SEROPREVALENCE OF *BRUCELLA* ANTIBODIES IN GOATS IN OJU LOCAL GOVERNMENT AREA OF BENUE STATE, NIGERIA

BY

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DECLARATION

I declare that the work in this thesis entitled "**Seroprevalence of *Brucella* antibodies in goats in Oju Local Government Area of Benue State, Nigeria**" has been carried out by me in the Department of Veterinary Public Health and Preventive Medicine under the supervision of Professor I. Ajogi and Dr. A. A. Dzikwi. 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.

Gabriel Ogbaji IJALE ---------- ----------

Signature Date

CERTIFICATION

This thesis entitled "**SEROPREVALENCE OF *BRUCELLA* ANTIBODIES IN GOATS IN OJU LOCAL GOVERNMENT AREA OF BENUE STATE, NIGERIA**" by Gabriel Ogbaji IJALE meets the regulations governing the award of the degree of Master of Science in Veterinary Public Health and Preventive Medicine of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my late parents, Mr and Mrs S. O. I. Alo

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ABSTRACT

In a study to determine the seroprevalence of *Brucella* antibodies in goats in Oju Local Government Area (LGA) of Benue State, 241 blood samples for sera were collected from goats sold at the Ihiejwu goat market and from some communities within the LGA. Similarly, epidemiological factors for brucella infection in such goats were studied and risk factors to brucella infection among goats and goat owners were examined through the use of a structured questionnaire. The serum samples were subjected to Rose Bengal Plate Test (RBPT) and Indirect Enzyme-Linked Immunosorbent Assay (iELISA) to determine the presence of *Brucella* antibodies. The RBPT gave a prevalence of 13.7% while the iELISA confirmed the presence of *Brucella* antibodies in 7.5% of the 241 sera tested. Based on age, 6.98% of the 43 goats below 12 months old were positive while 7.22% of the 97 goats between 13 - 36 months old were positive and 7.92% of the 101 goats above 36 months old were positive using the iELISA. Of the 49 male goats sampled, 6.12% were positive while 7.81% of the 192 female goats were positive with the iELISA. Breed-based prevalence rates gave 7.04% of the 213 Kano brown goats and 10.71% of the 28 West African dwarf breed with the iELISA. There was no significant association between brucella infection and age, sex or breed of goat sampled ($p > 0.05$). The questionnaire analysis revealed that none of the 33 goat owners vaccinated his/her goats against brucellosis and that 36% observed abortion and retained placenta in their goats. Nine percent of the owners admitted having joint pains while 3% each admitted having persistent fever and profuse sweating at night. Risk factors associated with brucellosis such as lack of vaccination and isolation of newly purchased goats, improper disposal of after-birth materials into the bush among others were established in this study. None of the owners could recognize brucellosis as a disease

affecting their goats. From the study, it appeared that the goat owners were neither aware of brucellosis as a disease affecting their goats nor as a zoonosis. Awareness campaign on brucellosis should be carried out in Oju LGA and Benue State in general to enlighten goat owners on this important zoonosis. A National Policy on the control of brucellosis is recommended.

TABLE OF CONTENTS

	Page
Title page	i
Declaration	ii
Certification	iii
Dedication	iv
Acknowledgement	v
Abstract	vi
Table of contents	viii
List of tables	xii
List of figures	xiii
List of appendices	xiv
List of abbreviations	xv
1.0 INTRODUCTION	1
1.1 Definition of the Problem	1
1.2 Statement of the Research Problem	2
1.3 Justification of the Study	4
1.4 Aim of the Study	5
1.5 Objectives of the Study	5
1.6 Research Questions	5
2.0 LITERATURE REVIEW	6
2.1 History	6
2.2 Description of the Causative Agent	6
2.2.1 Aetiology	6
2.2.2 Morphology	7

2.3 Epidemiology 7
2.3.1 Geographical distribution 7
2.3.2 Modes of transmission 8
2.3.3 Survival of *Brucella species* in the environment 9
2.3.4 Susceptibility and risk factors 11

2.4 The Disease 12
2.4.1 Pathogenesis 12
2.4.2 Clinical findings 14
2.4.3 Pathology 15

2.5 Immune Response 16
2.5.1 Humoral immunity 16
2.5.2 Cell mediated immunity 17

2.6 Diagnosis of Caprine Brucellosis 17
2.6.1 Direct agent detection methods 17
2.6.2 Serological methods 19
2.6.3 Molecular detection and typing methods 27
2.6.4 Tests based on cell mediated immunity 29
2.6.5 Cross-reactions in serological tests 30

2.7 Vaccination 31
2.7.1 *Brucella melitensis* Rev.1 vaccine 31
2.7.2 Vaccination with reduced doses of Rev.1 32
2.7.3 Conjunctival vaccination with Rev.1 33
2.7.4 Adverse effects of vaccination with Rev.1 vaccine 35
2.7.5 Handling Rev.1 vaccine 36
2.7.6 Other vaccines 36

2.8 Control and Eradication of Brucellosis..... 38
2.8.1 Immunization to reduce rate of infection..... 38
2.8.2 Test and slaughter..... 39
2.8.3 Prevention of spread among animals..... 40

2.9 *Brucella* Infection in Humans..... 41
2.10 Socio-economic Importance of Caprine Brucellosis..... 43
2.11 The Situation of Caprine Brucellosis in Nigeria..... 45

3.0 MATERIALS AND METHODS..... 46
3.1 Study Area..... 46
3.2 Study Design..... 48
3.3 Sample Size..... 48
3.4 Sample Collection and Processing..... 49
3.4.1 Sample collection and handling..... 49
3.4.2 Laboratory procedure..... 50

3.5 Questionnaire..... 52
3.6 Data Analysis..... 53

4.0 RESULT..... 54
4.1 Study Population..... 54
4.2 Questionnaire Responses..... 63
4.2.1 Diseases that affect goats in Oju LGA..... 63
4.2.2 Action taken when diseases occur in the goats..... 63
4.2.3 Occurrence of signs of brucellosis in the goats in Oju LGA..... 63
4.2.4 Occurrence of symptoms of brucellosis in the goat owners in Oju LGA..... 63
4.2.5 Vaccination of goats with *Brucella* vaccine..... 64
4.2.6 After-birth materials disposal by goat owners in Oju LGA..... 64
4.2.7 Cleaning of goat pens in Oju LGA..... 66

4.2.8 Isolation of newly purchased goats in Oju LGA..... 68
4.2.9 Consumption of goat milk in Oju LGA..... 68
4.2.10 Use of protective clothing by goat owners in Oju LGA..... 68

5.0 DISCUSSION..... 70

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS..... 75
6.1 Summary..... 75
6.2 Conclusion..... 75
6.3 Recommendations..... 76

REFERENCES..... 77

APPENDIX..... 96

LIST OF TABLES

Table	Page
4.1 Age-based seroprevalence of *Brucella* antibodies in goats in Oju LGA by iELISA	57
4.2 Sex-based seroprevalence of *Brucella* antibodies in goats in Oju LGA by iELISA	58
4.3 Breed-based seroprevalence of Brucella antibodies in goats in Oju LGA by iELISA	59
4.4 Biodata of goat owners interviewed in Oju LGA	60
4.5 Source of goats sold at the goat market in Oju LGA	61
4.6 Average number of goats sold per week per goat owner in Oju LGA	62

LIST OF FIGURES

Figure	Page
3.1 Map of Benue State showing Oju LGA in lemon green colour.....	47
4.1 Seroprevalence of brucellosis in goats in Oju LGA using RBPT and iELISA..	56
4.2 Disposal of after-birth materials by goat owners in Oju LGA.....	65
4.3 Cleaning of goat pens in Oju LGA.....	67
4.4 Goat owners' responses with regard to caprine brucellosis in Oju LGA.....	69

LIST OF APPENDICES

Appendix	Page
I Questionnaire to assess the level of awareness of caprine brucellosis among goat owners and handlers in Oju LGA.....	96

LIST OF ABBREVIATIONS

RBPT:	Rose Bengal plate test
iELISA:	Indirect enzyme-linked immunosorbent assay
LGA:	Local Government Area
LGAs:	Local Government Areas
LPS:	Lipopolysaccharide
S-LPS:	Smooth lipopolysaccharide
cELISA:	Competitive enzyme-linked immunosorbent assay
NK:	Natural killer
CFT:	Complement fixation test
PCFIA:	Particle concentration fluorescence immunoassay
MRT:	Milk ring test
GD:	Gel diffusion
RID:	Radial immunodiffusion
NH:	Native hapten
EU:	European Union
SAT:	Serum agglutination test
FPA:	Fluorescence polarisation assay
kDa:	kiloDalton
PCR:	Polymerase chain reaction
AP-PCR:	Arbitrary primed polymerase chain reaction
DNA:	Deoxyribonucleic acid
PFGE:	Pulsed-field gel electrophoresis
RE:	Restriction enzymes
OMP:	Outer membrane protein
IFN:	Interferon
CFU:	Colony forming unit
HRP:	Horseradish peroxidase

CHAPTER ONE

INTRODUCTION

1.1 Definition of the Problem

Brucellosis is a zoonotic and contagious systemic bacterial disease primarily of ruminants but also affecting man and other livestock and is characterized by inflammation of the genital organs and foetal membranes, abortion, sterility and formation of localized lesions in the lymphatic system and joints (WHO, 1971; CDC, 2005).

Brucellosis is caused by Gram negative and facultative non-motile intracellular bacteria of the genus *Brucella* (Corbel, 1997). Six most common species in the genus *Brucella* are *Brucella abortus, B.melitensis, B. suis, B. neotomae, B. ovis*, and *B. canis*. The first three species are called "classic brucella" (Alton, 1990). Other species are *Brucella maris* isolated from marine mammals, *B. cetaceae* (cetaceans) and *B. pinnipediae* (pennipeds) (Foster *et al*, 2002).

Goats and sheep are the natural reservoirs of *B. melitensis* and the sources of infection for goats are foetuses, foetal membranes, vaginal secretions and the environment (Alton *et al.,* 1988). The most common route of transmission is via the gastrointestinal tract following ingestion of contaminated pasture, forage and water (PAHO, 2001). All breeds of goats are susceptible to infection by *B. melitensis*. Infection by *B. suis* (Paolicchi *et al.*, 1993) and *B. abortus* (Luchsinger and Anderson, 1979) has occasionally been found in goats.

The clinical signs in goats are similar to those observed in other species of animals and the main sign is abortion, which occurs most frequently in the third or fourth month of pregnancy (Fensterbank, 1987a). Infection of the mammary gland is common and in

chronically infected herds, the signs of the disease are generally not very apparent (Alton, 1985).

Humans get infected by consuming unpasteurized milk and milk products of infected animals and by direct contact when handling brucella infected products with bruised skin (Grillo *et al*., 1997). Unhygienic attitude of animal handlers also leads to the spread of the disease (Ajogi *et al.*, 2002). Infection by inhalation of airborne agents is also possible (PAHO, 2001). Goats, cattle, pigs, sheep, horses and dogs play an important role in the transmission of this disease to man (EU Commission, 2001). The disease is primarily an occupational risk in exposed professions such as veterinarians, farmers, laboratory technicians, abattoir workers, and others who work with animals and their products and people living near infected premises may also contract infection (PAHO, 2001).

Collard (1962) documented the first serological evidence of human brucellosis in Nigeria where *Brucella* antibodies were demonstrated in the sera of healthy persons in various parts of the country.

1.2 Statement of the Research Problem

In Nigeria, the disease is endemic (Eze, 1985) and recent studies suggest increasing trend in the prevalence of the disease (Ocholi *et al.*, 2004 and Junaidu *et al.,* 2008 and 2010). *Brucella melitensis* had been isolated from sheep and goat milk in Nigeria (Bale, 1980). There were also serological reports of brucellosis in small ruminants in various parts of Nigeria (Cadmus *et al.*, 2006; Junaidu *et al.*, 2008; Bertu *et al.*, 2010 and Junaidu *et al.*, 2010) who reported prevalence rates of 0.86%, 7.96%, 16.1% and 10.9% respectively.

The disease is important as a major cause of economic losses in the livestock industry and Nigeria's food animals' population is estimated at 15.2 million cattle, 23 million sheep and 28 million goats (FAO, 2006). The role of brucellosis in limiting livestock production and its economic impact on the livestock industry in Nigeria is widely recognised (Esuruoso, 1979; Rikin, 1988; Ajogi and Akinwumi 2001). Losses in livestock are in terms of abortion, infertility, low conception and survival rates of neonates (Osori, 1976; Oyedipe *et al*., 1981).

As a result of compulsory pasteurization of milk products and strict control of the disease in livestock, the incidence of brucellosis has steadily declined in most industrialized countries during the last 50 years unlike in developing countries where people still consume unpasteurized milk, milk products and have unprotective contact with animals (PAHO, 2001).

Although brucellosis is a notifiable disease in Nigeria, the incidence, prevalence, and distribution of the disease are difficult to determine as the system of disease surveillance and reporting is fragmentary and inefficient (Ocholi *et al.*, 1993).

Brucellosis in humans is hardly diagnosed in hospitals in Nigeria despite suggestions that the magnitude of infections may be greater than appreciated (Njoku, 1995; Rajis *et al.*, 2003). In the case of Benue State, there is no known report of brucellosis in hospital patients in the entire area (Ofukwu *et al.*, 2004).

An abattoir based study on the serodiagnosis of swine brucellsosis in Makurdi, Benue State (Ngbede *et al.,* 2013) was carried out. However, no record of brucellosis has been reported from Oju Local Government Area of Benue State as no such documented study has been

carried out. This study seeks to determine the prevalence of *Brucella* antibodies in goats in Oju LGA of Benue State.

