *et al.,* 2004). Furthermore there is no planned control programme for brucellosis in small ruminants in Bauchi State. To the best of my knowledge there is no study to determine the status of brucellosis in small ruminants covering the whole of

Bauchi State. Consequently, this study became imperative to evaluate the prevalence of brucellosis in Bauchi State.

Thus, the zoonotic importance of the disease and the scarcity of literature on brucellosis in sheep and goats in Bauchi State and the risk of transmission to humans, justified this study.

1.4 Research Questions

1 What is the sero-prevalence of brucellosis in small ruminants in Bauchi State?
2 Is the prevalence of brucellosis different in sheep and goats in Bauchi State?
3 Does sex have effect on the prevalence of brucellosis in small ruminants in Bauchi State?
4 What is the level of awareness of brucellosis among small ruminant farmers in Bauchi State?

1.5 Aim and Objectives

The aim of the study was to conduct a sero-epidemiological study of brucellosis in small ruminants in Bauchi State. This was accomplished by the following specific objectives:

1. To determine and compare the sero-prevalence of brucellosis in sheep and goats in Bauchi State both in households and abattoirs.
2. To determine the sex distribution of brucellosis in sheep and goats in Bauchi State.
3. To assess knowledge and awareness of brucellosis among small ruminant farmers in Bauchi State.

CHAPTER TWO

LITERATURE REVIEW

2.1 Small Ruminants

Small ruminants are commonly found everywhere as poultry, though not so numerous. The world population of sheep and goats are 1078.2 and 861.9 million respectively (FAOSTAT, 2006) with variations in distribution around the globe. In Nigeria, they are estimated to be about 56.6 million, with goats outnumbering sheep by three to two folds (Jibril, 2010). While some seasonal movement of pastoral sheep place, the great majority of small ruminants are sedentary village livestock and their pattern of distribution mirror that of human settlement (RIMS, 1992). Pastoral animals are generally more productive than the village stock (Jibril, 2010).

West African Dwarf goats are kept in forest zones and Middle Belt, Red Sokoto are kept throughout the north while Sahel goats are restricted to a strip along the frontier with the Republic of Niger. Although pastoral Sahel goats are found in the northern semi-arid zone, the higher number of goat is kept in villages (Blench, 1999).

2.2 Small Ruminants Production Systems

Livestock production system in sub-Saharan Africa can be divided into two major types. Namely: traditional and modern, which can be differentiated mainly through three production factors, land capital and labour (Ibrahim, 1998; Jibril, 2010). They can further be classified as, solely livestock system where animal feeds are largely obtained from rangelands, pastures and annual forages with a small percentage purchased from non-livestock farming activities, or mixed farming system in which some portion of the

feed that are fed to animals come from crop by-products (Kruska *et al*., 2003). The most common production system in Nigeria is seasonal confinement (Ugwu, 2007), where livestock are allowed to graze during non-cropping season and are confined during cropping season. Sheep are predominantly kept by Fulani pastoralists under semi- intensive management, goats are kept mainly by crop farmers whose management practices include housing overnight and tethering by the day during wet season and allowing them to roam around freely in the dry season (Blench, 1999).

2.2.1 Traditional livestock production system

Considering livestock farming as a source of income and food product, the traditional livestock production system can be sub divided into pastoral, agro pastoral, agricultural and urban or peri urban (Ibrahim, 1998). In agro pastoral system, rangeland is used but crop residues are incorporated. Crop residues; forage and household waste are used for feeding small ruminants in the agricultural system. Urban system provides sheep and goats with grains, protein concentrates and household waste, more labour is requires in cutting and carrying the grass to the animals (Ibrahim, 1998).

2.2.2 Modern livestock production system

The modern livestock production system can be divided into the ranching, feedlot and station system (Ibrahim, 1998). They are characterized by high inputs and are all sedentary. Land is important in the ranching system while the feedlot system is capital intensive and requires large amounts of feeds. Sometimes feedlot system required cut and carry forages to provide feed and this activity requires labour (Ibrahim, 1998).

2.3 Brucellosis

Brucellosis is one of the world's most widespread zoonosis (Alton *et al*., 1988), caused by various bacteria in the genus *Brucella* which affects cattle, sheep, goats, pigs and some other animals. It leads to abortion, infertility and lowered milk yields in affected animals (Osori, 1976; Oyedipe *et al*., 1981). It can be passed to human via direct contact with livestock or through drinking unpasteurized milk from an infected animal. Infected livestock are clinically characterized by one or more of the following signs; abortion, retained placenta, Orchitis, epididymitis and, rarely, arthritis (Alton *et al*., 1988).

In people, the main symptom is intermittent fever, also referred to as undulant fever and it has tendency to be misdiagnosed as drug-resistant malaria in tropical countries. Brucellosis can cause variety of other symptoms like joint pain, fatigue and depression (WHO, 2006). It also causes substantial losses to livestock producers in a region where it is endemic. In most developed countries, where test and-slaughter programmes are being practiced, with adequate compensation for farmers and financial incentives for disease-free herds have more or less eliminated brucellosis in livestock (WHO, 2006).

2.3.1 Description of the causative agent

Brucellae are Gram-negative, facultative, intracellular bacteria showing a wide range of species-specificity and causing important diseases in both humans and animals. At present, six species are recognised: *B. abortus* (affecting mainly cattle), *B. melitensis* (sheep and goats), *B. suis* (swine), *B. neotomae* (desert rats), *B. ovis* (sheep), *B. canis* (dog. A couple of new species: *B. microti* (isolated from common voles)), *B. ceti* (cetaceans), *B. pinnipedialis* (pinnipeds) and *B. inopinata* (isolated from a human

patient) have been proposed but not yet accepted by the Taxonomy Subcommittee on *Brucella* by Blasco, (2010).

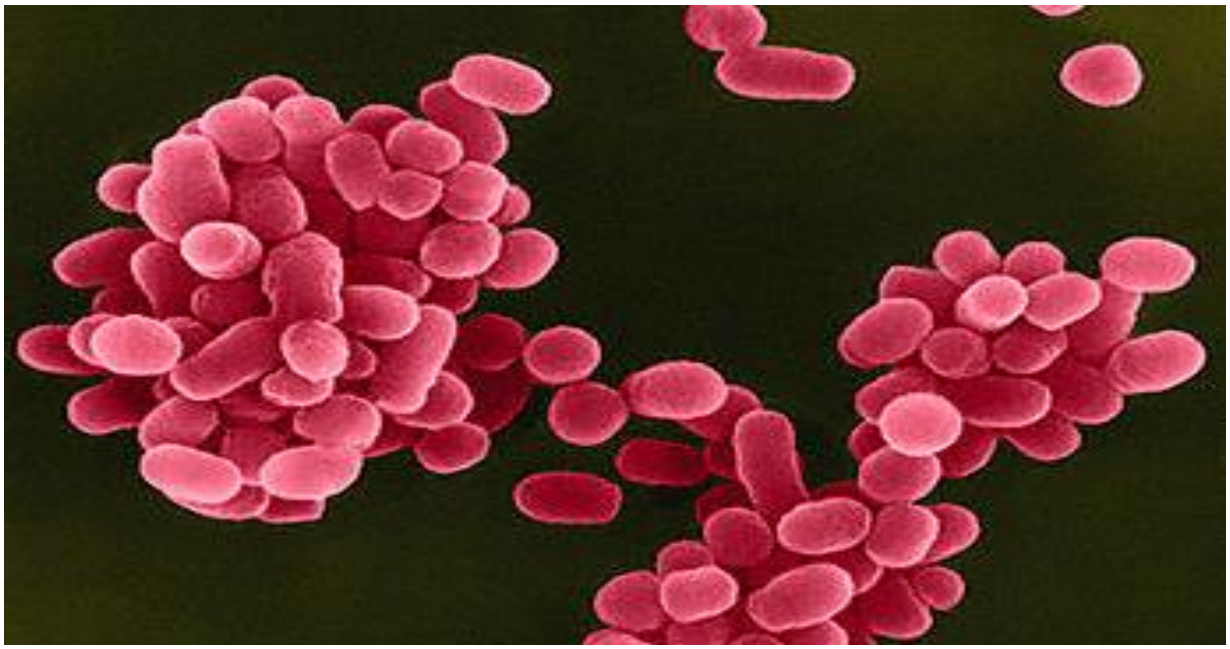

Plate I: Electron Microscopy of *B. abortus* (Dennis Kunkel Microscopy, Inc., 2004). Source: Zandraa, (2008)

2.3.2 Epidemiology

In sheep and goats, B. *melitensis* is nearly always the infecting species. B. *ovis* can also infect sheep but is of little significance in relation to human disease WHO (2006). The mode of transmission of B. *melitensis* in sheep and goats is similar to that in cattle but sexual transmission probably plays a greater role. The transmission of disease is facilitated by mixing of flocks and herds belonging to different owners and by purchasing animals from unscreened sources. The sharing of male breeding stock also promotes transfer of infection between farms Transhumance during hot season grazing is a significant promoting factor in some areas as is the mingling of animals at markets

or in humans and animals fairs. In cold climates, it can be the custom to house animals in close space and this also facilitates transmission of infection WHO (2006).

