SEROPREVALENCE OF BOVINE BRUCELLOSIS IN TARABA STATE, NIGERIA

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ZARIA, NIGERIA

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLOUNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIRMENTS FOR THE AWARD OF MASTER OF SCIENCE IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

DEPARTMENT OF VETERINARY PUBLIC HEALTH
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MAY, 2011

DECLARATION

I hereby declare that the work in this thesis titled "SEROPREVALENCE OF BOVINE BRUCELLOSIS IN TARABA STATE, NIGERIA" has been performed by me in the Department of Veterinary Public Health and Preventive Medicine under the supervision of Professor I. Ajogi, Dr. A.M. Raji, and Dr. M. Bello.

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 at any university.

HAMZA, ISA MOHAMMED

_____	_____	_____
Name of student	Signature	Date

CERTIFICATION

The thesis titled "SEROPREVALENCE OF BOVINE BRUCELLOSIS IN TARABA STATE, NIGERIA", by Hamza, Isa Mohammed meets the regulations governing the award of the degree of Master of Science in Veterinary Public Health and Preventive Medicine of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to:

My late Parents

Alhaji Inuwa Hamza and Hajiyya Fadimatu,

And my Wife and Children

Aishatu Isa, Fadimatu, Abubakar, Na'Imatu, Bilkisu and Umar.

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ABSTRACT

A total of 540 sera and 162 milk samples were obtained from 540 cattle in 90 herds from 9 of the 16 Local Government Areas (LGAs) of Taraba State. The 540 serum samples were screened for *Brucella abortus* antibodies using Rose Bengal Plate Test (RBPT). Out of these, 80 (14.8%) were positive for the test and were further subjected to Indirect- ELISA Test which gave 69 (12.8%) positives as confirmatory. The confirmation rate by IELISA was 86.3% (69/80). The overall individual animal and herd prevalences for *Brucella* agglutinins in milk was 9.9% (16/162) and 16.7% (15/90) respectively, using the Milk Ring Test (MRT). Of the 376 female and 164 male cattle tested, 42 (11.2%) and 27 (16.5%) were positive for brucellosis respectively. The difference in the infection rates were statistically significantly higher in males than in females (df = 1, X^2 =4.51, Odds ratio 1.57, CI: 0.93 -2.64 $P<0.05$). The result also showed age – based prevalence rate of 24.9% in cattle older than 3 years compared to 7.1% in age group below 3 years old. The difference in infection rates between the age-groups was statistically significant (df = 1, X^2 =33.32, Odds ratio 0.23, CI: 0.14 - 0.39 $P<0.05$). An overall individual animal prevalence of 2.8% (69/2475) and overall herd prevalence rate of 61.11% (55/90) were obtained. There were differences in infection rates between LGAs with highest prevalence obtained in Takum 20% (12/60) and the least in Zing 5.0% (3/60). Questionnaire on brucellosis awareness among herdsmen and butchers, showed only 22.2% (22/99) had knowledge of brucellosis. Of all the herds studied, none had ever been vaccinated against brucellosis and 9% of 99 respondents said they experienced retained placenta in their cattle. It was concluded that an overall seroprevalence of 12.8% and 16.7% of bovine brucellosis in Taraba State was determined during the study in both serum and milk respectively. There was general poor knowledge and awareness of brucellosis among stakeholder; this is of public health concern. A control policy for this disease is recommended in Taraba State particularly in occupationally risk groups. Further work is required in the areas of isolation and characterization of the organism responsible for brucellosis in Taraba State.

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LIST OF ABBREVIATIONS

RBPT:	Rose Bengal Plate Test
I-ELISA:	Indirect Enzyme-Link ImmunoSorbent Assay
HP :	Herd Prevalence
IAP :	Individual Animal Prevalence
OR :	Odds Ratio
CI :	Confidence Interval
HPR	Herd Prevalence Rate
IPR	Individual Prevalence Rate

CHAPTER 1

INTRODUCTION

1.1 DEFINITION OF THE PROBLEM

Brucellosis is an infectious disease occurring in many species of animals and man attributable to bacterial organism of the genus *Brucella*. *Brucellae* are small, non-motile, aerobic, facultative intracellular, Gram-negative coccobacilli. The ability of *Brucella* to replicate and persist in host cells is directly associated with its capacity to cause persistent disease and to circumvent innate and adaptive immunity (Fichi, 2003). The species of *Brucella* and their major hosts are *B. abortus* (cattle), *B. melitensis* (goats), *B. suis* (swine) and *B. ovis* (sheep) (Radostits *et al.*, 2000). Others are *Brucella maris* isolated from marine mammals, *B. cetaceae* (cetaceans) and *B. pinnipediae* (pennipeds) (Foster *et al*., 2002).

Brucellosis is one of the world's major zoonotic problems. Though it has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand (Geering *et al*., 1995), yet it remains an uncontrolled problem in regions of high endemicity such as the Africa, Mediterranean, Middle East, parts of Asia and Latin America (Refai, 2002). Considering the damage done by the infection in animals in terms of decreased milk production, abortions, weak offsprings, weight loss, infertility and lameness, it is one of the most serious diseases of livestock (Ajogi, 1987). It is also a major impediment for the livestock trade. Death may occur as a result of acute metritis, followed by retained fetal membranes (Radostits *et al.*, 2000).