1.3 Justification of the Study

Recent studies suggest increasing trend in the prevalence of the disease in various parts of the country and epizootiological investigations also revealed that results obtained varied depending on the region, area or animal group sampled (Bertu *et al*., 2010; Junaidu *et al.,* 2010; Talabi *et al.*, 2012; Ngbede *et al.,* 2013 and Kaltungo *et al.,* 2013). This trend makes it necessary to determine the prevalence of the disease in Oju LGA of Benue State where goat is the major livestock kept and which has a well established goat market with an excess of two hundred goats on market days (market holds every five days). The market is being patronized by people from other LGAs within the State and neighbouring States including Nassarawa, Enugu and Cross River. In Oju, chevon is the most consumed meat and it is used in all festivities.

There is no documented data on brucellosis in goats in Oju LGA, Benue State. The dearth of such data makes it difficult to determine the true situation of the disease in the area. Hence, the need to carry out research work on the seroprevalence of *Brucella* antibodies in goats in Oju to fill this information gap and provide baseline data to help veterinarians, animal scientists, goat owners and handlers and to assist regulatory bodies in making policies that will help to prevent and control the disease.

Caprine brucellosis being a zoonosis par excellence, has a direct impact on public health and livestock production and therefore is a major threat to humanity. It also adversely influences international trade of animals and animal products.

1.4 Aim of the Study

To determine the seroprevalence of *Brucella* antibodies in goats in Oju LGA.

1.5 Objectives of the Study

i. To detect the presence of *Brucella* antibodies in goats in Oju LGA using the Rose Bengal Plate Test and Indirect Enzyme-Linked Immunosorbent Assay.

ii. To determine the association between the infection and epidemiological variables such as age, sex and breed.

iii. To determine risk factors associated with *Brucella* infection in goats in Oju LGA using a structured questionnaire.

iv. To assess the level of awareness of caprine brucellosis among goat owners and handlers in Oju LGA using a structured questionnaire.

1.6 Research Questions

i. Are goats in Oju infected with *Brucella species*?

ii. Is there any relationship between the prevalence of *Brucella* antibodies and factors such as age, sex and breed of goats in Oju LGA?

iii. What are the risk factors associated with the Brucella species infection in Oju LGA?

iv. Are goat owners and handlers in Oju LGA aware of caprine brucellosis as a potential zoonosis?

CHAPTER TWO

LITERATURE REVIEW

2.1 History

Caprine brucellosis is a zoonotic infection with important effects on both public health and animal health and production and is widespread in many areas of the world (EU Commission, 2001).

Brucella melitensis, the main aetiologic agent of brucellosis in goats, was the first species described in the genus *Brucella*. It was first isolated by Bruce in 1887 (Alton, 1990) from the spleens of soldiers dying of Mediterranean fever on the island of Malta. Bruce called it *Micrococcus melitensis*. The origin of the disease remained a mystery for nearly 20 years until it was discovered that goats were the source of infection for human populations (Alton and Forsyth, 1996). The popular name "undulant fever" originates from the characteristic undulance (or "wave-like" nature) of the fever which rises and falls over weeks in untreated patients. In the 20th century, this name, along with "brucellosis" (after Brucella, named for Dr. Bruce), gradually replaced the 19th century names of "Mediterranean fever" and "Malta fever" (Alton, 1990).

2.2 Description of the Causative Agent

2.2.1 Aetiology

Brucellosis in goats is primarily caused by *Brucella melitensis*, and rarely by *Brucella abortus* (Luchsinger and Anderson, 1979) or *Brucella suis* (Paolicchi *et al*., 1993).

2.2.2 Morphology

The morphology of *Brucella species* has been described by many authors (Corbel and Brinley-Morgan, 1984; Alton *et al.,* 1988; Macmillan, 1990; Enright, 1992; Marin *et al.,* 1996a, 1996b; and Garin-Bastuji *et al.,* 1997). *Brucellae* are coccobacilli or short rods 0.6 to 1.5 μm long by 0.5 to 0.7 μm in width. They are arranged singly and less frequently in pairs or small groups. The morphology of *Brucella* is fairly constant except in old cultures, where pleomorphic forms may be evident. *Brucellae* are non-motile. They do not form spores, flagella, or pili. True capsules are not produced. *Brucellae* are Gram-negative and usually do not show bipolar staining. They are not truly acid-fast but resist decolouration by weak acids, thus stain red by the Stamp's modification of Ziehl-Neelsen method, which is sometimes used for the microscopic diagnosis of brucellosis from smears of solid or liquid specimens.

2.3 Epidemiology

2.3.1 Geographical distribution

Brucellosis is worldwide in distribution but more common in countries with poorly standardized animal and public health programmes (Capasso, 2002). New *Brucella* strains or species may emerge and existing *Brucella* species adapt to changing social, cultural, travel and agricultural environment (Godfroid *et al.,* 2005).

Brucella melitensis infection appears to occur endemically in the Mediterranean region, especially along its northern and eastern shores, stretching through Central Asia as far south as the Arabian peninsula and as far east as Mongolia (FAO/OIE/WHO, 1997). Parts of Latin America are also seriously affected, especially Mexico, Peru and northern Argentina (EU Commission, 2001). The disease also occurs in Africa and India. However, North

America (except Mexico) is believed to be free, as are Northern Europe (except for sporadic incursions from the south), Southeast Asia, Australia and New Zealand (FAO/OIE/WHO, 1997).

In Nigeria, the disease is endemic (Eze, 1985). *Brucella melitensis* had been isolated from sheep and goat milk in Nigeria (Bale, 1980). There were also serological reports of brucellosis in small ruminants in various parts of Nigeria (Cadmus *et al*., 2006; Bertu *et al*., 2010; Junaidu *et al*., 2010; Talabi *et al.*, 2012 and Kaltungo *et al.,* 2013).

2.3.2 Modes of transmission

Generally, transmission occurs in the same way in sheep and goats as in cattle with materials secreted/discharges from the female genital tract forming the main supply of organisms for transmission to other animals and man (Alton, 1990). Therefore, in most circumstances, the primary route of dissemination of *Brucella* is the placenta, foetal fluids and vaginal discharges by infected nanny goats and ewes after abortion or full-term parturition (Alton, 1990). Very large numbers of organisms are shed at the time of parturition or abortion. In goats, secretion of the organisms from the vagina is prolonged and copious (2 to 3 months generally). Shedding of *Brucella* is also common in the udder secretions and semen. *Brucella* organism may be isolated from various tissues such as lymph nodes from the head and those associated with reproduction, and sometimes from arthritic lesions (Alton *et al.,* 1988). The persistent infection of the mammary glands and supramammary lymph nodes leads to a constant or intermittent shedding of the organisms in the milk in succeeding lactations. It provides an important source of infection for man and young animals (Grillo *et al.,* 1997).

The modes of infection are direct or indirect. Animals become infected directly by infected aerosols or by uptake of infected material and on grazing pastures where infected animals mix with brucellosis-free animals or get in touch with contaminated premises, manure, materials among others (EU Commission, 2001). Dogs have been shown to be mechanical and biological vectors of brucellosis (Joint FAO/WHO Expert Committee on Brucellosis, 1986).

Persistent infection of mammary glands is associated with constant or intermittent shedding of the organisms in the milk in succeeding lactations (Philippon *et al*., 1971).

Similar to *B. abortus* infection in cattle, *B. melitensis* can be transmitted from the dams to lambs or kids. These lambs or kids may have infections in the lymph nodes draining the gastro-intestinal tract and may shed *Brucella* organisms in the faeces. It is also probable that a self-cure mechanism similar to that suggested in cattle takes effect in most of the infected kids and lambs (Grillo *et al.,* 1997).

2.3.3 Survival of *Brucella species* in the environment

The ability of *Brucella* to persist outside mammalian hosts is relatively high compared with most other non-sporing pathogenic bacteria, under suitable conditions (Alton and Forsyth, 1996). Numerous studies have assessed the persistence of *Brucella* under various environmental conditions and it was discovered that when pH, temperature and light conditions are favourable (for example pH>4, high humidity, low temperature and absence of direct sunlight), *Brucella* may retain infectivity for several months in water, aborted foetuses and foetal membranes, faeces and liquid manure, wool, hay, on buildings, equipment and clothes (Alton and Forsyth, 1996). *Brucellae* are able to withstand drying

particularly in the presence of extraneous organic material and will remain viable in dust and soil up to 72 days in damp soil and up to 144 days at 20^{o}C and 40% relative humidity (Alton and Forsyth, 1996). Survival is prolonged at low temperatures, especially below 0°C (Alton, 1985; Joint FAO/WHO Committee, 1986; Nicoletti, 1980).

At low concentration in liquid media, *Brucellae* are fairly heat-sensitive. Thus, dilute suspensions in milk are readily inactivated by pasteurisation or by boiling for upto 10 minutes (Davies and Casey, 1973). *Brucellae* do not persist for a long time in ripened fermented cheese. The optimal fermentation time to ensure safety is not known, but is estimated at 3 months (Nicoletti, 1989)

Brucellae are fairly sensitive to ionising radiation and are readily killed by normal sterilising doses of gamma-rays under conditions which ensure complete exposure, especially in colostrum (Garin-Bastuji *et al.,* 1990a).

In contrast to dairy products, the survival time of *Brucella* in meat seems extremely short, except in frozen carcasses where the organism can survive for years. The number of organisms per gram of muscle is small and rapidly decreases with the pH drop of the meat (Carrere *et al.,* 1960; Davies and Casey, 1973; Plommet *et al.,* 1988 and Nicoletti, 1989).

Most commonly available disinfectants readily kill *Brucella* in aqueous suspensions at normally recommended concentrations (phenol 10g/l, formaldehyde, xylene 1ml/l) except in the presence of organic matter or at low temperature which drastically reduces the efficacy (Joint FAO/WHO Expert Committee on Brucellosis, 1986). Diluted hypochlorite solutions, ethanol, isopropanol or iodophores and better, substituted phenols are effective

for decontamination of the exposed skin but the alkyl quaternary ammonium compounds are not recommended (Joint FAO/WHO Expert Committee on Brucellosis, 1986).

2.3.4 Susceptibility and risk factors

Brucella infection causes disease only in adult (sexually mature) females and males. Young animals may be infected but do not show any clinical sign and generally show only a weak and transient serological response, however, susceptibility increases after sexual maturity and especially with pregnancy (EU Commission, 2001).

The goat was originally considered the principal host of *B. melitensis*, notably in Latin America, where sheep were not significantly infected even when kept in close contact with goats but in many other areas the disease is more important in sheep. Most breeds of goat are fully susceptible to infection while in sheep, milking breeds appear more susceptible than those kept for meat production (Corbel and Brinley-Morgan, 1984).

Any of the wild ruminants that come in contact directly or indirectly with infected sheep or goats seem liable to infection with *B.melitensis* and to maintain the infection in the natural environment. It has been reported in the alpine ibex in Italy (Ferroglio *et al*., 1998) and in chamois in the Southern French Alps (Garin-Bastuji *et al*., 1990b).

While it is obvious that individual factors influence the outcome of *Brucella* infection in small ruminants (self-limited infections, acute infection, latent persistence among others) no studies seem to have demonstrated the exact nature and role of individual factors compared with other favourable or unfavourable factors (Garin-Bastuji and Blasco, 1997).

The system of husbandry and environmental conditions greatly influence the spread of infection (Radostits *et al*., 2006), thus lambing/kidding in dark, crowded enclosures is more favourable to spread than lambing/kidding in the open air in a dry environment. The spread of infection between flocks generally follows the movement or gathering of infected animals and the main risk for introducing the disease into a previously non-infected area is by purchase of infected animals (Radostits *et al*., 2006). In several countries there is a strong correlation between the prevalence of brucellosis in small ruminants and the practice of transhumance. Intermingling of flocks may occur under nomadic or semi-nomadic conditions of husbandry and also in static village flocks where animals are taken daily for grazing on common pastures (Radostits *et al*., 2006).

2.4 The Disease

2.4.1 Pathogenesis

Pathogenically, *B. melitensis* infection in goats and sheep is similar to *B. abortus* infection in cattle (OIE Manual, 1996). *Brucellae* are facultative intracellular parasites of the reticuloendothelial system. The virulence of *Brucella* varies considerably according to species, strain and the size of infecting inoculum. As in other forms of brucellosis, the pathogenesis depends upon localization in lymph nodes, udder, and uterus after an initial bacteraemia. Invading *Brucella* usually localize in the lymph nodes draining the invasion site resulting in the hyperplasia of lymphoid and reticuloendothelial tissues and the infiltration of inflammatory cells. Survival of the first line of defence by the bacteria results in a local infection and escape of *Brucellae* from the lymph nodes into the bloodstream (Enright, 1992).

When the bacteria prevail over the body defences, a bacteraemia is generally established. This bacteraemia is detectable after 10 to 20 days and persists from 30 days to more than 2 months. Localization in the placenta leads to the development of placentitis, with subsequent abortion. After abortion, uterine infection persists for up to 5 months and the mammary gland and associated lymph nodes may remain infected for years (Alton, 1990; Grillo *et al*., 1997).

Although placentitis impairs the normal function of the placenta, *Brucella* endotoxins may also play a role in inducing abortion as the endotoxins may cause production of high concentration of cortisol that decreases progesterone production and increases oestrogen production. Decreased progesterone and increased oestrogen levels induce a premature parturition (Enright *et al*., 1984).

The udder is a very important predilection site for *B. melitensis*. Infection in lactating non-pregnant goats is likely to lead to colonisation of the udder with excretion of *B.melitensis* in the milk (Renoux *et al.,* 1953). In goats, about two thirds of acute infections acquired naturally during pregnancy lead to infection of the udder and excretion of the bacteria in the milk during the current and subsequent lactation (Alton, 1962, 1985). Greatly reduced milk yield follows abortion, and infection of the udder following a normal birth also leads to a considerable reduction in yield. In spite of this, clinical signs of mastitis are seldom detectable in naturally infected goats (Alton, 1990). Orchitis, epididymitis, hygroma and arthritis are seen in males following localization in the reproductive organs, associated lymph nodes and joints (Fensterbank, 1987a).