Brucellosis occurs worldwide but it is well controlled in most developed countries. The disease is rare in industrialized nations because of routine screening of domestic livestock and animal vaccination programmes (Corbel, 1997; Maloney and Fraser, 2004). Clinical disease is still common in the Middle East, Asia, Africa, South and Central America, the Mediterranean Basin and the Caribbean. Additional losses result from human infection with its prolonged misery, debility and generalized aching, which may last for months or years (Hoover and Friedlander, 1997; Abdou, 2000) Sheep and goats and their products are the main sources of infection (Young, 1995). Animal brucellosis is an enormous problem worldwide and is endemic in most areas of the world, with the exception of countries such as the UK and Australia, which have eradicated the disease by strict veterinary hygiene measures (Young, 1995; Solera *et al*., 1999)

Human brucellosis is widely distributed all over the world, with regions of high endemicity such as the Mediterranean, Middle East, Latin America and parts of Asia (Corbel, 1997; López-Merino, 1989). The true incidence of human brucellosis is unknown. Reported incidence in endemic-disease areas varies widely, from <0.01 to >200 per 100,000 population (López-Merino, 1989).

2.3.3 Susceptibility

Host susceptibility is variable and is associated with the reproductive status. Thus, in the field, all intermediate stages between typical acute infection and complete resistance

may be observed (EC, 2001). In addition, vaccinal immunity may modify the parasite-host relationship. In goats, the infection may vary in duration from very short periods of mild infection which is rapidly eliminated (especially in vaccinated animals) to persistence for years may result in the excretion of the organism in the milk may continue for two or more lactations. The situation in sheep is very different, though this varies with the susceptibility of the breed (Alton, 1990). After recovery from infection with *B. melitensis* sheep are very resistant to reinfection (Alton, 1990). Werschilova and Striedter (1938) demonstrated a strong resistance to reinfection, even during pregnancy, up to 8 to 9 months, after which it began to decline, although some resistance was still demonstrated after 2 years. Durán-Ferrer (1998) reported a long-lasting immunity after an experimental infection (EC, 2001)**.**

2.3.4 Transmission

Infected animals shed the organisms in uterine discharges following abortion and subsequent parturition, and also in the colostrum and milk (FAO, 2003). Brucellosis is a flock problem; it is spread primarily by ingestion of contaminated materials, venereal infections can also occur, but this is mainly seen with *B. suis* infections (FAO, 2003). Congenital (*in utero*) or perinatal infections may also occur, with the ensuing development of latent infections. Spread between herds usually occurs by the introduction of asymptomatic chronically-infected animals (FAO, 2003).

Transmission of brucellosis to humans occurs through breaks in the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted foetuses or placentas (FAO, 2003). Food-borne infection occurs following ingestion of raw milk and other dairy products, but rarely from eating raw meat from infected animals (FAO, 2003). Occupational airborne infection in laboratories and abattoirs has also been

documented, accidental inoculation of live vaccines (such as *B. abortus* Strain 19 and *B. melitensis* Rev.1) can also occur, resulting in human infections (FAO, 2003). There are also case reports of venereal infection in humans (FAO, 2003).

2.3.5 Incubation period

The incubation period is usually one to four weeks, but occasionally, it may be as long as several months (Fiori *et al*., 2000; Mantur *et al*., 2007). It is generally accepted that *B. melitensis* causes more severe infection than *B. abortus* (Troy *et al*., 2005). The incubation period and the infective period could not be defined for *Brucella* as it could last as long as the life-span of the animals (OIE, 2009).

2.3.6 Diagnosis

The diagnosis of brucellosis is made by the isolation of *Brucella* species, but this method is successful in only 40 to 70% of cases (Yagupsky, 1999). Therefore, laboratory diagnosis of brucellosis very often relies on detecting specific serum antibodies (Wright and Smith, 1897). Several serological tests have been used for the diagnosis of human brucellosis. The serum agglutination test (SAT) for brucellosis, developed by Wright and Smith, (1897) is still the reference to which other tests are compared (Yagupsky, 1999). Other notable tests that have been developed since then are the Rose Bengal test, complement fixation test, indirect Coombs test, enzyme immunoassay (ELISA) and more recently, an immunocapture-agglutination test (Brucellacapt) (Ordun˜a *et al*., 2000; Concepcio´n Go´mez *et al*., 2008).

The presumptive bacteriological diagnosis of B. *melitensis* can be made by means of the microscopic examination of smears from vaginal swabs, placentas or aborted foetuses

stained with the Stamp modification of the Ziehl-Neelsen method (Alton *et al*., 1988) or detection of bacterial genome by application of polymerase chain reaction (EC, 2001).

Tests currently used for the serological diagnosis of *B. melitensis* infections in sheep and goats were initially developed for the diagnosis of *B. abortus* infections in cattle. Although not formally validated for use in sheep and goats, these tests, and in particular the Rose Bengal plate agglutination test, the complement fixation test, and more recently the ELISA have been used for the serological diagnosis of brucellosis in sheep and goats (EC, 2001). A combination of tests shows a degree of sensitivity and specificity which appears sufficient to detect infected animals, and removal of those animals appears to contribute to disease control when vaccination is avoided or when the Rev.1 vaccine is only administered in young animals, particularly by the conjunctival route (EC, 2001). In other situations, especially when the vaccine is administered in adult animals, the results of these tests can sometimes be difficult to interpret because of antibodies induced by vaccination (EC, 2001). It is recommended that the existing diagnostic tests are improved and validated (EC, 2001).

2.3.6.1 Rose Bengal plate test

The RBPT is a very simple and inexpensive single-step agglutination test, using antigen with a dye attached, buffered at pH 3.65. Its major disadvantage is its susceptibility to false positive reactions due to strain 19 vaccination. This susceptibility probably follows from the high sensitivity of the RBPT to IgM antibody (Alton *et al*., 1988). Where vaccination is practiced, it is commonly used as a screening test, sera positive to the RBPT being retested by CFT to give a definitive diagnostic result. A few animals may be negative to the RBPT but positive to the CFT. Differences in sensitivity have

sometimes been experienced with different batches of commercial Rose Bengal antigen (Alton *et al*., 1988).

2.3.6.2 Competitive Enzyme Linked Immunosorbent Assay

In this test, *Brucella* antigen is immobilized on the plate as with the indirect ELISA. Following that, the serum under test and a monoclonal antibody directed against an epitope on the antigen are co-incubated. This anti- *Brucella* monoclonal antibody is conjugated to an enzyme, the presence of which is detected if it binds to the antigen. This will only occur if there is no antibody in the serum sample which is bound preferentially (EC, 2001).

2.3.7 Treatment

A variety of antimicrobial drugs have activity *in vitro* against *Brucella* species; however, the results of routine susceptibility tests do not always correlate with clinical efficacy. Consequently, beta-lactam antibiotics and macrolide antibiotics such as penicillin, cephalosporin and erythromycin respectively, are associated with unacceptably high rates of relapse when used to treat patients with brucellosis WHO (2006). Although newer macrolides, such as azithromycin and clarithromycin are more active *in vitro* than erythromycin, they are not superior over current regimens for treatment of brucellosis, and their role in therapy remains to be determined WHO (2006).

Treatment of choice in acute brucellosis consists of antibiotic therapy. The best results are achieved with rifampicin combined with doxycycline for at least 6 weeks. Treatment

generally needs to be prolonged or repeated in persistent forms before a cure is achieved (EC, 2001)

2.3.8 Prevention

It is practically more economical to prevent brucellosis than to treat, control or eliminate. The preventive measures include:

1. Careful selection of replacement animals. These, whether purchased or produced from existing stock, should originate from *Brucella*-free herds or flocks. Pre-purchase tests are necessary unless the replacements are from populations in geographically circumscribed areas that are known to be free of the disease WHO (2006).
2. Isolation of purchased replacements for at least 30 days. In addition a serological test prior to commingling is necessary.
3. Prevention of contacts and commingling with herds of flocks of unknown status or those with brucellosis.
4. If possible, laboratory assistance should be utilized to diagnose causation of abortions, premature births, or other clinical signs. Suspect animals should be isolated until a diagnosis can be made WHO (2006).
5. Herds and flocks should be included in surveillance measures such as periodic milk ring tests in cattle (at least four times per year), and testing of slaughtered animals with simple screening serological procedures such as the RBPT.
6. Proper disposal (burial or burning) of placentas and non-viable foetuses. Disinfection of contaminated areas should be performed thoroughly WHO (2006).

2.3.9 Control

The control of brucellosis in animals is to reduce the impact of a disease on human health and the economic consequences. The elimination of the disease from the population is not the objective of a control, and it is implicit that some "acceptable level" of infection will remain in the population WHO (2006). Control programmes have an indefinite duration and will need to be maintained even after the "acceptable level" of infection has been reached, so that the disease does not re-emerge WHO (2006). In many countries, methods for the control of brucellosis are backed by governmental regulation. Certain principles can be used;

1. The reduction of exposure to *Brucella* spp.
2. The increase of the resistance to infection of animals in the populations.

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

In regions with high prevalence of the disease, the only way of controlling this zoonosis is by vaccination of all susceptible hosts and elimination of infected animals (Briones *et al*., 2001). The most commonly used vaccines against bovine brucellosis are *B. abortus* strain 19 and the recently USDA approved strain RB51; the latter unlike strain 19 does not interfere with serological diagnoses (Moriyon *et al*., 2004). The use of *B. abortus* strain 19 vaccine leads to the production of antibodies whose persistence depends mainly on the age of the animals at the time of vaccination. Based on test and slaughter coupled with control by vaccination, to ensure successful programs, there must be rigid control of the age at which strain 19 vaccination is allowed (Morgan, 1969). *B. melitensis* strain Rev1 although highly infectious to humans, is considered as the best vaccine available for the control of ovine and caprine brucellosis, especially when

administered at the standard dose by the conjunctival route. However, the Rev1 vaccine shows a considerable degree of virulence and induces abortions when administered during pregnancy (Morgan, 1969).

Also, the antibody response to vaccination cannot be differentiated from the one observed after field infection, which impedes control programs. Attempts have been made to develop new live attenuated rough *B. melitensis* vaccines, which are devoid of the O-side chain. Those vaccines await further evaluation in field experiments (Adone *et al*., 2008; Blasco, 1997; Garin-Bastuji *et al*., 1998). Vaccination alone will not eradicate *Brucella* as the immunity produced by *Brucella* vaccines are not absolute and can be circumvented by increasing the level of infection. It is obvious, therefore, that a policy of vaccination is more likely to succeed if combined with good measures of husbandry (Morgan, 1969). Live human vaccines *B. abortus* strain 19-BA and strain 104M are being used only in the former Soviet Union and China, respectively (Acha and Szyfres 2003). Control programmes in place include the proper application of vaccination. The programme requires a well-functioning surveillance system, financial support and co-operation with livestock owners. The proper application of vaccination results in a suppression of the infection pressure and reduces the zoonotic spread of brucellosis (Acha and Szyfres 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Bauchi State (Fig. 3.1) is situated in the North East geopolitical zone of Nigeria between latitude 9.3 ^{o} and 12.3 ^{o} north of the equator and longitude 8.5 ^{o} and 11 ^{o} east of the Greenwich meridian. The State is bordered by seven States; Kano and Jigawa to the north, Plateau and Taraba to the South; Yobe and Gombe to the East and Kaduna to the West. The State is divided into three senatorial zones namely; Bauchi South, Bauchi Central and Bauchi North senatorial zones and twenty local government areas (Bauchi State Diary, 2009) (Fig.1).

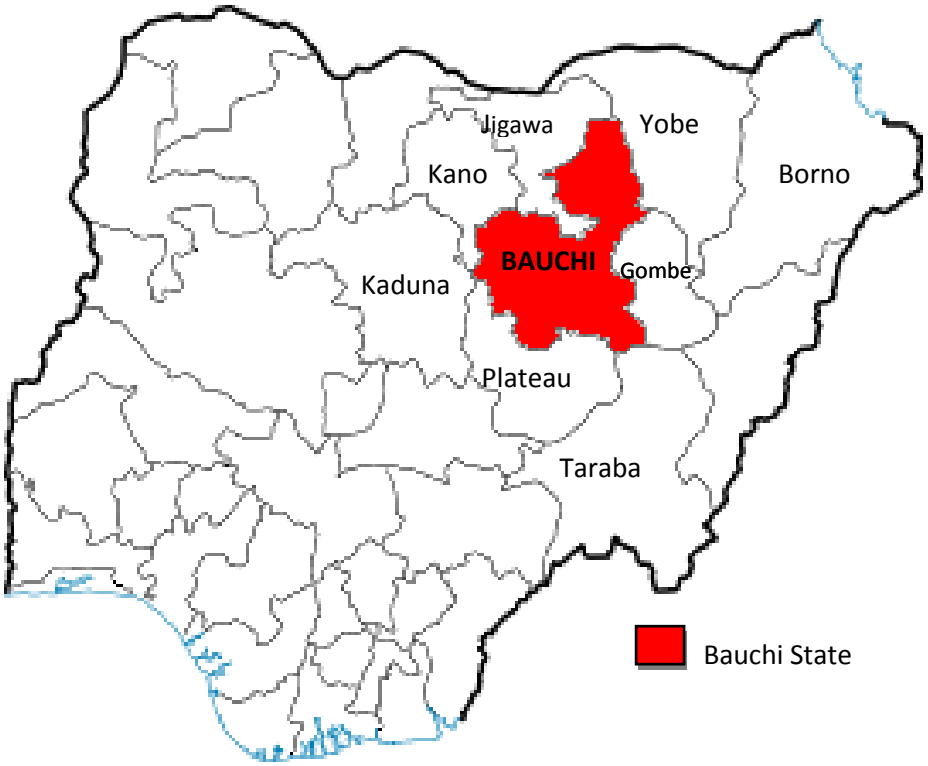

Figure 3.1: Map of Nigeria showing Bauchi State Among the 36 States of the Federation (Source: www.bauchistategov.org)

Figure 3.2: Map of Bauchi State Showing the Senatorial Districts and 20 Local Governments Areas (Source: www.bauchi-states.blogspot.com)

3.2 Study Design

3.2.1 Sample size determination

The estimated population of sheep and goats sampled were calculated using the formula:

$$n = \frac{1.96^2 P_{exp} (1-P_{exp})}{d^2}$$

(Thrusfield, 1997).

Where

n = required sample size

P_{exp} = expected prevalence; 14.5% for sheep and 16.1% for goats (Bertu *et al.,* 2010).

d = desired absolute precision (0.05).

Thus n = 191 for sheep and 207 for goats, which now gives 398

The sample size was increased to 739.

3.2.2 Sampling method

A multistage sampling method was adopted for this study. The state was divided into 3 senatorial zones and 2 Local Government Areas (LGAs) were randomly selected from each zone. The LGAs are Bauchi, Dass, Ningi, Darazo, Katagum and Gamawa. Thereafter, household flocks and abattoirs were conveniently selected for sampling.

3.2.3 Sampling from the Households

Sheep and goats meant for sampling were observed and examined for possible signs of brucellosis such as hygroma and orchitis. The sexes of the animals were recorded before blood sample collections. Inclusion criteria used was that animals over 3 months of age

were considered for the study. Total of 400 small ruminants which comprised of 262 sheep and 138 goats were sampled from households during the study.

3.2.4 Sampling from the Abattoirs

Blood sample was collected from 62 slaughtered sheep and 277 goats from the major abattoirs, i.e.: Bauchi main abattoir, Azare main abattoir, Ningi main abattoir, Dass main abattoir, Gamawa main abattoir and Darazo main abattoir.

3.2.5 Sample Collection

Five millilitres of blood was aseptically collected by jugular venepuncture from each animal using 10 ml, 21 gauge hypodermic needle syringes (Zarinject®) from the households. The sample were transferred into a clean plain and properly labelled 5 ml sample bottles (Tarcel®) devoid of anticoagulants.

Blood Samples from the abattoir were collected directly into the sampling bottles immediately after slaughter. Blood was allowed to stay undisturbed for 15-30 minutes, for separation of sera, which was then immediately transferred into a clean polypropylene tube using a Pasteur pipette. The sera were transferred into 2 ml serum vials (Sarstedt screw cap micro tube®) and stored in a deep freezer at -20 ^{0}C in Bauchi. Thereafter, it was transported in a cooler with ice pack from Bauchi to Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria and stored at -20 ^{0}C until used.

3.3 Laboratory Procedure

Sera stored were thawed to room temperature (37 ^{0}C) and subjected to Rose Bengal Plate Test (RBPT) and Competitive Enzyme-linked Immunosorbent assay (cELISA) as outlined by OIE Terrestrial Manual (2009), at the Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria. Antigens and the test kits were obtained from Veterinary Laboratory Agency (VLA), Weybridge, United Kingdom.

3.3.1 Procedure for Rose Bengal Plate Test (RBPT)

The test was carried out as follows:

A drop of serum sample was placed on a clean grease free ceramic tile and equal volume of antigen was placed near each serum spot.

The serum and antigen were mixed thoroughly using a sterile wire loop to produce a circular or oval zone approximately 2cm in diameter.

The mixture was agitated gently by shaking for 4 minutes at ambient temperature (25 ^{0}C) by gently rocking the slide. Agglutination were recorded as positive (+) and negative (-) as described by WHO (2006).