The tropism of *Brucella* to the male or female reproductive tract is thought to be by erythritol which stimulates the growth of the organism but *Brucella* has also been seen in the reproductive tract of animal with no detectable level of erythritol (Enright, 1992).

2.4.2 Clinical findings

2.4.2.1 Acute brucellosis

Although a variety of clinical signs have been described in artificially infected animals, the main clinical manifestations of brucellosis in goats are, as in all female ruminants, reproductive failure with abortion and birth of weak offspring (Alton and Forsyth, 1996). Abortion generally occurs during the last 2 months of pregnancy and is followed in some cases by retention of foetal membranes. In the male, localisation of the organisms in the testis, epididymis and accessory sex organs is common, and bacteria may be shed in the semen (Alton, 1990). This may result in acute orchitis and epididymitis and later in infertility. Arthritis is also observed occasionally in both sexes. There is no evidence that the clinical features of *B. melitensis* infection in sheep and goats vary according to the biovar involved (Fensterbank, 1987b).

2.4.2.2 Chronic brucellosis

Animals generally abort once, although reinvasion of the uterus occurs in subsequent pregnancies and *Brucella* organisms are shed with the membranes and fluids. Non-pregnant animals exposed to small numbers of the organisms may develop self-limiting, immunising infections or they may become latent carriers. Persistent infection of the mammary glands and supramammary lymph nodes is common in goats with constant or intermittent shedding of the organisms in the milk in succeeding lactations, while the self-limiting nature of the disease in sheep, which is seldom accompanied by prolonged excretion of the bacteria, has

been observed (Alton, 1990; Duran-Ferrer, 1998). The inflammatory changes in the infected mammary gland reduce milk production by an estimated minimum of 10% (EU Commission, 2001).

2.4.3 Pathology

Brucella infected animals generally develop granulomatous inflammatory lesions which frequently are found in lymphoid tissues and organs such as the reproductive organs, udder and supramammary lymph nodes and sometimes joints and synovial membranes. The lesions when present are not pathognomonic. Lesions that could include necrotising placentitis, palpable testicular alterations, necrotising orchitis and epididymitis with subsequent granuloma, necrotising seminal vesiculitis and prostitis could be observed. Acute mastitis with palpable nodules and the production of clotted and watery milk may occur (Fensterbank, 1987a).

Some aborted foetuses may have an excess of blood-stained fluids in their body cavities, enlarged spleen and liver while others may appear normal. Infected foetal membranes show changes affecting part or all of the foetal membrane. The necrotic cotyledons lose their blood-red appearance becoming thickened and dull-grey in colour (Seifert, 1996).

Histopathologic lesions include placentitis, trophoblast necrosis, chorioallantoic ulceration, presence of *Brucella* organisms in the chorionic villi, endometritis, lymphoid hyperplasia in lymph nodes and spleen, and lymphocytic mastitis and interstitial pneumonia (Meador *et al*., 1988).

2.5 Immune Response

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

2.5.1 Humoral immunity

Following infection by natural exposure, a serological response can be expected within 2 to 4 weeks, but the response is variable and may be absent altogether (Elberg, 1959). Invasion of the pregnant uterus can be expected to produce a large and persistent rise of antibodies, but this may be delayed until after abortion or parturition at the normal time. Invasion of the lactating udder causes a lesser serological response, and localisation confined to a small number of lymph nodes may fail to stimulate any response at all, or only a minimal one (Alton and Elberg, 1967).

The pattern of the serological response in terms of immunoglobulin production has not been extensively studied in sheep and goats, but available information suggests close similarity to that in cattle. There is production of IgM followed within a week or two by a predominance of IgG, with both isotypes falling to a low level in the more chronic stage of infection but with IgG predominating (Elberg, 1981, 1996).

The serological response is transient and sometimes missing in young sexually immature animals. The *B. melitensis* Rev.1 vaccine strain when applied under standard conditions with a full dose via the subcutaneous route in young replacement animals may induce a

long lasting serological response to the agglutination test that seriously interferes with serological screening of infected animals (Alton, 1990 and MacMillan, 1990).

2.5.2 Cell mediated immunity

Following phagocytosis and processing of *Brucella* organism by the macrophages, the T-lymphocytes (thymus derived lymphocyte) are responsible for the cell mediated immune response (Holland and Pickett, 1958). After gaining entrance into the body, the organisms encounter the cellular defences of the host and the fate of the invading bacteria is mainly determined by the cellular defences of the host, in particular macrophages and T-lymphocytes. The most widely used correlates of cell mediated immunity are lymphocyte stimulation (Smith, 1990), macrophage inhibition (Jiang *et al.,* 1999), delayed-type hypersensitivity and g-interferon induction (Schurig *et al.,* 2002). Others are neutrophils (Cunning *et al*., 1985) and Natural Killer (NK) cells (Yabu *et al*., 1991

2.6 Diagnosis of Caprine Brucellosis

Disease existence may be suspected from herd history and signs observed including occurrence of one or several abortions in the third trimester of pregnancy or birth of weak kids but differential diagnosis of abortion may be difficult clinically and require laboratory confirmation (Morgan, 1970).

2.6.1 Direct agent detection methods

2.6.1.1 Direct smear

Microscopic examination of smears made from vaginal swab, placenta, aborted foetus, milk and semen stained by modified Kosier staining, modified Ziehl-Neelsen (Stamp *et al*.,

1950) and fluorescent antibody techniques (Janney and Berman, 1962) may be diagnostic for brucellosis.

2.6.1.2 Cultural isolation

The only unequivocal method for the diagnosis of brucellosis in small ruminants is based on the isolation of *Brucella species* (Alton *et al.,* 1988). The isolation of *Brucella species* on appropriate culture media is recommended for an accurate diagnosis. Vaginal swabs and milk samples are the most suitable samples to isolate *Brucella species* from sheep and goats.

The spleen and lymph nodes (iliac, mammary and prefemoral) are the most reliable samples for isolation purposes in necropsied animals (Marin *et al.,* 1996a).

Brucella melitensis does not require serum or CO_2 for growth and can be isolated on ordinary solid media under aerobic conditions at 37°C. However, the use of selective media is needed for isolation purposes because of the overgrowing contaminants usually present in field samples (Corbel, 1985). 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 at the concentration used in this medium may have inhibitory effects on some *B. melitensis* strains (Marin *et al.,* 1996b). Thus, its sensitivity for the isolation of *B. melitensis* from naturally infected goat 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 both the Farrell's and the modified Thayer-Martin's media (Marin *et al.,* 1996b).

2.6.1.3 Animal inoculation

Guinea pigs are more susceptible to *Brucella* than any other laboratory animal (Avong, 2000). However, mice are also susceptible and may be substituted for guinea pigs (Avong, 2000). A suspension of suspected material (foetal, lung, abomasal content, placenta, blood, uterine discharge and milk) is injected intraperitoneally and the animal necropsied three to six weeks later. If positive, *Brucella* is recovered from the spleen by culturing on to appropriate media (Alton *et al.,* 1988).

2.6.2 Serological methods

2.6.2.1 Rose Bengal Plate Test (RBPT)

The RBPT was developed more than 30 years ago for the diagnosis of bovine brucellosis. Despite the scanty and sometimes conflicting information available (Trap and Gaumont, 1975; Fensterbank and Maquere, 1978; Farina, 1985; MacMillan, 1990; Alton, 1990; Blasco *et al.,* 1994a, 1994b), this test is internationally recommended for the screening of brucellosis in small ruminants (Joint FAO/WHO expert committee on Brucellosis, 1986; Garin-Bastuji and Blasco, 1997). One of the conflicting issues is on the sensitivity of the RBPT and it concerns the standardisation of the antigen. The European Union regulations require antigen suspensions in lactate buffer at pH 3.65 ± 0.05 that are able to agglutinate at a dilution of 1:47.5 (21 IU/ml) of the International Standard anti-*B. abortus* serum (ISaBS) but give a negative reaction at a dilution of 1:55 (18.2 IU/ml) of the same serum. These standardisation conditions, which seem to be suitable for the diagnosis of *B. abortus* infection in cattle (MacMillan, 1990), limit the sensitivity of the test resulting in reduced performance for the diagnosis of *B. melitensis* infection in sheep (Blasco *et al.,* 1994a, 1994b). This accounts for the relatively low sensitivity of some commercial RB antigens

when diagnosing brucellosis in sheep and goats (Falade, 1978, 1983; Blasco *et al.,* 1994a) and for the fact that a high proportion of sheep and goats belonging to *B. melitensis*-infected areas give negative results in the RBPT but positive ones in the CF test (Blasco *et al.,* 1994a).These phenomena have raised serious questions over the efficacy of using the RBPT as an individual test in small ruminants. However, if the antigen is standardised differently to give a higher analytical sensitivity, the diagnostic sensitivity is much improved (MacMillan, 1997). Some workers claimed that, at least for sheep, the sensitivity of the RBPT test can be improved significantly when the antigens are standardised against a panel of sera from several *B. melitensis* culture positive and *Brucella*-free sheep, respectively, or when the volume tested is increased from 25µl to 75µl (Blasco *et al.,* 1994a).

2.6.2.2 Serum Agglutination Test (SAT)

The SAT antigen was developed by Welch and Mickle (1933). It is the most widely used serological test for the diagnosis of brucellosis and for conducting eradication programmes. The test was based on the agglutination of killed *Brucella* cells by specific *Brucella* antibodies. The sensitivity of the test is a function of the antigen in the cells. The test identifies the vast majority of infected animals but fails to differentiate antibodies resulting from infection and recent vaccination. The test may be negative in the early stages of infection and in long standing chronic infections (Morgan, 1967). These limitations result from the test not detecting IgG, a relevant antibody isotype, and its reaction is mediated mainly through the IgG2, IgM and IgA immunoglobulin fractions (Nielsen *et al.,* 1988).

2.6.2.3 Complement Fixation Test (CFT)

The CFT is the most widely used test for the serological confirmation of brucellosis in animals (MacMillan, 1990). As in cattle brucellosis, despite its 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). When testing a limited number of sera obtained from *B.melitensis* culture positive and *Brucella* free goats, the CFT provided the same sensitivity as the RBPT and indirect ELISA (iELISA) (Diaz-Aparicio *et al.,* 1994). However, under field conditions, the sensitivity of CFT has been reported to be somewhat lower (88.6%) than those of the RBPT (92.1%) and iELISA (100%) for diagnosing *B. melitensis* infection in sheep (Blasco *et al.,* 1994a, b). On the other hand, the CFT has many drawbacks such as complexity, variability of reagents, prozones, anticomplementary activity of sera, difficulty to perform with haemolysed sera, and subjectivity of the interpretation of low titres. Therefore, while the sensitivity of RBPT is sufficient for the surveillance of free areas at the flock level, RBPT and CFT should be used together in infected flocks to obtain accurate individual sensitivity in test-and-slaughter programmes. Moreover, an important drawback of both RBPT and CFT is their low specificity when testing sera from sheep and goats vaccinated subcutaneously with Rev.1 (Fensterbank *et al.,* 1982; Jimenez de Bagues *et al.,* 1992; Diaz-Aparicio *et al.,* 1994). However, when the Rev.1 vaccine is applied conjunctivally (Fensterbank *et al.,* 1982), the interference problem is significantly reduced in all serological tests (Jimenez de Bagues *et al.,* 1992; Diaz-Aparicio *et al.,* 1994).

2.6.2.4 Enzyme immunoassay

The enzyme immunoassays in use in brucellosis diagnosis are the indirect enzyme-linked immunosorbent assay (iELISA), competitive enzyme-linked immunosorbent assay (cELISA) and particle concentration fluorescence immunoassay (PCFIA) (EU Commission, 2001).

Engvall and Perlman (1971, 1972) and Vanweemen and Schurs (1971, 1972) were the pioneers of Enzyme-Linked Immunosorbent Assay (ELISA) which utilizes enzymes as signal modifier in the primary binding site.

The indirect ELISA (iELISA) is the major enzyme immunoassay in use in brucellosis diagnosis (Nicoletti and Tanya, 1993). ELISAs are methods that involve the immobilisation of one of the active components of the reaction on a solid phase, and iELISAs are those in which the antigen is bound to a solid phase, usually a polystyrene microtitre plate so that antibody, if present in a sample, binds to the immobilized antigen and may be detected by an appropriate anti-globulin-enzyme conjugate which in combination with a chromogenic substrate gives a coloured reaction indicative of the presence of antibody in the sample (Marin *et al.,* 1999).

The competitive ELISA (cELISA) is gaining prominence in publications on brucellosis diagnosis (Gorrell *et al.,* 1984; Rylatt *et al.,* 1985; Sutherland *et al.,* 1986; Macmillan *et al.,* 1990; Greiser-Wilke *et al.,* 1991; Nielsen *et al.,* 1991; Marín *et al.,* 1999). In this test, *Brucella* antigen is immobilised on the plate as in 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.

The Particle Concentration Fluorescence Immunoassay (PCFIA) is a commercially produced immunofluorescence assay which has been used in the USA and elsewhere (Nicoletti and Tanya, 1993). The technique has much in common with the competitive ELISA in that antigen is immobilised onto polystyrene, except that in this case it is initially in the form of sub-micron particles held in suspension. Serum is co-incubated with an anti-*Brucella* conjugate in the presence of the antigen coated particles, and they compete for binding. In this case, the antibody is labelled with a fluorescent probe. The unreacted reagents are separated from the polystyrene particles which are themselves concentrated by filtration through a membrane at the bottom of each well (Greenlee *et al.,* 1994; Reynolds 1987).