3.3.2 Procedure for Competitive-emzyme Linked Immunosorbent Assay (cELISA) Test

The cELISA was carried out as recommended by the supplier:

1. Conjugate solution was prepared and then diluted with diluting buffer to working strength according to the instructions on the ampoule label.
2. Twenty microliters of test serum were added to each microliter plate well.
3. Twenty microliters of the negative control were added to wells A11, A12, B11, B12, C11 and C12.

4. Twenty microliters of the positive control were added to wells F11, F12, G11, G12, H11 and H112.
5. The remaining wells have no serum, acting as conjugate control.
6. One hundred microliters of the prepared conjugate solution were immediately dispensed into all wells. This now gives a final serum dilution of 1/6.
7. Plate was then vigorously shaken (on the microtiter plate shaker) for two minutes in order to mix the serum and conjugate solution. The plate was then covered with the lid and incubated at room temperature (21.1±6.1 ^{0}C) for 30 minutes.
8. The contents of plate were discarded by agitating the plate.
9. The plate was rinsed 5 times with washing solution and was then thoroughly dried by tapping on absorbent paper towel.
10. The microplate reader was switched on, and allowed to stabilize for 10 minutes.
11. Substrate and chromogen solution were immediately prepared by dissolving one tablet of urea H_2O_2 in 12 ml of distilled water, Ophenylenediamine (OPD) tablet was also added and mixed. 100 µl of the solution was then added to each well.
12. Plate was left at room temperature for 15 minutes.
13. One hundred microliters of stopper solution were added to all wells to slow the reaction.
14. Condensation was removed from the bottom of the plate with absorbent paper towel and plate was read using ELISA reader at 450nm.

3.3.3 Interpretation

The lack of colour development indicates that the sample tested was positive. A positive/negative cut-off was calculated as; 60% of the mean of the optical density (OD) of the 4 conjugate control wells. All test samples that gave an OD equal to or below this value were regarded as positive.

3.4 Questionnaire

Data collected from the respondents were age, sex, educational status, protective measures, awareness on route of transmission, and clinical signs of brucellosis using open ended structured questionnaire. The contents of the questionnaire were interpreted into local language for those who could not understand English. Names of the respondents were not recorded for confidentiality.

3.5 Data Analysis

Data generated at the end of the study were analysed using Chi-square (Snedecor and Cochran, 1980) to test for association of brucellosis with sex, zones and, between household and abattoir samples using 5% level of significance.

CHAPTER FOUR

RESULTS

4.1 Sero-prevalence of Brucellosis in Small Ruminants in Bauchi State

The overall prevalence of brucellosis in sheep and goats in six local government areas of Bauchi State are shown in (Table 4.1). Out of a total of 324 sheep tested, 114 (35.2%) and 77 (23.8%) were positive for brucellosis by RBPT and cELISA respectively. And out of 415 goats sampled, 98 (23.6%) and 56 (13.5%) were positive by RBPT and cELISA respectively.

4.2 Sero-prevalence of Brucellosis in Small Ruminants in Bauchi State based on source of sample

Out of the 400 samples obtained from the house hold flocks138 (34.5%) and 88 (22.0%) were positive for brucellosis by RBPT and cELISA respectively, while out of the 339 samples collected from abattoirs 74 (21.8%) and 45 (13.3%) were positive by RBPT and cELISA respectively.

4.3 Sero-prevalence of Brucellosis in Small Ruminants in Bauchi State based on Sex

Out of the 293 samples that were from the male animals 84 (28.7%) and 55 (18.8%) were positive for brucellosis by RBPT and cELISA respectively, while from the 446 samples collected from the females 128 (28.7%) and 78 (17.5%) were positive by RBPT and cELISA respectively.

4.4 Sero-prevalence of Brucellosis in Sheep in Bauchi State based on Sex

324 samples were collected from sheep, 97 of them were rams and 227 were ewes. Among the rams, 37 samples (38.1%) and 29 (29.9%) were positive for brucellosis by RBPT and cELISA respectively, while among the ewes 77 (33.9%) and 48 (21.1%) were positive by RBPT and cELISA respectively.

4.5 Sero-prevalence of Brucellosis in Goats in Bauchi State based on Sex

415 samples were collected from goats, 196 of them were bucks and 219 were does. Among the bucks, 47 samples (24.0%) and 26 (13.3%) were positive for brucellosis by RBPT and cELISA respectively, while among the does 51 samples (23.3%) and 30 sample (13.7%) were positive by RBPT and cELISA respectively.

4.6 Sero-prevalence of Brucellosis of Sheep and Goats in the LGAs Studied

The prevalence of brucellosis in sheep and goats in the six local government areas (LGA) of Bauchi state are shown in Table 4.6. The highest prevalence of brucellosis were found in Dass and Katagum LGAs with 21 samples (24.7%) and 36 samples (25.9%) being positive respectively using cELISA, while the lowest prevalence rates were found in Gamawa and Ningi with 10 samples (8.1%) and 8 samples (10.9%) being positive respectively.

4.7 Sero-prevalence of Small Ruminant Brucellosis in the Three Zones of Bauchi State

Prevalence of brucellosis in the three senatorial zones of Bauchi state is presented in Table 4.7. The highest prevalence was found in sheep and goats from Bauchi South 59 (19.0%), while Bauchi Central 28 (16.9%) had the lowest prevalence.

4.8 Mode of Feeding of the Animals among Household Flocks in the six Local Government Areas of Bauchi State

Table 4.8 shows the type of feeding system employed by small ruminant farmers. Greater percentage (55.4%) of farmers allowed there animals to feed outside.

4.9 Level of Awareness of brucellosis among Small Ruminant Farmers in the six LGAs of Bauchi State

Out of 220 small ruminant farmers that responded to the questionnaire only 80 (36.4%) were aware of brucellosis, while 140 (63.6%) did not know about the existence of the disease.

4.10 Level of Awareness on Signs of Brucellosis in Small Ruminant Households in the six LGAs of Bauchi State

The common signs of brucellosis seen in small ruminant household flocks were abortions, retained placenta and Orchitis with 130 (59.1%), 34 (15.5%) and (4.1%) respectively.

4.11 Level of Awareness on Transmission of Brucellosis from Animals to Humans by small Ruminant Farmers in Bauchi State

Out of 220 small ruminant farmers who responded only 53 (24.1%) were aware that the disease can be transmitted to human beings, while 140 (63.6%) were not aware of the transmission of the disease.

4.12 Level of awareness on the need for protection against brucellosis by small ruminant farmers in the six LGAs of Bauchi State

One hundred and seventy four (79.1%)responded that they washed their hands after handling small ruminants, 4.1% used gloves and 16.4% do nothing, while handling small ruminants.

4.13 Consumption of Milk by Small Ruminant Farmers from their animals

Sixty eight 68 (30.9%) of the farmers said they consume milk and 152 (69.1%) do not consume milk from the small ruminants.

4.14 Mode of Milk intake by small ruminants farmers

Most of the farmers drink small ruminant milk after boiling 41 (60.3%), 4 (5.9%) drink fermented milk while 23(33.8%) drink raw milk.

Table 4.1 Sero-prevalence of Brucellosis in Small Ruminants in Bauchi State

Animal species	N	No (%) Positive RBPT	No (%) Positive cELISA	P-value	OR	95% C.I on OR	
						Lower	Upper
Sheep	324	114(35.2)	77(23.8)	0.0003	1.998	1.366	2.924
Goats	415	98(23.6)	56(13.5)				
Total	739	212(28.7)	133(17.9)				

RBPT = Rose Bengal Plate Test.
cELISA = Competitive Enzyme Linked Immunosorbent Assay.
C.I = Confidence interval.
OR = Odds Ratio.
n = number of animals sampled.

Table 4.2 Sero-prevalence of brucellosis in small ruminants in Bauchi State Based on Source of Samples

Source of samples	N	No (%) Positive RBPT	No (%) Positive cELISA	P-value	OR	95% C.I on OR	
						Lower	Upper
Households	400	138 (34.5)	88 (22.0)	0.0021	1.843	1.244	2.730
Abattoirs	339	74 (21.8)	45 (13.3)				
Total	739	212 (28.7)	133 (17.9)				

RBPT = Rose Bengal Plate Test.
cELISA = Competitive Enzyme Linked Immunosorbent Assay.
C.I = Confidence interval.
OR = Odds Ratio.
n = number of animals sampled.

Table 4.3 Sero-prevalence of Brucellosis in Small Ruminants in Bauchi State Based on Sex.

Sex	n	No (%) Positive RBPT	No (%) Positive cELISA	P-value	OR	95% C.I on OR	
						Lower	Upper
Male	293	84 (28.7%)	55 (18.8%)	0.6957	1.090	0.7443	1.597
Female	446	128(28.7%)	78 (17.5%)				
Total	739	212 (28.7)	133 (17.9)				

RBPT = Rose Bengal Plate Test.
cELISA = Competitive Enzyme Linked Immunosorbent Assay.
C.I = Confidence interval.
OR = Odds Ratio.
n = number of animals sampled.

Table 4.4 Sero-prevalence of Brucellosis in Sheep in Bauchi State Based on Sex

Sex	N	No (%) Positive RBPT	No (%) Positive cELISA	P-value	OR	95% C.I on OR	
						Lower	Upper
Male	97	37 (38.1)	29 (29.9)	0.1164	1.590	0.9277	2.727
Female	227	77 (33.9)	48 (21.1)				
Total	324	114 (35.2)	77 (23.8)				

Table 4.5 Sero-prevalence of Brucellosis in Goats in Bauchi State Based on Sex

Sex	N	No (%) Positive RBPT	No (%) Positive cELISA	P-value	OR	95% C.I on OR	
						Lower	Upper
Male	196	47 (24.0)	26 (13.3)	1.0000	0.9635	0.5478	1.695
Female	219	51 (23.3)	30 (13.7)				
Total	415	98 (23.7)	56 (13.5)				

RBPT = Rose Bengal Plate Test.
cELISA = Competitive Enzyme Linked Immunosorbent Assay.
C.I = Confidence interval.
OR = Odds Ratio.
n = number of animals sampled.

Table 4.6 Distribution of Small Ruminant Brucellosis by RBPT and cELISA Test in the Six LGAs of Bauchi State

Locations (Local Gov. Areas)	Total number of animals sampled	No (%) Positive RBPT	No (%) Positive Celisa
Bauchi	226	46 (20.4)	38 (16.8)
Dass	85	31 (36.5)	21 (24.7)
Gamawa	123	33 (26.8)	10 (8.1)
Katagum	139	42 (30.2)	36 (25.9)
Darazo	93	50 (53.8)	20 (21.5)
Ningi	73	10 (13.6)	8 (10.9)
Total	739	212 (28.7)	133 (17.9)

Table 4.7: Sero-prevalence of Brucellosis in the Three Senatorial Zones of Bauchi State

Zones	Total number of animals sampled	No (%) Positive RBPT	No (%) Positive cELISA
Bauchi South	311	77 (24.8)	59 (19.0)
Bauchi North	262	75 (28.6)	46 (17.6)
Bauchi central	166	60 (36.1)	28 (16.9)
Total	739	212 (28.7)	133 (17.9)

Table 4.8: Mode of Feeding of the Animal Among Household Flocks in the Six Local Government Areas of Bauchi State

LGAs	Mode of Feeding			Total
	At home (%)	Outside (%)	Both (%)	
Bauchi	32 (14.6)	2 (0.9)	25 (11.3)	59
Ningi	8 (3.6)	1 (0.5)	20 (9.1)	29
Dass	22 (10.0)	0 (0.0)	7 (3.2)	29
Darazo	11 (5.0)	5 (2.3)	15 (6.8)	31
Katagum	12 (5.5)	0 (0.0)	29 (13.2)	41
Gamawa	14 (6.4)	1 (0.5)	16 (7.3)	31
Total	98 (44.6)	9 (4.1)	113 (51.4)	220

Table 4.9: Level of awareness of Brucellosis Among Small Ruminant Farmers in the Six LGAs of Bauchi State

LGAs	Awareness on Brucellosis		Total
	Yes (%)	No (%)	
Bauchi	11 (5.0)	48 (21.8)	59
Ningi	7 (3.1)	22 (10.0)	29
Dass	6 (2.7)	23 (10.5)	29
Darazo	21 (9.6)	10 (4.6)	31
Katagum	16 (7.3)	25 (11.4)	41
Gamawa	19 (8.6)	12 (5.5)	31
Total	80 (36.4)	140 (63.6)	220

Table 4.10 The Common signs of Brucellosis in Small Ruminant Household Flocks in the Six LGAs of Bauchi State

LGAs	Signs of Brucellosis				Total
	Abortion (%)	Retained Placenta (%)	Orchitis (%)	None (%)	
Bauchi	29 (13.2)	8 (3.6)	1 (0.5)	21 (9.6)	59
Ningi	22 (10.0)	2 (0.9)	1 (0.5)	4 (1.8)	29
Dass	15 (6.8)	2 (0.9)	2 (0.9)	10 (4.6)	29
Darazo	22 (10.0)	7 (3.2)	2 (0.9)	0 (0.0)	31
Katagum	17 (7.7)	13 (5.9)	1 (0.5)	10 (4.6)	41
Gamawa	25 (11.4)	2 (0.9)	2 (0.9)	2 (0.9)	31
Total	130 (59.1)	34 (15.5)	9 (4.1)	47 (21.4)	220

Table 4.11 Level of Awareness on Transmission of Brucellosis from Animals to Humans by Small Ruminant Farmers in the Six LGAs of Bauchi State

LGAs	Awareness on Human Transmission		Total
	Yes (%)	No (%)	
Bauchi	3 (1.4)	56 (25.5)	59
Ningi	3 (1.4)	26 (11.8)	29
Dass	4 (1.8)	25 (11.4)	29
Darazo	21 (9.6)	10 (5.0)	31
Katagum	5 (2.3)	36 (16.4)	41
Gamawa	17 (7.7)	14 (6.4)	31
Total	53 (24.1)	167 (75.9)	220

Table 4.12: Level of Awareness on the Need for Protection Against Brucellosis by Small Ruminant Farmers in the Six LGAs of Bauchi State

LGAs	Farmers Hygienic Practices			Total
	Wash Hand (%)	Use Hand gloves (%)	Do nothing (%)	
Bauchi	45 (20.5)	2 (0.9)	12 (5.5)	59
Ningi	23 (10.5)	0 (0.0)	6 (2.7)	29
Dass	25 (11.4)	1 (0.5)	3 (1.4)	29
Darazo	24 (10.9)	2 (0.9)	5 (2.3)	31
Katagum	30 (13.6)	1 (0.5)	10 (5.0)	41
Gamawa	28 (12.7)	3 (1.4)	0 (0.0)	31
Total	174 (79.1)	9 (4.1)	36 (16.4)	220

Table 4.13 Consumption of Milk by Small Ruminant Farmers from their Animals

LGAs	Consumption of Small Ruminant Milk by Farmers		Total
	Yes (%)	No (%)	
Bauchi	23 (10.5)	36 (16.4)	59
Ningi	4 (1.8)	25 (11.4)	29
Dass	10 (4.6)	19 (8.6)	29
Darazo	10 (4.6)	21 (9.6)	31
Katagum	5 (2.3)	36 (16.4)	41
Gamawa	16 (7.3)	15 (6.8)	31
Total	68 (30.9)	152 (69.1)	220

Table 4.14: Mode of Milk Intake by Small Ruminant Farmers

LGAs	Mode of Milk intake			Total (%)
	Raw (%)	Boiled (%)	Fermented (%)	
Bauchi	11(16.2)	12 (17.7)	0 (0 .0)	23 (33.8)
Ningi	2 (2.9)	9 (13.2)	2 (2.9)	4 (5.9)
Dass	3 (4.4)	6 (8.8)	1 (1.5)	10 (14.7)
Darazo	5 (7.4)	5 (7.4)	0 (0.0)	10 (14.7)
Katagum	2 (2.9)	3 (4.4)	0 (0.0)	5 (7.4)
Gamawa	0 (0.0)	15 (22.1)	1 (1.5)	16 (23.5)
Total	23(33.8)	41 (60.3)	4 (5.9)	68

CHAPTER FIVE

DISCUSSION

The study was designed and conducted to determine the sero-prevalence of brucellosis in small ruminants in six Local Governments Areas of Bauchi state. All sera from the small ruminants were subjected to Rose Bengal Plate Test (RBPT) as a screening test and the Competitive Enzyme Linked Immunosorbent Assay (cELISA) as confirmatory test.

This study reveals a brucellosis sero-prevalence of 35.2% by RBPT and 23.8% by cELISA in sheep; and 23.6% by RBPT and 13.5% by cELISA in goats. The sero-prevalence in the present study is comparable to the reports by previous workers from other states in Nigeria (Okoh (1980); Falade and Shonekan, (1981); Okewole *et al*. (1988); Brisibe *et al*. (1993); Ogundipe *et al*. (1994); Shehu *et al*. (1999) and Bale *et al*. (2003). While some previous works reported higher prevalence, others reported lower prevalence compared to this findings. For example, in goats, Bale *et al*. (2003) reported a high prevalence of 34.8% in seven government farms in northern Nigeria. Ojo *et al*. (2007) reported higher prevalence of 45.8% in goat flock in Abeokuta which also disagreed with the current prevalence of 13.5% in goats in Bauchi State. Although Falade and Shonekan, (1981); Ogundipe *et al*. (1994) and Shehu *et al*. (1999) reported lower prevalence of 4.8%, 9.0% and 5.9% respectively, compared to the current study. The prevalence in sheep (23.8%) in Bauchi State disagreed with findings of Okoh (1980) who reported 14.5% in Kano, and Bale *et al*. (1982), reported 14.1% in a study conducted in Northern Nigeria. However, Bale *et al*. (2003) reported brucellosis in seven government farms in northern Nigeria revealed a prevalence of 15.9% in sheep which is lower compared to the findings of the current study. Lower prevalence was

also reported by Okewole *et al*. (1988) in northern Nigeria, Brisibe *et al*. (1993) in Maiduguri and Shehu *et al*. (1999) in Bauchi both reported 6.6%. This can be attributed to increase in animal population and lack of adequate brucellosis control in the study area. To the best of our knowledge, this appears to be the first serological study of small ruminant brucellosis that involved all the three senatorial zones of Bauchi state. There is no documented information on any previous work of this nature that has been conducted in small ruminants in the state. The high prevalence is not surprising since small ruminants are not being vaccinated against brucellosis in Bauchi State and the country at large.