2.6.2.5 Milk Ring Test (MRT)

Fleischaner developed the *Brucella* Milk Ring Test in 1937 for detecting the presence of *Brucella* antibodies in milk using stained *Brucella* antigens. However, this test has limitations which include false positive reactions which can be produced by vaccination of adult animals in the absence of infection. Reactions to the test reappear during the drying-off period in pregnant animals and the presence of mastitis also causes reaction to be produced to the MRT because of the passage of antibodies from the blood stream. This test is also affected by fat globules and time of sampling (Morgan, 1967).

2.6.2.6 Gel Precipitation Test

As with the RBPT and CFT, the specificity of the iELISA is quite low when testing sera from goats and sheep subcutaneously vaccinated with the live *B. melitensis* Rev.1 vaccine (Jimenez de Bagues *et al*., 1992, Diaz- Aparicio *et al*., 1994, Marin *et al*., 1999). Under these conditions, only the Gel Diffusion (GD) or the Radial immunodiffussion (RID) tests with Native Hapten (NH) as antigen (Diaz and Moriyon, 1989) are specific enough to discriminate the immune responses of sheep (Marin *et al*., 1999) and goats (Diaz-Aparicio *et al*., 1994) infected with *B. melitensis* from those due to Rev.1 vaccination. As demonstrated by the contrasting results of the iELISA and the cELISA, the superior diagnostic specificity of the latter in vaccinated sheep (Marin *et al*., 1999) can be due to the elimination by the competing anti-C monoclonal antibody of the low avidity antibodies, supposed to be dominant in the sera from vaccinated animals. This antibody avidity, rather than epitopic differences in the antigens used, is also likely to account for the high specificity of the NH precipitation tests. The higher specificity of the precipitation tests with NH may result from the higher threshold avidity required in precipitation tests as compared to that of iELISA. This could explain why NH fails to react with sera from vaccinated animals in the former but not in the latter assay, if low avidity antibodies are predominant at a given time after vaccination (Marin *et al.,* 1999).

Many serological responses occurring in healthy animals (particularly in the Rev.1 immunised ones) in endemic areas may be the consequence of mere secondary antigenic contacts and do not correspond always to established infections in the host. In fact, under these conditions, the RBPT, CFT and iELISA tests have been reported to be highly unspecific to detect exclusively the bacteriologically positive animals (Blasco *et al*.,

1994b). Accordingly, an overkilling of healthy animals took place in the eradication campaigns in EU countries fifteen years ago, with the ensuing disagreement among veterinarians and owners (EU Commission, 2001). Having in mind that more additional research and field studies are needed for a proper confirmation, the NH precipitation test (Diaz and Moriyon, 1989; Diaz-Aparicio *et al*., 1994; Marin *et al*., 1999) again appears to be the most specific serological test to detect brucellosis in animals, particularly after Rev.1 mass vaccination programmes in endemic situations. The avidity hypothesis commented above could account for the high specificity of the NH tests to detect exclusively the truely infected animals (Marin *et al.,* 1999).

2.6.2.7 Antiglobulin (Coombs') Test

The antiglobulin test or Coombs' test (Coombs *et al.,* 1945) was developed to detect antibodies which, although they combine with cellular antigens of *Brucella*, do not give rise to agglutination. The presence of these so-called "incomplete agglutinins" can be detected by using an antibody directed against the IgG fraction of the animal species being tested (MacMillan, 1990). Early in 1955, the test was described as the most effective immunological method for the detection of brucellosis in goats (Esteban, 1959).The classical time-consuming methodology of the test in man has been considerably improved by its adaptation to a microtitre plate format (Otero *et al.,* 1982).

Farina (1985) reported that the Coombs' test could be useful to check sera from animals that give negative, suspicious or non-conclusive responses to SAT, due to the presence of incomplete antibodies in these sera. There is evidence (Alton, 1990; Unel *et al.,* 1969) suggesting that the antiglobulin test is effective in diagnosing brucellosis in sheep and also in goats, but because of the complexity of the technique its use may be restricted to special

situations like the detection of antibodies in anticomplementary sera. Its use is not recommended in bovines vaccinated with strain S19 (MacMillan, 1990) or in small ruminants immunised with Rev.1 vaccine (Farina, 1985), because of its low specificity as compared to the CFT.

2.6.2.8 Immunocapture Test

The immunocapture test is a one step technique, very easy to perform, able to detect antibodies of medium to high affinity against *Brucella* and suitable for simple standardisation and automation (Orduna *et al*., 2000). The test is based on a blue coloured cellular antigen of *Brucella melitensis* (strain 16M) and on anti-total species immunoglulin coated polystyrene microtitre plates of 96 U-wells. The test was initially evaluated for the serodiagnosis of human brucellosis and has shown high sensitivity and specificity both in the first stages of the disease and, in particular, in chronic cases as well as in relapses and re-infections (Gomez *et al*., 1999; Orduna *et al*., 2000). Moreover, the immunocapture test and Coombs' test have a similar performance in the diagnosis of human brucellosis but the immunocapture test is more sensitive than the Coombs' test and usually shows higher titres (Orduna *et al*., 2000).

2.6.2.9 Fluorescence Polarisation Assay (FPA)

The principle of fluorescence polarisation was described by Perrin in 1926. It is a simple technique for measuring antigen/antibody interaction and may be performed in a laboratory setting or in the field. It is a homogeneous assay in which analytes are not separated and it is therefore very rapid (Corbel and MacMillan, 2000).

The premise for the Fluorescence Polarization Assay (FPA) is that a small molecule in solution randomly rotates at a rate inversely proportional to its size. Plane polarized light allows measurement, using an attached fluorochrome of the rate of rotation through a given angle. Thus a small molecule rotating at a high rate will revolve through the angle rapidly resulting in a low polarization value. If an antibody is attached to the small molecule, the increased size will cause a decrease in the rate of rotation resulting in a higher polarization value. The FPA is a homogeneous assay, requiring no steps to remove unreacted reagents, and can therefore be performed in minutes, even outside the laboratory, and is very cost effective (Nielsen *et al.,* 1996).

Based on this, Nielsen *et al.,* (1996) developed the FPA for the detection of antibodies to *B. abortus* in cattle, using as antigen a small molecular weight fragment of O-polysaccharide prepared from *B. abortus* lipopolysaccharide (average 22 kDa) conjugated with fluorescein isothiocyanate. This antigen is added to diluted serum or whole blood and a measure of the antibody content is obtained in about 2 minutes using a fluorescence polarisation analyser.

Little information is available on the usefulness of the FPA for the serodiagnosis of ovine and caprine brucellosis. Residual vaccinal antibodies would not cause reactivity in most cases in the cELISA and in the FPA. Based on these data, the FPA appears to be as efficient as other commonly used serological tests and may prove to be a useful tool for the presumptive diagnosis of *B. melitensis* infection in small ruminants (Nielsen *et al.,* 1996).

2.6.3 Molecular detection and typing methods

In the case of tissues or fluids 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 on pure cultures (Fekete *et al.,* 1990a, 1990b; Baily *et al.,* 1992; Herman and De Ridder, 1992; Romero *et al.,* 1995a; Da Costa *et al.,* 1996).

Others showed that PCR could be a potentially useful tool when used alone (PCR, AP-PCR, rep-PCR, ERIC-PCR) or in combination with labelled probes to differentiate some *Brucella* species and biovars (Fekete *et al.,* 1992b, Bricker and Halling, 1994, 1995; Cloeckaert *et al.,* 1995; Mercier *et al.,* 1996; Ouahrani-Bettache *et al.,* 1996; Tcherneva *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 micro-organisms, could potentially increase the rate of detecting animals infected with *Brucella.* However few studies have been performed with clinical or field samples (Fekete *et al.,* 1992a; Leal-Klevezas *et al.,* 1995; Romero *et al.,* 1995b; Matar *et al.,* 1996; Rijpens *et al.,* 1996) and till now, no technique has been demonstrated to be sensitive enough to replace bacteriology on all kinds of biological samples.

Detection of restriction fragment length polymorphism is considered a reproducible and easily performed method within a laboratory. However, laboratory reproducibility has been problematic and this makes the universal application of the method unlikely (Bricker *et al.,* 2003). Alternatively, pulsed-field gel electrophoresis (PFGE) of total genomic DNA digested with restriction enzymes (RE) that cut infrequently has been a useful technique for discriminating among the species and among several isolates in the same species of *Brucella* (Jensen *et al.,* 1995, 1999) but a common problem is incomplete cell lysis or incomplete protease digestion (Faber, 1996).

Major Outer Membrane Protein (OMP) genes of *Brucella* have attracted attention as they exhibit sufficient polymorphism to allow differentiation between *Brucella* species and some of their biovar (Cloeckaert *et al.,* 1995). Studies of the RFLP profiles of two closely related genes OMP2a and OMP2b, encoding and potentially expressing the *Brucella* species major OMP of 36 kDa (Fitch *et al.,* 1990) and 25 kDa (Wergifosse *et al.,* 1995) have been useful for the differentiation of *Brucella* species, biovars and strains and for the differentiation among isolates from marine mammal host (Cloeckaert *et al*., 1996).

2.6.4 Tests based on Cell Mediated Immunity

2.6.4.1 Brucellin Test

Brucella cytoplasmic antigens, known also as brucellin (Jones *et al.,* 1973) have been used with variable success for the allergic diagnosis of brucellosis in sheep and goats (Fensterbank, 1982, 1985; Ebadi and Zowghi, 1983; Loquerie and Durand, 1984; Blasco *et al.,* 1994b). Cold saline protein extracts of the rough *B. melitensis* 115, a strain devoid of the O-chain polysaccharide were used as allergen (Jones *et al.*, 1973).

2.6.4.2 Interferon-gamma test

Tests for the in-vitro detection of Cell Mediated Immunity (lymphocyte transformation and proliferation assays) showed a lack of acceptable efficacy in order to be applied for the large scale routine diagnosis of *Brucella* infection (Nicoletti and Winter, 1990).

However, in the last two decades, the recognition of the role of some cytokines, such as interferon (IFN) gamma, in immunity against intra-cellular agents has enabled the development of an in-vitro test with useful diagnostic applications. The test was first

designed for the diagnosis of bovine tuberculosis (Rothel *et al.,* 1990, Wood *et al.,* 1990, 1991).

Brucellae are facultative intracellular bacteria that survive and replicate in both phagocytic and non-phagocytic cells (Dubray, 1985). Phagocytes play a key role in initiating T-cell responses by processing and presenting antigens. In this path, IFN-gamma is one of the most important T-cell stimulated cytokines in the course of an infection. It is a potent activator of macrophages and monocytes and up-regulates their metabolic activities to produce oxidative metabolites and other microbicidal molecules. Based on these data, the usefulness of IFN-gamma assay in the diagnosis of small ruminant brucellosis (Garrido, 1992; Duran-Ferrer, 1998) and bovine brucellosis (Weynants *et al.,* 1995) has been explored.

2.6.5 Cross-reactions in serological tests

All smooth *Brucella* strains show complete cross-reaction with each other in agglutination tests with unabsorbed polyclonal antisera, a cross-reaction which does not extend to non-smooth variants. Cross-reactions between non-smooth strains can be demonstrated by agglutination tests with unabsorbed anti-R sera. The smooth *Brucella* antigens used in classical tests depend on S-LPS, and therefore, they should not react with antibody against the rough strain. Serological cross-reactions have been reported between smooth *Brucella* and various other Gram negative bacteria *Escherichia coli* O:116 and O:157, *Salmonella* group N(O:30) of Kaufmann-White, *Pseudomonas multophilia*, *Vibrio cholerae* and especially *Yersinia enterocolitica* O:9. These organisms can induce significant levels of antibodies which cross-react with S-LPS *Brucella* antigens in diagnostic tests (EU Commission, 2001).

2.7 Vaccination

2.7.1 *Brucella melitensis* Rev.1 vaccine

The live attenuated *Brucella melitensis* Rev.1 strain is presently recognised as the best available vaccine for the prophylaxis of brucellosis in sheep and goats (Blasco, 1997b). Numerous independent field and controlled experiments confirm its value for this purpose (Alton and Elberg, 1967; Alton, 1990; Elberg, 1981 and 1996; Blasco, 1997a). Moreover, "correctly standardised Elberg 101 strain Rev.1 vaccine should continue to be considered as the basis of brucellosis control in small ruminants where vaccination is applied, until new safer and effective versions of *B. abortus* and *B. melitensis* vaccines, based on rough strains, are tested under controlled experimental and field conditions and shown to be at least equivalent to the Rev.1 vaccine" (WHO, 1997). The ability of the vaccine (Rev.1 strain) to produce a high level of immunity against both artificial and natural challenge has been convincingly demonstrated both for sheep and goats (Alton, 1990).

It has been well established that a large proportion of vaccinated animals is protected against infection (Elberg, 1959, quoted by Garrido, 1992), and in those vaccinated animals where infection occurred, it is often transitory. Hence, the period of *Brucella* excretion from the udder or vagina is shorter, the degree of microbial contamination of the surroundings is reduced and, consequently, disease transmission within and between herds is significantly reduced (Garrido, 1992).

Erratic administration of vaccines or their use without adequate quality control is not effective. Adequate protection is only possible if the vaccine quality is good and if the vaccines are administered to at least 80 % of the animals at risk (Garrido, 1992).

The duration of immunity conferred by vaccination with Rev.1 was investigated by vaccinating Maltese goats when they were 4 to 12 months of age and challenging some at 2 ½ years old (Alton, 1966) and others at 4 ½ years old (Alton, 1968) after vaccination. Those challenged at 4 ½ years old were as resistant as those challenged at shorter intervals after vaccination, and it was concluded that immunity could be considered lifelong. Good results of protection were obtained when young goats were vaccinated at 4 months of age (conjunctival or subcutaneous route) and challenged 8.5-12.5 months after (Fensterbank *et al.,* 1987a).