Based on the current study, brucellosis may be said to be endemic in the study area and the prevalence is high in small ruminants in the state. The disease is widely spread in the LGA surveyed. This high prevalence in small ruminants may be attributed to the fact that there are no vaccination schedules against brucellosis in the LGAs. Currently, there are no prevention or control measures for brucellosis in the state at large. As long as small ruminants continue to remain unvaccinated, they will become infected and serve as reservoirs of the organism and become a threat to cattle and human populations. The small ruminants in the study areas are under semi-intensive regiment, hence they mix with other animals especially in grazing areas and water points. Most of the farmers keep small and large ruminants together and this practice serve as means of brucellosis transmission between farms, households, and communities. The prevalence is widespread among sheep and goats in the LGAs of the three senatorial zones. This may be due to differences in management practices and awareness of brucellosis among the farmers. Introduction of new animals into a flock without screening for brucellosis may contribute to the spread of infection in the study area.

The higher prevalence of brucellosis in sheep (23.8%) compared to goats (13.5%) in this study is contrary to the findings of Bale *et al*. (1982) who reported a prevalence of 16.1 % in goats and 14.1 % in sheep. The results also disagreed with findings of Falade and Shonekan, (1981); Brisibe *et al*. (1993); Bale *et al*. (2003) and Ojo *et al*. (2007). But the results of this research agreed with findings of Okewole *et al*. (1988) who reported higher prevalence rates in sheep (1.8- 14.1%) compared to goats (1.5% and 12.1%)respectively.

Although Kramer *et al*. (1967) carried out their study only in sheep in Eastern Nigeria and in goats in northern Nigeria; they reported a marginally higher prevalence of 1.0% in goats compared to 0.9% in sheep. Okoh (1980) reported a prevalence of 14.5% in Kano, Falade and Shonekan (1982) reported 2.6% prevalence in sheep in Ibadan, Falade and Sellers (1974) reported 4.3% prevalence in goats in 10 States of Nigeria, while Adams and McKay (1966) reported zero prevalence in both sheep and goats in government farms in eastern Nigeria.

The variation in prevalence of brucellosis in sheep and goats from the three senatorial zones in the state might be due to the introduction of non-indigenous sheep (Sudan white, Balami) from the neighbouring states like Yobe and Jigawa States which share common boundary. These variations were not statistically significant ($P > 0.05$). This may be responsible for the high prevalence in Bauchi north as infection is simply transmitted within the flocks under semi intensive management practice commonly practiced in the zone.

Bauchi south and Bauchi central zones being the zones with high human population may have a higher demand for animal protein than rural settings. The semi intensive management practice in Bauchi south of cattle and sheep could favour the spread of brucellosis from cattle to sheep or vice versa. Susceptible animals are known to be infected following grazing on contaminated pastures (Kabagambe *et al*., 2001). Bauchi central seem to have higher prevalence compared to Bauchi south though it is the zone with the lowest population of small ruminants. The farmers are restocking their farms from neighbouring states and areas which may be responsible for the relative high prevalence of brucellosis in the zone. This is because either the animals were brought in with the infection or the area is highly contaminated causing immediate spread of infection of introduced animals since no consideration was given to the brucellosis status of the introduced animals or the environment.

Balami and especially the Sudan white breeds of sheep are mostly brought in from different states by traders and individuals that can afford them. They are mostly kept under intensive management for fattening. They are commonly found in urban and semi-urban settlements in the state.

The disease brucellosis is mostly associated with reproductive system especially in female animals. This raised the need to compare prevalence of brucellosis between the sexes. The results showed higher prevalence (29.9%) in rams compared to ewes (21.2%). The difference was however not statistically significant ($P > 0.05$). On the contrary prevalence of brucellosis was found to be higher (13.7%) in does than in bucks (13.3%). This shows that sex may play a role in the prevalence of brucellosis.

This study revealed that brucellosis is prevalent in reared and slaughtered animals. The prevalence is higher in field sampled animals (22.0%) compared to those from abattoirs and slaughter slabs (13.3%). The high prevalence found in samples collected from the field may be due to large sample size compared to the sample size obtained from the abattoir and slaughter slabs.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

1. This study revealed a 17.9% total prevalence of small ruminant brucellosis in Bauchi State using cELISA.

2. Based on cELISA test there was no statistically significant difference in prevalence between infection and the sexes in sheep.

3. The results from the questionnaires indicated that most of the small ruminant farmers were not aware of the disease, and the few who were aware did not know that it is zoonotic.

6.2 Recommendations

Based on the findings of this study the following are recommended:

(a) There is need to constitute control and surveillance programmes for brucellosis and brucellosis surveillance units should be established in all the three senatorial zones of the state.

(b) Farmers and abattoir personnel should be educated on personal hygienic practices and improved sanitary conditions especially when handling aborted materials.

(c) The practice of keeping small ruminants and large animals like cattle together should be discouraged.

(d) Further studies should be carried out in all the other LGAs not covered by this study.

(e) More work should be done to isolate *Brucella* and characterize them using molecular techniques.

(g) Milk samples should be examined in future studies.

References

Abdou , A. E. (2000). Fifty years of veterinary public health activities in the Eastern Mediterranean Region. *East Mediterranean Health Journal*, 6: 796- 807.

Adams, L. G. (2002). The Pathology of brucellosis reflects the outcome of the battle between the host genome and the Brucella genome. *Veterinary Microbiology*, 90: 553-561.

Adams, J. W. and McKay, J. (1966) Brucellosis in government owned herd in eastern Nigeria. *Nature* (London) (212): 217.

Adone, R., Francia, M. and Ciuchini, F. (2008). Evaluation of *Brucella melitensis* B115 as rough phenotype vaccine against *B. melitensis* and *B. ovis* infections. *Vaccine*, 26: 4913–4917.

Acha, N.P. and Szyfres, B. (2003). *Zoonosis and Communicable Diseases Common to Man and Animals*, third edition., volume. 1. *Pan American Health Organization* (PAHO), Washington, DC.

Ajogi, I. (1997). Prevalence of brucellosis in Nigeria: A chronology of serological investigation. *Journal of Medical and Allied Science*, 1(1): 23-26.

Ajogi, I. and Akinwumi, J.A. (2001). Cash-flow model of the cost of brucellosis traditionally managed cattle herds in Nigeria. *Bulletin of Animal Health Production in Africa*, 49: 169-173.

Alton, G. G., Jones, L. M., Angus, R. D. and Verger, J. M. (1988). *Techniques for the brucellosis laboratory*, Institut National de la Recherche Agronomique Paris. p. 29

Alton, G. G. (1990), *Brucella melitensis*. 383 - 409. *In:* Nielson, K. and Duncan J. R. (ed'.), *Animal brucellosis*. CRC Press, Incorporation ., Boca Raton, Florida.

Ate, I. U., Andrew, P.I.R., Nok, J. and Tekdek, L.B. (2007). Seroprevalence of brucellosis in puerperal cows and its public health implications in Zaria, Nigeria. *Journal of Animal and Veterinary Advances*, 6 (7): 863-866.

Bale, J.O., Nuru, S., and Addo, P.B. (1982). Serological study of sheep and goat brucellosis in Northern Nigeria. *Bulletin of Animal Health Production in Africa*, 30:73–79.

Bale, J. O. and Kumi-Diaka, J. (1981). Serological and bacteriological study of bovine brucella from livestock investigation and breeding centers in Nigeria. *British Veterinary Journal*, 137: 256-261.

Bale, J. O and Kwanashie, G. G. (1985). Seroprevalence of brucellosis among horses in Northern Nigeria. *Journal of Animal Production and Research*, 4: 161-164.

Bale, J. O. (1991). Brucellosis: A threat to livestock production and human health in Nigeria. Paper presented at a symposium at *National Animal Production Research Institute*, Zaria Nigeria.

Bauchi State Government of Nigeria Diary, 2013.

Bawa, E. K., Adekeye, J. O., Oyedipe, E. O., Umoh, J. U. and Adenowo, T. K. (1987). Seroprevalence of bovine brucellosis in Kaduna State. *Nigerian Veterinary Journal*, 16: 59-60.

Bertu, W. J., Ajogi, I., Bale, J. O., Kwaga, J. K. P. and Ocholi, R. A. (2010). Sero-epidemiology of brucellosis in small ruminants in Plateau State, Nigeria. *African Journal of Microbiology Research*, 4 (19): 1935-1938.

Blasco, J.M. (1997). A review of the use of B. melitensis Rev 1 vaccine in adult sheep and goats. *Preventive Veterinary Medicine*, 31, 275–283.

Blasco, J. M. (2010) Control and eradication strategies for *Brucella Melitensis* Infection in sheep and goats *Biological Medical Science*, 1: 145–165.

Blench, R. (1999). Traditional livestock breeds geographical distribution and dynamics in relation to the ecology of West Africa. Overseas Development Institute, working paper, 122. Stag place, London.

Brisibe, F. D., Nawathe, R. and Bot, C. J. (1993) "Serological prevalence of brucellosis in sheep,goats and human beings in Maiduguri Metropolis," *Tropical Veterinarian, 11: 27–33.*

Briones, G., Inon de Iannino, N., Roset, M., Vigliocco, A., Paulo, P.S. and Ugalde, R.A. (2001). *Brucella abortus* cyclic beta-1,2-glucan mutants have reduced virulence in mice and are defective in intracellular replication in HeLa cells. *Infection and Immunity*, 69, 4528–4535.

Durán-Ferrer, M. (1998). Comparación entre métodos inmunológicos de diagnóstico de la brucelosis ovina por *(Brucella Melitensis)* y eficacia de la inmunización de ovejas adultas con la vacuna Rev.1 por vía conjuntival. *PhD Thesis*, University of Murcia, Spain.

Concepcio´n Go´mez, M., Jose´, A., Nieto, C, R., Paloma, G., A´ngeles, M. E., Mun˜oz, A. and Lo´pez, C. (2008). Evaluation of seven tests for diagnosis of human brucellosis in an area where the disease is endemic. *American Society for Microbiology*. 1031–1033.

Corbel, M. J. (1997). Brucellosis: an overview. *Emerging Infectious Diseases*, (3): 213-221.

Ehizibolo, D. D., Gusi, A.M., Ehizibolo, P. O., Mbuk, E. V. and Ocholi, R. A. (2011). Serologic Prevalence of Brucellosis in Horse Stables in Two Northern States of Nigeria. *Journal of Equine Science*, 22(1): 17-19.

Esuruoso, G. O and Van Blake, H. E. (1972). Bovine brucellosis in two Southern States of Nigeria: An investigation of selected herds. *Bulletin of Epizootic Diseases in Africa*, 20:269-274.

Esuruoso, G. O. (1974). Bovine brucellosis in Nigeria. *Veterinary Record*, 95:54-58.

EC (2001). Brucellosis in sheep and goat *(Brucella melitensis)*: In Health & Consumer Protection Directorate-General.

Eze, E. N. (1978). Isolation of Brucella from the Nigerian livestock and the typing of such isolates. *Bulletin of Animal Health and Production in Africa*, 26:29.

Falade, S., Ojo, M. O. & Sellers, K.C. (1974). A serological survey of caprine brucucellosis in Nigeria. *Bulletin of Animal Health and Production in Africa*. 22, 335.

Falade, S. and Shonekan, A. O. (1981). A serological survey of *Brucella abortus* infection in Nigerian sheep. *Nigerian Veterinary Journal*, 2:50-52.

FAO (2003). Guidelines for coordinated human and animal brucellosis surveillance. *Animal Production and Health Paper* 156: 2.

FAO (2005). Capacity building for surveillance and control of diseases. Rome Italy, Pp 55-66.

FDL. (2011). Nigerian National Livestock Survey. Federal Department of Livestock and pest Control Services, Abuja, 2: 89.

Fiori, P. L., Mastrandrea, S., Rappelli, P., Cappuccinelli, P. (2000). *Brucella abortus* infection acquired in microbiology laboratories, *Journal of Clinical Microbiology*, 38: 2005- 2006

Food and Agricultural Organisation (FAO) (2003). *Guidelines for coordinated human and animal production and health papers*. 156: pp: 15-25.

Food and Agricultural Organisation (2006). FAOSTAT. Database. *Food and Agricultural Organisation* Rome, Italy.

Garin-Bastuji, B., Blasco, J.M., Grayon, M. and Verger, J.M. (1998). *Brucella melitensis* infection in sheep: present and future. *Veterinary Research*, 255–274.

Hale, M. and Ajogi, I. (1997). Brucellosis in Nigeria: review of recent development. *Israel Journal of Veterinary Medicine*, 52 (4): 125-133.

Hoover, D. L. and Friedlander, A. M. (1997). *Brucellosis*. In: Zajtchuk R, Ed. *Textbook of military medicine*: medical aspects of chemical and biological warfare. Washington DC, US Department of the Army: Surgeon General & Borden Institute; 2: 513-21.

Ibrahim, H. (1998). Small ruminant production techniques. *ILRI Manual* 3. ILRI (International Livestock Research Institute) Nairobi, Kenya. Pp. 207.

Jibril, A. (2010). Effects of different levels of protein intake on live weight changes, testicular function and haematology in Yankasa rams. *MSc. Thesis* submitted to Ahmadu Bello University, Zara, Nigeria.

Kabagambe, E. K., Elzer, P. H., Geagham, J. P., Opuda-Asibo, J., Scholl D. T. and Miller, J. E. (2001). Risk factors for brucella seropositivity in goat flocks in eastern and western Uganda. *Preventive Veterinary Medicine*, 52: 91-108.

Kadohiri, M., McDermott, J. J., Shoukri, M. M. and Kyule, M. N. (1997). Variation in the prevalence of antibody to brucella infection in cattle by farm area and district in Kenya. *Epidemiology of Infection*, 118: 35-41.

Kramer, J.W., Nduaka, O. and Uzoukwu, M. (1967) Serological survey of diseases of cattle, sheep and goats in the Eastern provinces of Nigeria. *Bulletin of Epizootic Disease in Africa*, 15: 25–29.

Kruska, R.L. Reid, R.S. Thornton, P.K. Henninger, N. and Kristjanson, P.M. (2003) Mapping livestock-oriented agricultural production systems for the developing world. *Agricultural Systems*, 77: 39–63

Lòpez-Merino, A., (1989). Brucellosis in Latin America. *In: Brucellosis: Clinical and laboratory aspects of infection*.Young, E.J. and Cobel. J. M. (eds).Chemical Rubber Company (CRC) Press, Boca Raton. 151-161.

Maloney, G. E. and Fraser, W. R. (2004) *CBRNE - Brucellosis*. Omaha, Nebraska, eMedicine, (http://www.emedicine.com/emerg/topic 883.htm accessed 1 October 2006).

Mantur, B. G., Amarnath, S. K. and Shinde, R. S. (2007). Review of clinical and laboratory features of human brucellosis. *Indian Journal of Medical Microbiology*, 25:188.

Map of Bauchi State [Imge] (n.d.). Retrieved January 15,2010, from http://www.bauchi-students.blogspot.com/home.htm.

Map of Nigeria. [Imge] (n.d.). Retrieved November 18, 2011 from http://www.bauchi-students.blogspot.com/p/events.html

McDermott, J. J and Arimi, S. M. (2002). Brucellosis in sub-Saharan Africa: Epidemiology, control and Impact. *Veterinary Microbiology*, 90 (1-4): 111-134.

Morgan W., MacKinnon, D., Lawson, J. and Cullen, G. (1969): The Rose Bengal plate agglutination test in the diagnosis of brucellosis. *Veterinary Record*, 85: 636–7.

Moriyon, I., Grillo, M.J., Monreal, D., Gonzalez, D., Marin, C., Lopez-Goni, I., Mainar-Jaime, R.C., Moreno, E. and Blasco, J.M. (2004). Rough vaccines in animal brucellosis: structural and genetic basis and present status. *Veterinary Research*, 35, 1–38.

Nicoletti, P. (1984). Control of brucellosis in tropical and sub-tropical regions. *Preventive Veterinary medicine*, 2: 193-196.

Nigeria galleria.com, 2011 retrieved 18th November, 2011 from http://www.nigeriagalleria.com/Nigeria/States_Nigeria/Bauchi_State.html.

Ocholi,. R. A., Kalejaiye, J. O. and Okewole, P. A. (1993). Brucellosis in Nigeria review. *Tropical Veterianrian*, 11:15-26.

Ocholi, R. A., Bertu, W. J. Kwaga, J. K. P., Ajogi, I., Bale, J. O. and Okpara, J. (2004). Carpal bursitis associated with *Brucella abortis* in a horse in Nigeria. *Veterinary Record*,155: 566-7.