The Rev.1 vaccine is a useful tool for the control of brucellosis in sheep and goats and to stop the infection of human beings. Its administration should be related to the epidemiological situation in order to be compatible with an eradication policy based on test-and-slaughter. The degree of attenuation of Rev.1 strain is not enough to allow its use without any restriction (Fensterbank *et al.,* 1987a). Due to residual virulence it may induce abortions and also lead to persistent immune responses, which could interfere with classical methods of serological diagnostic tests. With the general aim to minimise these adverse effects, different routes like the conjunctival route instead of subcutaneous route and/or reduction of the dose of vaccine have been studied in the recent decades (EU Commission, 2001).

2.7.2 Vaccination with reduced doses of Rev.1

The vaccination of pregnant animals with a full Rev.1 dose administered subcutaneously is followed by abortion in many animals and by a long-lasting immune response (Fensterbank *et al*., 1982). A reduction of the vaccine dose induces a shorter and less intense antibody response following vaccination in the animals (Gasca *et al*., 1985; Sales-Henriques *et al*.,

1992 and Delgado *et al*., 1995). Accordingly, 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; Kolar, 1995; Al-Shamakh, 1995; Uysal, 1995 and Delgado *et al*., 1995). This method of immunisation has demonstrated its efficacy in well-controlled experiments (Gasca *et al.,* 1985). However, field trials and controlled experiments (Blasco, 1997a) have demonstrated that reduced doses of Rev.1 vaccine may induce vaginal excretion and abortion in pregnant sheep and goats after field infections (Alton, 1970; Crowther *et al.,* 1977; Fensterbank *et al*., 1982, Jimenez de Bagues *et al.,* 1989). It was noted that the level of protection was poor in goats which received a dose of 10^4 CFU (Alton, 1970).

2.7.3 Conjunctival vaccination with Rev.1

2.7.3.1 Young animals

When Rev.1 vaccine is administered in young replacement animals by the conjunctival route (0.5-2 x 10^9 CFU contained in a small volume of 30-50µl inoculated in the conjunctival sac), the protection conferred is similar to that induced by the classical subcutaneous method but the serological response evoked is significantly reduced. Thus, this route of vaccine administration is compatible with eradication programmes based on test and slaughter principles (Fensterbank *et al.,* 1985; Jimenez de Bagues *et al.,* 1992; Diaz-Aparicio *et al.,* 1994; Marin *et al.,* 1999).

2.7.3.2 Adult animals

The induction of abortion when vaccinating pregnant sheep and goats means that there is no entirely safe strategy for mass vaccination with Rev.1 vaccine. When vaccinating pregnant sheep and goats, the conjunctival method results in fewer abortions compared to the subcutaneous method (Zundel *et al.,* 1992). However, a significant proportion of sheep and goats vaccinated conjunctivally with standard doses of Rev.1 vaccine aborted (Jimenez de Bagues *et al*., 1989 and Zundel *et al.,* 1992). Most of these vaccine-induced abortions take place between 40 and 60 days after vaccination, depending mainly on the stage of pregnancy at the time of vaccination. The percentage of abortions obtained when sheep are vaccinated during the last month of pregnancy is significantly lower than when vaccinated at two months of pregnancy (Jimenez de Bagues *et al*., 1989). When administered conjunctivally, a reduced Rev.1 dose induces fewer abortions than the standard dose (Jimenez de Bagues *et al*., 1989 and Zundel *et al*., 1992).

However, the experimental studies that are available are inconclusive at determining the maximum dose which is compatible with an acceptable degree of safety. The minimum dose explored under controlled studies is 1 x10^8 CFU (Zundel *et al*., 1992) and the authors concluded that its innocuousness was not yet sufficient to propose the indiscriminate vaccination of sheep and goats with this dose by the conjunctival route whatever the age or physiological status of the animal. Under experimental conditions, the minimum dose applied by the conjunctival route that has been demonstrated as effective against brucellosis and *Brucella* infection are 4 x 10^8 CFU in lambs (Fensterbank *et al.,* 1985), 1.1 x 10^7 CFU in kids (Fensterbank *et al.,* 1985) and 4.5 x 10^8 CFU in ewes (Duran-Ferrer, 1998). The

minimum dose administered by the subcutaneous route that demonstrated its efficacy was 1-2 x 10^5 CFU (Gasca *et al.,* 1985).

2.7.4 Adverse effects of vaccination with Rev.1 vaccine

2.7.4.1 Young animals

When Rev.1 is administered by the standard method (1-2 x 10^9 CFU subcutaneously) in young (3-6 months old) replacement animals, it induces a solid and durable immunity but also a long-lasting serological response, making the interpretation of serological tests, conducted after vaccination, difficult (Fensterbank *et al.,* 1982; Jimenez de Bagues *et al.,* 1992; Diaz-Aparicio *et al.,* 1994). This causes obvious problems for the application of combined vaccination and test and slaughter eradication programmes.

2.7.4.2 Adult animals

The vaccination of pregnant animals with standard Rev.1 doses administered subcutaneously is followed by vaccine induced abortion in many animals (Alton and Elberg 1967; Elberg, 1981; Jimenez de Bagues *et al.,* 1989; Zundel *et al.,* 1992 and Blasco, 1997).It has been stated that the capability of the Rev.1 strain to induce abortion is a phenomenon that depends on dose and on time of pregnancy when the females are vaccinated. Reduction of the dose increases the degree of safety, and the risk of abortion is minimized when animals are vaccinated at the end of pregnancy or during lactation (Jimenez de Bagues *et al*., 1989 and Elberg, 1996).

The vaccination of adult animals with standard Rev.1 doses administered subcutaneously induces a long-lasting serological response, making it difficult to discriminate the serological response evoked, when test-and slaughter eradication programmes are

simultaneously operated (Jimenez de Bagues *et al.,* 1992; Marin *et al.,* 1999). In general, the residual immune response induced by the vaccination is of less intensity and duration when the dose is reduced and/or the conjunctival route is used.

2.7.5 Handling Rev.1 vaccine

The Rev.1 vaccine strain and other *Brucella* vaccines can cause infection in humans and should therefore be handled and used with care (Blasco and Diaz 1993).

2.7.6 Other vaccines

2.7.6.1 B. suis S2

The *B. suis* S2, a classically obtained Brucella attenuated strain with smooth LPS, was apparently successfully used in controlling brucellosis following oral administration to small ruminants in field conditions in China (Xin, 1986) and Libya (Mustafa and Abusowa, 1993). However, this vaccine showed no protective effect against *B. melitensis* in sheep in fully controlled experimental conditions (Verger *et al.,* 1995).

2.7.6.2 B. abortus RB51

Another classically obtained, live attenuated but rough (S-LPS lacking) Brucella strain is the *B. abortus* RB51 strain vaccine (Schurig *et al.,* 1991). The strain RB51 has been reported to be equally effective as S19 vaccine in protecting against *B. abortus* in cattle, without inducing anti-O chain antibodies as detected by serological tests (Palmer *et al*., 1997). In contrast, it has been demonstrated that this vaccine does not confer protection against *B. ovis* in controlled experiments in rams (Jimenez de Bagues *et al.,* 1995). RB51 efficiency (compared with S19) and its innocuousness remain controversial and it could

infect humans and it is highly resistant to rifampicin, one of the antibiotics of choice for treating human brucellosis (Tittarelli *et al.,* 2008).

2.7.6.3 VTRMI

VTRM1 is a live rough strain obtained by transposon mutagenesis from smooth *B. melitensis* 16M. Mutagenesis resulted in a truncated gene for mannosyltransferase, an enzyme necessary for the synthesis of the S-LPS and accordingly, VTRM1 does not induce antibodies against the S-LPS and does not interfere with classical serological tests. A study done (Adone *et al.,* 2005) indicated that these rough mutants were able to induce a very good level of protection against *B. melitensis* infection, similar to that provided by Rev.1 and superior to that of RB51, without inducing antibodies to O-antigen. In addition, all *B. melitensis* mutants were able to stimulate good production of gamma interferon. The characteristics of these strains encourage further evaluation of them as alternative vaccines to Rev.1 in primary host species (Adone *et al.,* 2005).

2.7.6.4 RfbK strain

The mutant rfbK is a live rough strain obtained by transposon mutagenesis from smooth *B. abortus* 2308 (Adams *et al.,* 1998). Mutagenesis resulted also in a truncated gene for mannosyltransferase. The rfbK strain vaccine gives protection against brucellosis in goats with a relative risk of abortion of 2.13 as against 4.45 for RB51 and 1.33 for Rev.1 (Villa *et al*., 2008).

2.7.6.5 Novel vaccines

Other interesting approaches to develop new generation vaccines, such as the construction of recombinant strains deleted in relevant diagnostic proteins, or DNA based vaccines, are

being currently investigated. It is probable that some of these vaccines will become candidates to replace the available classical vaccines in the near future. However, to date, none of these vaccines has been found to surpass the immunity conferred by the classical Rev.1 vaccine and accordingly, until new and more effective vaccines have been developed and tested properly under controlled conditions, the Rev.1 strain should continue to be the reference vaccine for the prophylaxis of brucellosis in small ruminants (EU Commission, 2001).

2.8 Control and Eradication

Because brucellosis is a disease of major economic and zoonotic importance, a strategy for its control in small ruminants is essential in endemic areas. The initial aim of the strategy selected will be the reduction of infection in the animal population to such a level that the impact of the disease on human health as well as on animal health and production will be minimized (Kaplan, 1966). Subsequent steps can include eradication from a region by test and slaughter and, following successful eradication, measures to prevent reintroduction of the disease.

2.8.1 Immunisation to reduce rate of infection

Control of brucellosis can be achieved by using vaccination to increase the population's resistance to the disease. Vaccination practically eliminates the clinical signs of brucellosis and is accompanied by a reduced contamination of the environment as well as exposure of the population at risk to the infectious agent (Nicoletti, 1993).

However, in many countries, where the animals were kept under extensive conditions with nomadic or semi-nomadic husbandry, this approach was impractical and failed to reduce

the incidence and prevalence of the disease, because the development of herd immunity was very slow (Kolar, 1995; Blasco, 1997b). In addition, the unvaccinated adult animals remain unprotected and the infection can spread (Kolar, 1995).

Mass vaccination of flocks helps to rapidly establish a relatively immune population, and reduces the level of abortions and excretors of *Brucella*, thus reducing contamination of the environment and disease transmission (Kolar, 1995).

Provided that the prevalence of the disease is moderate, financial resources are available, and a well functioning surveillance by the veterinary service is in place, vaccination of young animals can be combined with a test and slaughter policy in a long term action to control brucellosis in small ruminants (WHO, 1998).

2.8.2 Test and slaughter

It is usually accepted that a programme of eliminating brucellosis by test and slaughter policy is justified on economic grounds only when the prevalence of infected animals in an area is about 2% or less (Nicoletti, 1993).

For the implementation of such a programme, it is essential that the flocks are under strict surveillance and movement control. Animals must be individually identified and an efficient and well organised veterinary service for surveillance and laboratory testing must be in place (Alton, 1988; Nicoletti, 1993).

The flock size and the prevalence of brucellosis are the most important factors of this strategy which has been shown to be ineffective and unreliable when attempted in large flocks with a high prevalence of brucellosis (Kolar, 1995). The limited reliability of the

diagnostic tests used which are unable to reveal all infected animals and which may give false negative results due to incubation period, latency or due to criteria used to interpret the results must also be considered. These drawbacks apply more to sheep and goat flocks than to cattle herds where a test and slaughter strategy has been more effective (Kolar, 1984; Nicoletti, 1993).

Before embarking on the implementation of such a strategy it is necessary to ensure that the epidemiological situation is favourable, the necessary facilities and financial resources are available, a pool of healthy replacement animals is available and that the resources exist for continuing surveillance for a considerable period (Nicoletti, 1993). It is also essential that full co-operation of farmers is available as slaughter of seropositive animals can be resisted by owners because of lack of clinical signs, inadequate compensation or lack of replacement animals (Nicoletti, 1993).

2.8.3 Prevention of spread among animals

In areas where the brucellosis-free status has been established or where such a status is suspected on epidemiological grounds, the risk of importing the disease by means of animal movement must be curtailed (EU Commission, 2001). Movement of potentially infected animals into such areas must be prohibited or importation permitted only from certified brucellosis-free farms or areas. This applies to the transport of animals and of certain animal products within countries as well as between countries, following the general principles and procedures as specified in the International Zoo-sanitary Code of the OIE which also describes the essential testing of animals and quarantine measures (WHO/MZCP, 1998).

It should be noted that there have been incidents of spread of brucellosis by serologically negative animals originating from inadequately certified and supervised sources (WHO, 1986 and Schnurrenberger *et al.,* 1987).

2.9 *Brucella* Infection in Humans

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, Lopez-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 (Lopez-Merino, 1989).

Humans are accidentally infected and almost always dead-end hosts of *Brucella* infections. The disease is primarily an occupational risk in exposed professions. Thus, veterinarians, farmers, laboratory technicians, abattoir workers, and others who work with animals and their products are mostly at risk. The primary source is the animal and infection is contracted either by direct or indirect contact through the skin or mucous membranes or ingestion of contaminated products, especially fresh dairy products (Alton, 1990). The maximum danger is during the lambing or kidding period. Dairy products are the main sources of infection for people who do not have direct contact with animals (EU Commission, 2001).

Much of the milk which is consumed is now rendered safe by pasteurisation or boiling. Cream and ice cream prepared from goat milk has been incriminated in the transmission of *Brucella* infection to humans (Flores-Castro and Baer, 1980). The prevalence of human brucellosis acquired from dairy products is seasonal, reaching a peak soon after kidding and

lambing (PAHO, 2001). Abattoir workers handling infected animals are also at risk, especially from the contents of uteri and udders (Alton, 1990). The handling of raw wool has been identified as a potential source of infection of the workers involved (Alton, 1990). Furthermore, *Brucella* infection is easily acquired by laboratory workers (PAHO, 2001).

Humans are susceptible to *Brucella melitensis* and it often gives rise to a severe and long lasting form of the disease. After an incubation period of 8 to 20 days, illness occurs in different forms (Alton and Forsyth, 1996). The acute form of the disease gives symptoms such as lassitude, headache and muscular or joint pain, and drenching sweats, especially at night. The manifestations of brucellosis are sometimes more pronounced or limited to a specific system or organ. This is then termed a complication when it occurs in the course of acute infection, or localized brucellosis when occurring in the absence of other signs of systemic illness. The most common localisations are spondylitis, peripheral arthritis, especially of the hip, knee and shoulder, or epididymo-orchitis (EU Commission, 2001).

The disease presents a great variety of clinical manifestations, making it difficult to diagnose clinically. Therefore, the diagnosis must be confirmed directly by isolation of *Brucella* mostly from blood culture, or indirectly by the detection of immune response against its antigens. The diagnosis of brucellosis based exclusively upon *Brucella* isolation presents several drawbacks (Orduna *et al*., 2000). The slow growth of *Brucella* in primary cultures means that diagnosis may take more than 7 days (Ariza, 1996; Rodriguez-Torres and Fermoso, 1987 and Yagupsky, 1999). Besides, blood culture sensitivity is often low, ranging from 50-90 % depending on disease stage, *Brucella* species, culture medium, number of circulating bacteria and the culture technique employed (Gotuzzo *et al.*, 1986 and Yagupsky, 1999). Hence, serological tests play a major role in the diagnosis when the

agent cannot be detected by blood culture. Yet, the interpretation of these tests is often difficult, particularly in patients with chronic brucellosis, in re-infections and relapses, and in endemic areas where a high portion of the population carries antibodies against brucellosis (Orduna *et al*., 2000).

Various serological tests have been used for the diagnosis of human brucellosis. The most common tests used are serum agglutination test (SAT), Coombs anti-*Brucella* test, Rose Bengal test and complement fixation test (Orduna *et al*., 2000). During the last two decades, radioimmunoassays (Hewitt and Payne, 1984, Parrat *et al*., 1977) and in particular enzyme immunoassays (Ariza *et al*., 1992, Gazapo *et al*., 1989, Saz *et al*., 1987) have also been used.

The treatment of choice in acute brucellosis consists of antibiotic therapy (WHO, 1986). The best results are achieved with rifampicine combined with doxycycline for at least 6 weeks. Treatment generally needs to be prolonged or repeated in persistent forms before a cure is achieved (WHO, 1986). Infections with complications such as meningoencephalitis and treatment in children may require therapy of rifampicine and trimethoprim/sulphomethazolin (Lubani *et al*., 1989) while endocarditis may be treated with rifampicine, streptomycin and doxycycline for six weeks (Chan and Hardiman, 1993). A triple therapy of doxycycline together with rifampicine and cotrimoxazole has been used successfully to treat neurobrucellosis (McLean *et al*., 1992).

2.10 Socio-economic Importance of Caprine Brucellosis

The significance of brucellosis in man and livestock has been extensively investigated (Boycott, 1964; Ajogi, 1997) and it has major veterinary and human importance in affected

countries. The role of brucellosis in limiting livestock production and its economic impact on the livestock industry in Nigeria is widely recognised (Esuruoso, 1979; Rikin, 1988; Ajogi and Akinwumi 2001). Losses in livestock are in terms of abortion, infertility, low conception and survival rates of neonates (Osori, 1976; Oyedipe et al., 1981). Ajogi *et al*., (1998) estimated brucellosis losses of 12.6 million naira in the grazing reserves of Wase and Wawa-Zange of Nigeria.

As a zoonosis of great importance, there is also a significant loss in human productivity due to brucellosis (Madkour, 1989; WHO, 1986). It is of particular importance among the zoonoses not only because of the heavy economic losses and reduction in animal protein supplies but of its direct impact on human health in terms of human illness, physical incapacity and loss of manpower in addition to economic losses in medical care and reduction in productivity which remain a matter of concern in many parts of the world (Ajogi *et al*., 1998).

Costs due to brucellosis include production losses associated with infection in animals, the considerable cost of preventive programmes, and human disease (Elberg, 1996).There is further loss from restriction in international trade in animals and their products.

The occurrence of *B. melitensis* in goat and sheep population of countries that have eradicated *B. abortus* poses a threat for the continuing problem of brucellosis in cattle herds. Brucellosis contributes substantially to the low standard of living in many countries which led to the formation of the Joint FAO/WHO Expert Committee on Brucellosis which meets periodically to discuss and review current problems in human and animal population (Anon, 1977).

2.11 The Situation of Caprine Brucellosis in Nigeria

Detailed studies confirming the problem of caprine brucellosis in Nigeria's livestock have been documented by several authors (Falade, 1974; Falade *et al.*, 1974; Falade *et al*., 1975; Okon, 1980; Brisibe *et al*., 1993; Cadmus *et al*., 2006; Bertu *et al*., 2010; Junaidu *et al*., 2010 Talabi *et al.*, 2012 and Kaltungo *et al.,* 2013) with evidence of the spread of the disease in all parts of the country which is usually accompanied by severe economic losses.

Previous work on isolation of *Brucella* in Nigerian livestock has indicated that *Brucella abortus* has been the major species isolated from cattle, (Eze, 1978; Falade and Shonekan, 1981; Bale and Kumi-Diaka, 1981) and from sheep and goats, (Okon, 1980 and Ocholi *et al.*, 2005).

As a result of compulsory pasteurization of milk products and strict control of the disease in dairy animals, the incidence of brucellosis has steadily declined in most industrialized countries during the last 50 years (Cadmus *et al.*, 2006) unlike in Nigeria where people still consume unpasteurized milk and milk products.

Evidence of the presence of the disease in humans in Nigeria has also been published (Alausa, 1977; Alausa and Osoba, 1977; and Falade, 2002). Collard (1962) documented the first serological evidence of human brucellosis in Nigeria where *Brucella* antibodies were demonstrated in the sera of healthy persons in various parts of the country.

Although brucellosis is a notifiable disease in Nigeria, the incidence, prevalence and distribution of the disease are difficult to determine as the system of disease surveillance and reporting is fragmentary and inefficient (Ocholi *et al.*, 1993).

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study Area

This study was carried out in Oju LGA, Benue State, North-central geo-political zone of Nigeria. It shares geographical boundaries with Obi LGA to the North-west, Ado LGA to the West, Konshisha LGA to the North-east and Cross River State to the South. Oju LGA is located in the South Senatorial zone of Benue State and in the southern guinea savannah vegetation zone of Nigeria. It lies within latitude 6□ 50′ 43N and longitude 8□ 25′ 3E with an altitude of 180 metres (593 feet). Total annual rainfall range is 1500mm – 1750mm and the monthly mean temperature is 16 ^{o}C to 37 ^{o}C. It has an estimated population of 180,000 (National Population Commission, 2006). The inhabitants are the Igede speaking people of Benue State. It is home to the College of Education as well as home to a popular Christian mission at Egga called Elim. Agriculture is the mainstay of the economy and livestock production, which is mainly goat, is kept by semi-intensive management system.



Figure 3.1: Map of Benue State showing Oju LGA in lemon green colour

Source: Google maps (website, 2012)

3.2 Study Design

A cross-sectional study was performed in September and October 2013. Onyike (Ihiejwu) town which is the administrative capital of Oju LGA has a goat market and this goat market was used as the sampling unit as goat owners from the villages in the Local Government Area bring goats for sale at the market. Sampling by convenience was used and blood samples were collected after authorisation from market authorities and goat owners who consented. The market operates every five days and sampling was done on every market day for eight weeks. Blood samples were also collected from goat owners who consented in the communities of Ikachi, Ogengeng, Ikachi Road, Egga, Umoda and Zion Hill within the LGA. These 6 communities were selected by simple random sampling without replacement from a list of all the communities in the LGA.

3.3 Sample Size

Sample size was determined by using the formula of Thrusfield (1997) at 95% confidence level and prevalence rate of 10.1% as reported by Bertu *et al* (2010).

$$N = \frac{Z^2pq}{d^2}$$

Where N = sample size

Z = appropriate value for the standard normal deviation for the desired confidence = 1.96

p = prevalence

q = 1 – p

d = level of significance (0.04) decreased in this case to get an increased sample size

$$\text{Therefore } N = \frac{1.96^2 \times (0.101 \times 0.899)}{(0.04)^2}$$

$$= 218$$

However, a total of 241 blood samples were collected for this study.

Eighty six samples were collected from goats sold at the Onyike goat market, 21 from herds in Ikachi community, 38 from Ogengeng, 31 from Ikachi Road, 15 from Egga, 17 from Umoda and 33 from Zion Hill.

3.4 Sample Collection and Processing

3.4.1 Sample collection and handling

Goats were properly restrained and the age obtained by examining the mouth for appearance and wear of teeth. Sex was determined by looking at the perineum and between the legs on the ventral position for presence of vulva and scrotal sac for females and males respectively. Breed was ascertained based on their physical characteristics while information on the management system was obtained from the owners/handlers. Five millilitres of venous blood was aseptically collected from the jugular vein into a clean and well labelled sample bottle devoid of anticoagulant using sterile hypodermic needle and 10ml syringe. The blood samples were allowed to clot by laying the sample bottles in a slanting position for an hour and the sera obtained by decantation into new well labelled sample bottles with sample number, age, sex and breed. Serum samples were then transported to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria in a Coleman box with ice packs and stored at -20 □C in a freezer until used.

3.4.2 Laboratory Procedure

3.4.2.1 Rose Bengal plate test (RBPT)

The known *B. abortus* antigen, negative and positive controls for the test were obtained from ID.vet, France. The procedure was performed according to the manufacturer's instruction which is as described by Alton *et al* (1988). Thus, 30µl of antigen was placed on the test plate using a clean Pasteur pipette and the same volume of test serum was placed beside the antigen using another clean Pasteur pipette. The two were mixed thoroughly using a sterile applicator stick, rocked gently for four minutes and observed for agglutination. The formation of distinct pink granules (agglutination) was recorded as positive while the absence of agglutination was recorded as negative. The controls served as standards to determine what samples were positive or negative.

3.4.2.2 Indirect enzyme-linked immunosorbent assay (iELISA)

The Indirect ELISA kit including negative and positive controls was obtained from ID.vet, France. The test procedure was performed as instructed by the kit manufacturer.

This diagnostic kit is designed to detect antibodies directed against *Brucella melitensis, Brucella abortus* and *Brucella suis* and it can be used in caprine, ovine, bovine and porcine individual serum and plasma or in bovine pools (up to 10 sera or plasma).

Principle**:** Specimens to be tested and the controls are added to the microwells diluted at 1/20. Anti-Brucella antibodies, if present, form an antibody-antigen complex. A multi-species horseradish peroxidase (HRP) conjugate is added to the microwells. It fixes to the anti-Brucella antibodies, forming an antigen-antibody-conjugate-HRP complex. After washing in order to eliminate the excess conjugate, the substrate solution (TMB) is added.

The resulting colouration depends on the quantity of specific antibodies present in the specimen being tested:

- In the presence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution.
- In the absence of antibodies, no colouration appears.

The microplate is read at 450nm with a microplate reader.

Kit components**:** Microplates (12 x 8-well strips) coated with purified *Brucella* lipopolysaccharide (LPS), concentrated conjugate, positive and negative controls, dilution buffer 2, dilution buffer 3, wash concentrate, substrate solution (TMB) and stop solution.

Other materials used include mono and multi-channel micropipettors, disposable micropipettor tips, distilled water, manual wash system and 96-well microplate reader.

Testing Procedure**:** All reagents were allowed to come to room temperature and homogenized by inversion before use. Sera samples to be tested and the controls were added to the microwells diluted at 1/20.

The microplate was prepared by adding 190µl of dilution buffer two to all wells, 10µl of the negative control to wells A1 and B1, 10µl of the positive control to wells C1 and D1 and 10µl of each sera sample to the remaining wells before the microplate was incubated at 21□C for 45 minutes. After the incubation, the wells were emptied and each well washed three times with 300µl of the wash solution which was prepared by diluting the wash concentrate (20X) in distilled water. One hundred microlitre of the conjugate was added to each well and the plate incubated at 21□C for another 30 minutes, the conjugate was prepared by diluting the concentrated conjugate (10X) in dilution buffer three. Thereafter,

the wells were emptied and washed three times with 300µl of the wash solution. Substrate solution was added at 100µl per well and further incubated in the dark at 21□C for 15 minutes before the stop solution was added at 100µl per well in order to stop the reaction. A microplate reader (Optic ivymen system, Model 2100-C) was used to read and record the optical density at 450nm.

Interpretation**:** For each serum sample, the S/P percentage (S/P %) was calculated as follows using the sample and control values:

$$S/P\ \% = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100$$

Where S/P = sample/positive

OD = optical density

NC = negative control

PC = positive control

Serum samples with an S/P%:

- Less than or equal to 110% were considered negative
- Greater than 110% and less than 120% were considered doubtful
- Greater than or equal to 120% were considered positive.

3.5 Questionnaire

A structured questionnaire was designed and administered by face to face interview to thirty three goat owners within the study area between September and October 2013. The goat owners were randomly selected at the goat market and also from the randomly selected communities of Ikachi, Ogengeng, Ikachi Road, Egga, Umoda and Zion Hill. The

questionnaires were administered to those whose herds were sampled. Some of the questions had "yes" or "no" answer options while others had specific answers as options. The questions were explained to the respondents by the researcher using Igede and English languages and their responses were written down. The questionnaire had five sections (A-E). The biodata of the goat owner was contained in section A and Section B contained information on herd data. Section C contained information on health indicators for herd and Section D contained information on health indicators for herd owners while information on risk factors for herd and herd owners was contained in section E. A copy of the questionnaire is presented as Appendix I.