Ocholi, R. A., Kwaga, J. K. P., Ajogi, I. and Bale, J.O. (2005). Abortion due to *Brucella abortus* in sheep in Nigeria. *Review in Science and Technology. Office International des Epizootics, 24* (3): 973-979.

Office international des Epizootics (2009). Caprine and ovine brucellosis *Terrestrial manual*. Pp 2-3.

Ogundipe, G.A.T., Oyeyemi, M.O. and Ijagbone, I.F. (1994). Serolo-prevalence of Brucella abortus agglutinins in slaughtered cattle in Ibadan. *Tropical Veterinarian*, 12, 158-161.

Ojo, E. E. Oyekunle, M. A., Omtainse, S. O., Ocholi, R. A., Ogunleye, A. O. and Bertu W. J. (2007). Serological evidence of brucellosis in a goat flock with recurrent abortion in Abeokuta, Nigeria. *Tropical Veterinarian*, 25 (1): 26-33.

Okewole, P. A., Eze, E. N., Okoh, A. E. J., Oyetunde, I.L. and Odeyemi, N. S. (1988). Small ruminants brucellosis in some parts of Northern Nigeria. *Bulletin of Animal Health Production in Africa*, 36: 251-254.

Okoh, A. E. J. (1980). An investigation of abortion in sheep on Kano LIBC near Kano, Nigeria. *Bulletin of Animal Health Production in Africa*, 28: 135-136.

Omer, M. M., Abdelaziz, A. A., Abusalab, S. M. A and Ahmad. A. M. (2007). Survey of brucellosis among sheep goats camels and cattle in Kassala area, Eastern Sudan. *Journal of Animal Veterinary Advancement*, 6:635-637.

Ordun˜a, A. A., Almaraz, A., Prado, M. P. Gutie´rrez, A. Garcı´a-Pascual, A. Duen˜as, M. Cuervo, R. Abad, B. Herna´ndez, B. Lorenzo, M. A. Bratos, and A. R. Torres. (2000). Evaluation of an immunocapture-agglutination test (Brucellacapt) for serodiagnosis of human brucellosis. *Journal of Clinical Microbiology*, 38: 4000–4005.

Osinubi, M. O. V., Ajogi, I and Ehizibolo, D. O. (2004). *Brucella abortus* agglutinins in dogs in Zaria. *Nigerian Veterinary Journal*, 25:35-38.

Osori D. (1976). Seasonal variation in reproductive activity of indigenous cattle in Northern Nigeria. *PhD Thesis*, Ahmadu Bello University, Zaria.

Oyedipe ,E. O., Bavanendran, V. and Eduvie, L. O. (1981). Factors affecting the reproductive performance of Fulani Cattle .*National Animal Production Research Institute, Seminar.*

Rikin, V. M. (1988). Brucellosis of cattle in Nigeria: Proposal for a control programme under intensive and extensive husbandry system. *Aeta Veterinaria Scandinarian*, 11: 15-26.

RIMS (1992). Nigerian National Livestock Resource Survey. (Volume IV) Report by Resource Inventory and Management Limited (RIM) to Federal Department of Livestock and Pest Control Services. Federal Ministry of Agriculture and Natural Resources (FDLPCS) Abuja, Nigeria, pp. 440.

Santellano-Estrada, E., Infante, F., Diaz-Apraricio, E and Flores-Guetierrez, G. H. (2004). Use of an immune binding test on nitrocellulose paper to diagnose caprine brucellosis. *Veterinary Research Communication*, 28: 27-31.

Shehu, L. M. Yusuf, H. Kudi, A. C.and Kalla, D. U. (1999) Seroprevalence of brucellosis in ruminants in Bauchi and environs, *Nigerian Veterinary Journal*, 20: 1: 67–74.

Snedecor, G. W. and Cochran, W. G. (1980). *Statistical Methods*, 7th ed. Iowa State University Press, Ames, USA.

Solera, J., Lozano, E., Martinez-Alfaro, E., Espinosa, A. Castillejos, M. L. and Abad L (1999). Brucella spondylitis: review of 35 cases and literature survey. *Clinical Infectious Diseases*. 29: 1440-1449.

Thrusfield, M. (1997). *Veterinary Epidemiology*, 2nd ed. *Blackwell Science*, Oxford: 280-282.

Troy, S. B., Rickman, L. S. and Davis, C. E. (2005). Brucellosis in San – Diego: epidemiology and species related differences in acute clinical presentations. Medicine (Baltimore). 84: 174.

Ugwu, D. S. (2007). The role of small ruminants in the household economy of South-East Zone Nigeria. *Research Journal of Applied Science*, 2(6): 726-732.

Werschilowa, P. and Striedter, W. (1938) Etudes sur l'immunite dans la brucellose ovine par la methode de la surinfection. *Office International des Epizootics Bulletin*, 17, 563.

Winchell, J. M., Wolff, B. J., Tiller, R.,Bowen, M. D. and Hoffmaster, A. R. (2010). Rapid identification and discrimination of brucella isolates by use of Real-time

PCR and High Resolution Melt analysis. *Journal of Clinical Microbiology*, 48 (3): 67-702.

World Health Organization (WHO) (2006): Brucellosis in Humans and Animals. Produced in Collaboration with Food and Agricultural Organization (FAO) and Office International des Epizootics (OIE). WHO/CDS/EPR/2006. 7: 57-65.

Wright, A. E. and Smith, F. (1897). On the application of the serum test to the differential diagnosis of typhoid fever and Malta fever. *British Medical Journal*, 1: 1214–1215.

Yagupsky, P. **(**1999). Detection of brucella in blood cultures. *Journal of Clinical Microbiology*, 37: 3437–3442.

Young, E. J. (1991). Serologic diagnosis of human brucellosis: analysis of cases by agglutination test and review of the literature. *Review of infectious Diseases*, 13:359-372.

Young, E. J. (1995). An overview of human brucellosis. *Clinical Infectious Diseases*, 2: 283-90.

Zandraa ,J. (2008) New procedures for the diagnosis of human brucellosis in Mongolia p. 2.

Zygmunt, M. S., Blasco, J. M., Letesson, J. J. Cloeckaett, A. and Moriyon, I. (2009). DNA polymorphism analysis of Brucella, lipopolysaccharide genes reveals marked differences in O-polysaccharide biosynthetic genes between smooth and rough Brucella species and novel species-specific markers. *BioMed Central* (BMC) *Microbiology*, 9: 92.

APPENDIX 1

QUESTIONNAIRE SAMPLE

DEPARTMENT OF PUBIC HEALTH AND PREVENTIVE MEDICINE

FACULTY OF VETERINARY MEDICINE

AHMADU BELLO UNIVERSITY ZAIRA

S/NO. _____ QUESTIONNAIRE TO ASSESS THE LEVEL OF AWARENESS OF BRUCELLOSIS AMONG SMALL RUMINANT FARMERS IN BAUCHI STATE.

Section (A) Demographics information

1. Farm No. _____
2. Date_____
3. Age _____
4. Sex_____
5. Educational qualification
   (a) Islamic [ ] (b) Primary [ ] (c) secondary [ ] (d) Tertiary
6. Marital status
   (a) Married [ ] (b) Single [ ] (c) Divorced [ ] (d) Widow [ ]
7. Number of children
   (a) None [ ] (b) 1-5 [ ] (c) 6-10 [ ] (d) over 10 [ ]

Section B.

1. Type of farming system (a) Small scale [ ] (b) Large scale [ ]
2. Source of animal (a) local markets [ ] (b) known farm [ ] (c) others [ ]
3. Presence of other species of animal (a) Yes [ ] (b) No [ ]
4. Presence of other animals (a) Cattle [ ] (b) canine [ ] (c) feline [ ] (d) equine [ ] (e) none [ ]
5. Method of feeding (a) at home [ ] (b) outside [ ] (c) Both [ ]

Section C

1. Are you aware of the disease brucellosis (a) Yes [ ] (b) No [ ]
2. Have you seen one or more of the following conditions in the flock

(a) Abortion [ ] (b) Retained Placenta [ ] (c) Ochitis [ ] (d) None [ ]

3. Which among the following sign you see most?

(a) Abortion [ ] (b) Retained Placenta [ ] (c) Ochitis [ ] (d) None [ ]

4. How did you manage health problems in your flock

(a) Consult veterinarians/Animal Health workers [ ] (b) Handle by yourself [ ] (c) Do nothing [ ]

5. Did you know brucellosis can be transmitted to human from animal

(a) Yes [ ] (b) No [ ]

6. How did you handle yourself while /after handling your animal

(a) wash my hand with soap and/ or disinfectants [ ] (b) do nothing [ ]

(c) use hand gloves before handing placenta or aborted material [ ]

7. Do you drink milk from sheep or goat (a) yes [ ] (b) No [ ]
8. In which form (a) raw [ ] (b) boiled [ ] (c) fermented[ ]

APPENDIX II

cELISA TEST PLATE JUST BEFORE READINGS WITH ELISA READER.