3.6 Data Analysis

All the data obtained from this research were statistically analyzed using GraphPad Prism 4 for Windows. Results are presented using tables, bar charts and pie charts. Chi-square was used to determine association between the prevalence of antibodies and factors such as age, sex and breed and values of $p < 0.05$ were considered significant.

The seroprevalence of *Brucella* antibodies was determined by the formula

$$\text{Seroprevalence} = \frac{\text{Positive sample}}{\text{Total sample analysed}} \times 100$$

CHAPTER FOUR

RESULT

4.1 Study Population

The 241 serum samples used for this research were obtained from goats in Oju LGA of Benue State. Of the 241 samples, 49 were from male goats while 192 were from female goats. Twenty eight West African dwarf and 213 Kano brown breeds of goats made up this total. Forty three of the goats were below 12 months, 97 were between 13 - 36 months while 101 goats were above 36 months. All the goat owners practiced semi-intensive system of management.

All serum samples were subjected to RBPT which gave a seroprevalence of 33 (13.7%) and iELISA which confirmed the presence of *Brucella* antibodies in 18 (7.5%) of the goats in Oju LGA as shown by Figure 4.1.

Seroprevalence increased with age although there was no significant association between the infection and the age of the goats as the p-value was greater than 0.05 (Table 4.1).

The seroprevalence of *Brucella* antibodies by sex is presented in Table 4.2. The female goats had higher seroprevalence of 7.81% than the males that had a seroprevalence of 6.12%.

On breed distribution, West African dwarf breed had higher seroprevalence (10.71%) of *Brucella* antibodies than the Kano brown breed (7.04%) of goats (Chi-square value and p-value as 0.4828 and 0.4871 respectively). This is presented in Table 4.3.

The biodata of the goat owners interviewed, source of goats sold and the average number of goats sold per week per goat owner in Oju LGA are as shown in Tables 4.4, 4.5 and 4.6 respectively.

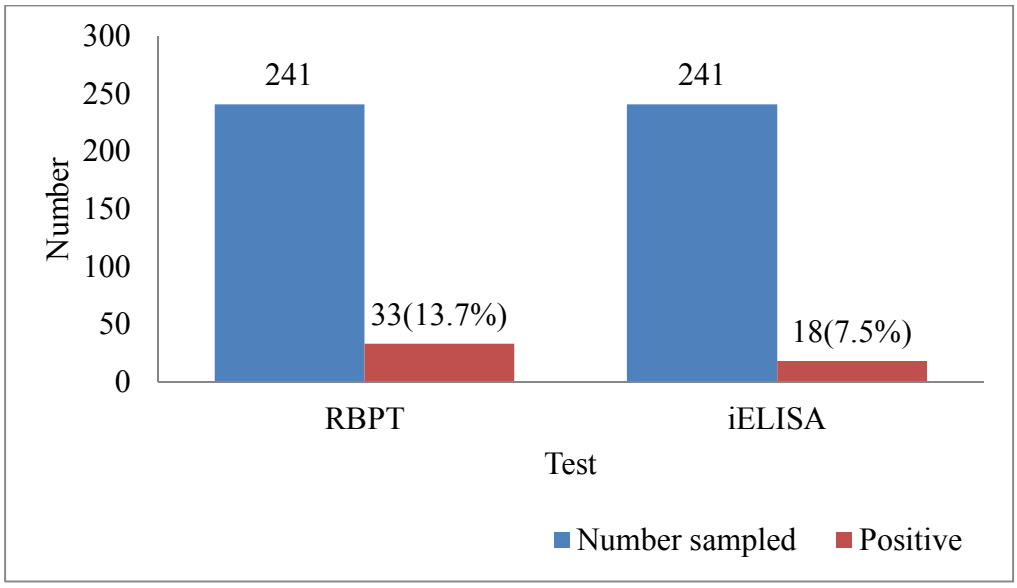


Figure 4.1: Seroprevalence of *Brucella* antibodies in goats in Oju LGA using RBPT and iELISA

Table 4.1: Age-based seroprevalence of *Brucella* antibodies in goats in Oju LGA by iELISA

Age (months)	Number tested	iELISA positive (%)	χ^2	(df)	p-value
0 - 12	43	3 (6.98)			
13 – 36	97	7 (7.22)	0.05386	2	0.9734
Above 36	101	8 (7.92)			
Total	241	18			

Table 4.2: Sex-based seroprevalence of *Brucella* antibodies in goats in Oju LGA by iELISA

Sex	Number tested	iELISA positive (%)	χ^2	(df)	p-value
Male	49	3 (6.12)	0.1613	1	0.6879
Female	192	15 (7.81)			
Total	241	18			

Table 4.3: Breed-based seroprevalence of *Brucella* antibodies in goats in Oju LGA by iELISA

Breed	Number tested	iELISA positive (%)	χ^2	(df)	p-value
Kano brown	213	15 (7.04)	0.4828	1	0.4871
West African dwarf	28	3 (10.71)			
Total	241	18			

Table 4.4: Biodata of goat owners interviewed in Oju LGA

Parameters		Number tested
Sex	Male	27
	Female	6
	Total	33
Age (years)	Less than 20	0
	Between 21 and 40	16
	Above 40	17
	Total	33
Marital status	Married	31
	Not married	2
	Total	33
Management system practiced	Intensive	0
	Semi-intensive	33
	Extensive	0
	Total	33

Table 4.5: Source of goats sold at the goat market in Oju LGA

Source of goats sold	Number of goat owners (percentage)
Rear goats	7 (21)
Buy from other herds	5 (15)
Buy from other markets	2 (6)
Rear + buy from other herds	8 (24)
Rear + buy from other markets	11 (34)
Total	33 (100)

Table 4.6: Average number of goats sold per week per goat owner in Oju LGA

Number of goat owners (percentage)	Average number of goats sold per week
17 (52)	□ 5
10 (30)	5 ≤ 10
3 (9)	10 ≤ 15
3 (9)	15 ≤ 20
33 (100)	

4.2 Questionnaire Responses

4.2.1 Diseases that affect goats in Oju LGA:

None of the owners recognized brucellosis as a disease affecting their goats (Figure 4.4). However, 18 (55%) and 15 (45%) of them said goat catarrh and helminthosis respectively, affect their goats.

4.2.2 Action taken when diseases occur in goats:

None of the goat owners called a veterinarian (no vet clinic present) when diseases occurred but 22 (67%) of them reported calling someone with a knowledge about animal diseases while 10 (30%) others treated the animals themselves and 1 (3%) respondent reported giving no treatment.

4.2.3 Occurrence of signs of brucellosis in the goats in Oju LGA:

Twelve (36%) of the owners said abortion and retained placenta had occurred in their goats and of this, 2 (17%) removed the retained placenta by traction while 10 (83%) waited for it to drop on its own. None of the respondents called a veterinarian's attention. One (3%) each, said epididymitis and repeat breeding respectively, had occurred while 58% did not notice abortion, retained placenta, epididymitis or repeat breeding in their herds (Figure 4.4).

4.2.4 Occurrence of symptoms of brucellosis in the goat owners in Oju LGA:

One (3%) each, of the goat owners has had persistent fever and profuse sweating at night respectively, 3 (9%) admitted having joint pains, while 28 (85%) did not have any of the symptoms (Figure 4.4).

4.2.5 Vaccination of goats with *Brucella* vaccine:

None of the goat owners interviewed in Oju LGA vaccinated his/her goats against brucellosis (Figure 4.4).

4.2.6 After-birth materials disposal by goat owners in Oju LGA:

Fourteen (42%) of the goat owners threw away after-birth materials into the bush while 19 (58%) buried them in a pit (Figure 4.2).

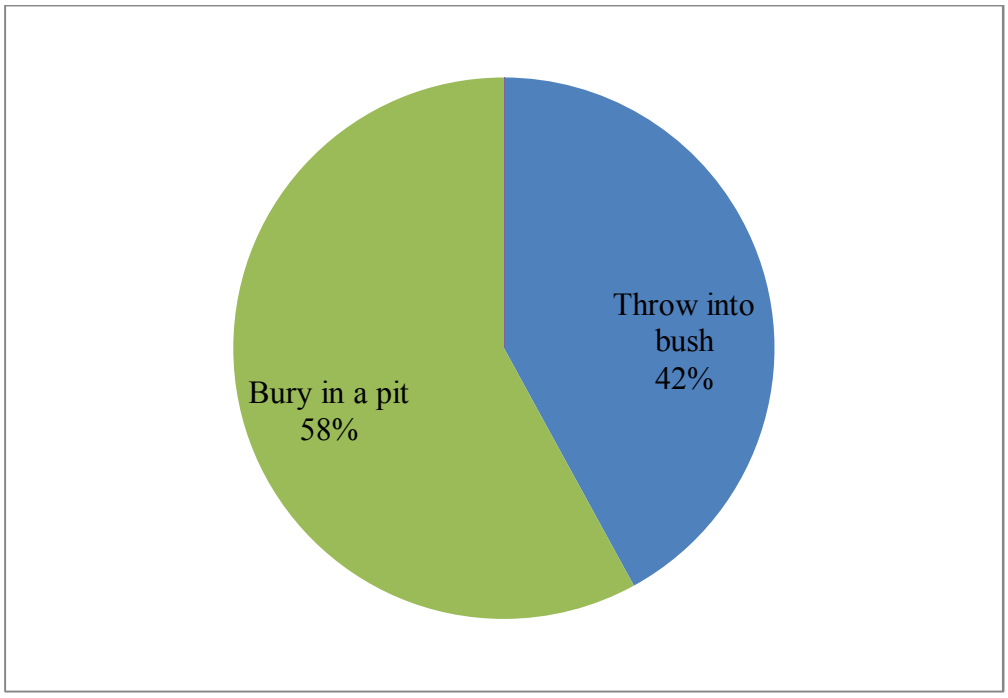


Figure 4.2: Disposal of after-birth materials by goat owners in Oju LGA

4.2.7 Cleaning of goat pens in Oju LGA:

All goat owners cleaned the goat pens by sweeping only. They neither washed such pens with detergents nor applied disinfectants (Figure 4.3).

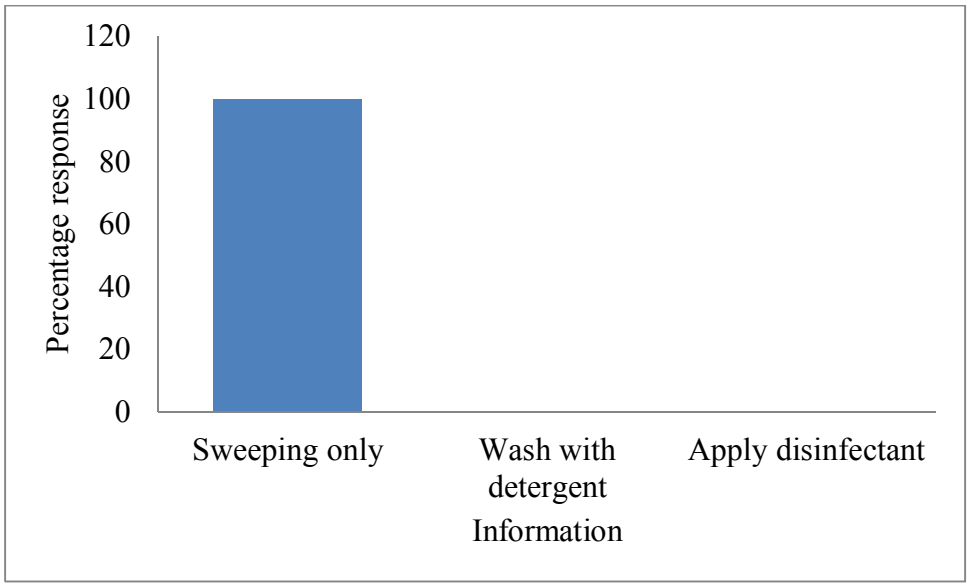


Figure 4.3: Cleaning of goat pens in Oju LGA

4.2.8 Isolation of newly purchased goats in Oju LGA:

All owners introduced newly purchased goats to the herd without isolation or examination by a professional (Figure 4.4).

4.2.9 Consumption of goat milk in Oju LGA:

None of the goat owners consumed goat milk (Figure 4.4).

4.2.10 Use of protective clothing by goat owners in Oju LGA:

All owners interviewed used bare hands when handling goats. None of them used coverall, laboratory coats, boots, hand gloves or face masks while handling their goats (Figure 4.4).

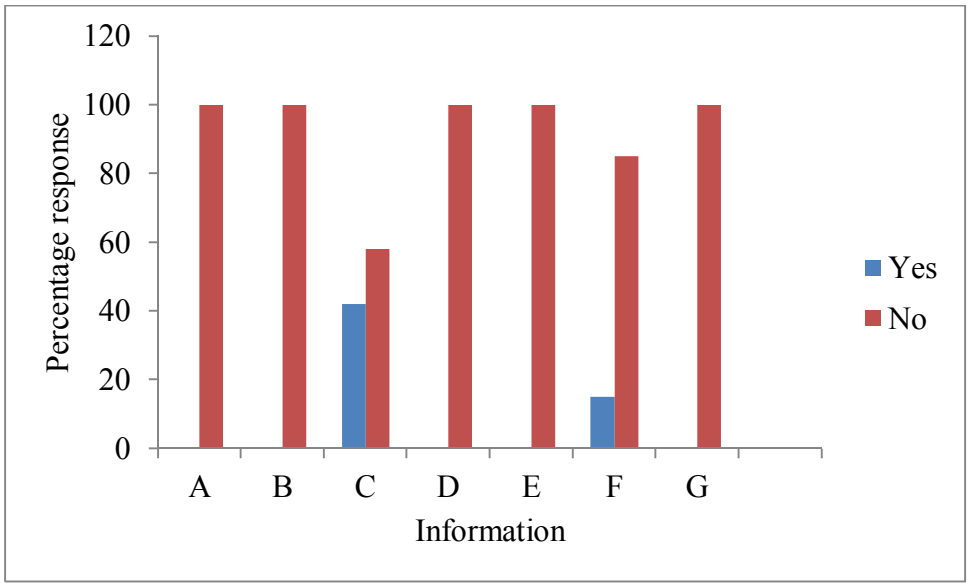


Figure 4.4: Goat owners' responses with regard to caprine brucellosis in Oju LGA

Key

A: Occurrence of brucellosis in the goats

B: Vaccination of goats with *Brucella* vaccine

C: Observation of abortion, retained placenta, epididymitis and repeat breeding

D: Isolation of newly purchased goats

E: Use of protective clothing during goat handling

F: Occurrence of persistent fever, profuse night sweating and joint pains in goat owners

G: Consumption of goat milk

CHAPTER FIVE

DISCUSSION

All serum samples were subjected to RBPT which gave a seroprevalence of 33 (13.7%) and iELISA which confirmed the presence of *Brucella* antibodies in 18 (7.5%) of the goats in Oju LGA. The higher seroprevalence obtained by the RBPT could be due to serological cross-reactions that have been reported between smooth *Brucella* and various other Gram negative bacteria such as *Escherichia coli* 0:116 and 0:157, *Salmonella* group N (0:30) of Kauffman-White, *Pseudomonas multophilia, Vibrio cholera* and especially *Yersinia enterocolitica* 0:9. These organisms can induce significant levels of antibodies which cross-react with S-LPS *Brucella* antigens in diagnostic tests (EU Commission, 2001) such as the RBPT, hence the use of iELISA which is a highly specific test than the RBPT (Nielsen *et al*., 1992) to detect *Brucella* antibodies. The result obtained by the iELISA would be confirmatory since the kit used in this study was designed to detect antibodies directed against *B. melitensis*, *B. abortus* and *B. suis*.

The lack of vaccination, the semi-intensive system of management practiced by the goat owners that participated in the study and the convergence of goats at the market for sale and subsequent return of unsold goats to the rest of the herd could be responsible for the spread of the infection in this locality. Furthermore, the purchased goats going to the herds of the buyer could introduce the infection in such herds if they were infected. While some previous researchers reported higher prevalences, others reported lower prevalences compared to this finding. Bertu *et al*. (2010) reported a similar rate of 7.0% from goats in Bassa LGA of Plateau State which is in the same geopolitical zone (North-Central Nigeria)

as Benue State. A slightly higher prevalence rate of 10.9% for *B. melitensis* in a study of the seroprevalence of brucellosis in goats in Sokoto State, North-West Nigeria was obtained by Junaidu *et al*. (2010) while a much higher value of 45.75% was obtained by Ojo *et al*. (2007) in a study of a goat herd in Abeokuta, Ogun State, South-West Nigeria. Much lower prevalence rates of 2.3% and 1.7% for Quanpan LGA and Shendam LGA respectively of Plateau State were obtained by Bertu *et al*. (2010). Tigist *et al*. (2011) reported a prevalence rate of 4.2% of caprine brucellosis in South Omo Zone of Southern Ethiopia. The implication of this seroprevalence is the risk of transmission of brucellosis by infected goats to other animals and man and the economic losses associated with it.

There was no significant association between brucella infection and age ($p > 0.05$) although the prevalence increased with age. Oloffs *et al*. (1998) had stated that brucella infection increased with age and most diseased animals carried the infection throughout their lives. Kaltungo *et al*. (2013) in a study of brucellosis in goats in Kaduna obtained the highest seroprevalence in goats aged between 1 to 3 years. In this study, more of the animals above one year were available for sampling and animals in this age range are actively involved in breeding. The presence of brucellosis in them means they could easily spread the infection since they freely mix with animals in other herds especially during breeding. Animals in this age group are more often sold out for slaughter and this poses a risk to humans as source of infection. Animals less than a year old that were positive could have obtained the infection by suckling from infected dams and through feed and water.

Although females had higher prevalence than males, there was no significant association between brucella infection and sex ($p > 0.05$). Studies carried out by Muma *et al*. (2006) indicated that *Brucella* antibody titres were not associated with sex. However, Radolf

(1994) had stated that allantoic factors including erythritol, possibly steroid hormones and other substances stimulate the growth of most of the *Brucellae*. Keppie *et al*. (1965) also stated that erythritol, which is a polyhydric acid, is found in higher concentration in the placenta and foetal fluids of females than in the seminal vesicles and testes of males and this could be responsible for the observed differences in prevalence rates between the sexes. However, *Brucella* has also been found in the reproductive tract of animals with no detectable level of erythritol (Enright, 1992). The higher seroprevalence in females could also be due to the fact that more females were available for sampling as they are kept for a longer time in the herd for the purpose of breeding.

Although the West African dwarf breed had higher prevalence rate than the Kano brown breed, there was no significant association between brucella infection and breeds of goats ($p > 0.05$). Ajogi *et al*. (2002) had reported that there is no association of brucella infection with breeds of animals.

From this study, both males and females were involved in goat business in Oju LGA with only adults of above 20 years of age being owners of the goats. The unmarried goat owners if infected with brucellosis could be at a great loss due to the reproductive failure caused by human brucellosis while the married owners could also sexually transmit the infection to their spouses if infected. Regardless of the sources of goats sold at the market, all goats sampled were in Oju LGA as at the time of this study. Fifty two percent of the goat owners sold less than 5 goats on each market day while 9% sold between 15 and 20 goats on each market day, this showed that goats were a good source of income to them. Unsold goats were returned to the rest of the herd and could serve as a source of infection to the herd if they became infected at the market.

The questionnaire revealed that all the goat owners in Oju LGA practiced semi-intensive system of management and introduced newly purchased goats into the herd without isolation and examination by a professional. The semi-intensive system of management greatly influences the spread of brucella infection (Radostits *et al*., 2006) as the disease spread among herds generally follows the movement or gathering of infected animals with susceptible ones. Furthermore, the introduction of new animals can serve as the main risk for introducing the disease into a previously non-infected herd/area. Intermingling of goat herds may occur under semi-intensive or extensive system of management and also in static village herds where animals are taken daily for grazing on common pastures (Radostits *et al*., 2006).

The primary route of dissemination of *Brucella* to other animals and man is the placenta, foetal fluids and vaginal discharges expelled by infected nanny goats after abortion or full-term parturition (Alton *et al*., 1988). The after-birth materials thrown into the bush by respondents could be a source of spread of the infection especially if stray dogs carry such materials to areas with healthy animals and humans. Dogs have been shown to be mechanical and biological vectors of brucellosis (Joint FAO/WHO Expert Committee on Brucellosis, 1986). Thus, should such after-birth materials be contaminated with *Brucella species,* the spread could be real. The fact that the owners handle such materials could further mean that the owners could also be at risk of coming down with the disease should such materials be contaminated with *Brucella* organisms. Kaltungo *et al*. (2013) similarly reported sheep and goat owners throwing away placentae into bushes. This habit clearly shows their ignorance of the epidemiology of the disease.

From the study, none of goat owners vaccinated his/her goats against brucellosis. This is a pointer to their unawareness of the disease or unavailability of the vaccine and it poses a risk to both the animal and human population. Vaccination when used exhaustively greatly decreases the prevalence of brucellosis in both animal and human population (Elberg, 1981 and 1996) and prevention of human brucellosis is dependent on the control of the disease in domestic livestock, especially where test and slaughter and mass vaccination programmes are in place (Nicoletti, 2001).

All goat owners interviewed cleaned their goat pens by sweeping only. No detergents or disinfectants were used. Also, owners used bare hands when handling goats with no use of protective clothing such as coverall, laboratory coats, boots, hand gloves or face masks. This puts them at risk of the disease as *Brucellae* can gain entry through intact skin or abrasions (Mantur and Amarnath, 2008). Use of bare hands can serve as a means of spread among animals and humans through direct contact if the organism is retained on the hands as *Brucella* may retain infectivity for several months in water, aborted foetuses and foetal membranes, faeces and liquid manure, wool, hay, on buildings, equipment and clothes (Alton and Forsyth, 1996).

The fact that none of the owners could recognize brucellosis as a disease affecting their herd could be attributed to the lack of veterinary presence in the locality as most of them refer to anyone with knowledge of animal diseases or treated the animals themselves.

The non-consumption of goat milk by the goat owners as observed in this study has eliminated the possibility and risk of infection by humans in the study area.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

A seroprevalence of 7.5% for *Brucella* antibodies in goats in Oju LGA was obtained in this study. There was no significant association between brucella infection and the epidemiological variables of age, sex and breed of goats sampled in this study ($p > 0.05$). The study has established risk factors such as lack of vaccination, introduction of newly purchased goats into the herd without isolation, semi-intensive system of management, disposal of after-birth materials in the bush, lack of use of protective clothing when handling goats and cleaning of pens by sweeping without disinfection. The fact that none of the goat owners could recognize brucellosis as a disease affecting their herds and do not vaccinate against the disease is a pointer to their unawareness of the disease and as a zoonosis. Transmission of brucellosis by goat milk consumption in the study area could not be established as all the respondents said they do not consume goat milk.

6.2 Conclusion

This study is the first of its kind to document the prevalence of *Brucella* antibodies in goats in Oju LGA of Benue State. Management practices and marketing of goats by goat owners as observed in this study supported the spread of the infection and posed risks to the owners and other handlers. Goat owners were unaware of brucellosis and therefore unaware of its zoonotic importance. There seems to be human brucellosis in the study area based on responses by owners. Goat owners in Oju LGA have been verbally informed of these

findings by the researcher; however, an awareness campaign on brucellosis to enlighten the populace on this important zoonosis should be carried out.

6.3 Recommendations

i. Aborted materials and placenta should be properly disposed of by deep burial or burning.

ii. More studies on the disease in both animals and humans should be carried out in the study area, other LGAs and the State for effective control and prevention of the disease.

iii. Public awareness programmes on zoonotic diseases, especially brucellosis, should be carried out in the Local Government Area.

iv. Government should introduce Animal care health delivery in the study area and a national policy on the control of brucellosis is recommended.

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APPENDIX

I Questionnaire to assess the level of awareness of caprine brucellosis among goat owners and handlers in Oju LGA

Instruction: Please tick the options as appropriate

A BIODATA

1.Respondent number.....

2. Sex [ ] male [ ] female

3. Age [ ] less than 20 years [ ] between 20 & 40 years [ ] above 40 years

4. Marital status [ ] married [ ] not married

5. Level of education [ ] primary [ ] secondary [ ] tertiary [ ] informal

B HERD DATA

6. Age of goat [ ] below 1 year [ ] 1- 3 years [ ] above 3 years

7. Sex [ ] male [ ] female

8. Breed of goat [ ] Kano brown [ ] Sokoto red [ ] West African dwarf

9. What is the source of the goats you sell?

[ ] I rear goats [ ] I buy from other herds [ ] I buy from other markets

10. On the average, how many goats do you sell per week?

[ ] less than 5 [ ] 5-10 [ ] 10-15 [ ] 15-20 [ ] greater than 20

11. Which markets do you sell your goats

[ ] Onyike market in Oju [ ] market outside Oju [ ] both

C HEALTH INDICATORS FOR HERD

12. What diseases commonly affect your goats?

[ ] brucellosis [ ] goat catarrh [ ] pneumonia [ ] helminthosis

13. What do you do when diseases occur

[ ] call a veterinary doctor

[ ] take the sick animals to a vet clinic

[ ] call someone with knowledge about animal diseases

[ ] treat them yourself

14. Has any of these signs occurred in your herd of goats?

Abortion [ ] yes [ ] no

Retained placenta [ ] yes [ ] no

Epididymitis [ ] yes [ ] no

Repeat breeding [ ] yes [ ] no

None [ ]

D HEALTH INDICATORS FOR HERD OWNERS

15. Have you had any of these symptoms?

[ ] persistent fever (remains for more than a week continuously or discontinuously)

[ ] profused sweating at night [ ] joint pain [ ] abortion or miscarriage [ ] none

16. What did you do? [ ] visited a hospital [ ] took drugs on my own [ ] use herbs

E RISK FACTORS FOR HERD AND HERD OWNERS

17. Management system [ ] intensive [ ] semi-intensive [ ] extensive

18. What diseases do you vaccinate your goats against?

[ ] brucellosis [ ] PPR [ ] CCPP.

19. If abortion and retained placenta have occurred, how were they handled?

[ ] remove with hand [ ] wait for it to drop [ ] call vet. doctor's attention

20. How do you dispose of after birth materials?

[ ] throw into bush [ ] throw into refuse dump [ ] bury in a pit [ ] burn

21. How do you clean the goat pens?

Sweeping only [ ] yes [ ] no

Wash with detergent [ ] yes [ ] no

Apply disinfectant [ ] yes [ ] no

22. What do you do to newly purchased goats?

[ ] isolate and observe for sometime before introducing into herd

[ ] immediately introduce into herd

[ ] call a vet. doctor to examine them

23. Do you drink goat milk? [ ] yes [ ] no

24. If yes, what type? [ ] fresh/raw [ ] boiled [ ] both

25. What do you wear when handling your goats?

Use bare hands [ ] yes [ ] no

Coverall/lab. coat [ ] yes [ ] no

Boots [ ] yes [ ] no

Hand gloves [ ] yes [ ] no

Face mask [ ] yes [ ] no