In animals, the spread of the infection is through ingestion of infected raw milk by the young, licking of vaginal discharges by the bulls, aborted fetuses by dams, and placentas (Ocholi, *et al*., 2005) as well as artificial insemination using infected semen, contaminated

pens, padlocks, feeds, water and pasture from infected uterine discharges, urine and faeces (Bale, 1999). Following infection *with B. abortus*, pregnant adult females' animals developed a placentitis usually resulting in abortion between the fifth and ninth month of pregnancy, neonatal mortality and infertility in both sexes (Alausa, 1983). Adult male cattle may develop hygroma, usually involving leg joints (Arthur *et al*., 1982).

From public health view point, brucellosis is considered to be an occupational disease that mainly affects slaughter-house workers, butchers, and veterinarians (Plommet *et al.*, 1998). Transmission typically occurs through contact with infected animals or materials with skin abrasions (Plommet *et al*., 1998). Symptoms in human brucellosis can be highly variable, ranging from non–specific, flu-like symptoms (acute form) to undulant fever, arthritis, orchitis and epididymitis (Plommet *et al.,* 1998). Although brucellosis is a notifiable disease in Nigeria, the precise incidence and prevalence of the disease is difficult to determine because of poor reporting system (Ocholi *et al*., 1993) and there has been no co-ordinated government efforts to monitor the spread and control of the disease in livestock in Nigeria (Bawa *et al*., 1987).

1.2 STATEMENT OF THE RESEARCH PROBLEM

Brucellosis is prevalent in all major livestock production systems in sub-saharan Africa, yet its presence often remains unrecognized through lack of awareness and absence of accessible laboratory diagnostic facilities (Henk and Sally, 2004). As a consequence, brucellosis remains a largely neglected disease with little attention to control and prevention except in South Africa where a successful control policy has been instituted (McDermott and Arimi, 2002). This was based on vaccination programmes combined with test and slaughter policies (Emsile and Nel, 2002).

Investigations have shown that infections as well as outbreaks have occurred in cattle in Nigeria (Ajogi, 1997), sheep and goats (Bale *et al*., 1982; Ocholi *et al*., 2005), horse (Ocholi *et al*., 2004), sheep, goats and human beings (Brisbe *et al*., 1993; EU, 2000) and camels (Okoh, 1979; Adamu and Ajogi, 1999).

Infection in human is often due to occupational exposure and is essentially acquired by the oral, respiratory or conjuctival routes, by veterinarians, farmers or slaughterhouse workers (Osman *et al*., 2005). Infection of dairy products constitutes the main risk to general public, causing acute febrile illness, undulant-fever which may progress to a more chronic form with serious complication affecting the musclo-skeletal, cardiovascular and central nervous systems (Corbel, 1997).

1.3 SIGNIFICANCE OF THE STUDY

There is an increasing demand on the cattle industry in Nigeria for more beef and dairy products so as to enhance the availability of adequate supplies of animal protein for the over growing population. The Nigeria's livestock resources indicated cattle population of about 18.4 million cattle (Anon, 1994). While 99.50% of these cattle production is still under the management of the Fulani pastoralist who are accustomed to extensive system of production (Rim, 1992). There are considerable reproductive wastages in the country in terms of high abortion rate at first conception, low conception rates, low calving rates, low calf-survival rates, reduced fertility, lowered milk yield and slow growth rate of calves and young cattle (Osori, 1976; Oyedipe *et al*., 1981). Several factors may be responsible for these reproductive wastages such as poor nutrition, disease, poor housing and other environmental factors directly or indirectly affecting livestock production (Nuru, 1982).

Other financial losses are due to reduced milk yield, reduction in draught power, research and veterinary expenses (Ajogi *et al.*, 1998).

In human, the disease often shows influenza-like signs with intermittent fever, malaise, generalized aching, bacteremia and granulomatous lesions in internal organs. Economic losses is enormous due to ill-health, loss of working hours as a result of mental suffering in affected people, inability to have enough funds with low morale to cattle breeding on affected herd owners due to abortion (Ajogi *et al.*, 1998). There are also reports on isolation and characterization of Brucella strains in Nigerian livestock (ocholi *et al*., 2004). Further reports, based on surveys at abattoir and slaughter slabs where prevalence's of between 0.4 to 8.6% were obtained (Nuru and Schnurrenberger, 1975; Chukwu, 1987a; Ogundipe *et al.*, 1993; Ajogi, 1997). The incidence of the disease in other animals species such as sheep, goats, pigs, camels, horses, dogs, Chicken and humans have been reported, even though less frequently, (Bale *et al.*, 1982, 1984; Brisebe *et al*., 1993; Ocholi *et al*., 1993; Halle and Ajogi 1997, Osinubi *et al*., 2004; Junaidu *et al*., 2006). The recent livestock population census survey in Taraba State reveals a population of 4,805,820 heads of cattle (MOA, 2008).

The detection of antibody has been a useful tool in the bovine brucellosis control and eradication programme established by many countries (Nielsen *et al.*, 1988). Brucellosis can be diagnosed definitively by isolation and identification of the causative organism and a number of serological tests have been developed and modified in various ways to increase their performance (Nielsen, 2002). The Rose Bengal Plate Test (RBPT) and serum agglutination test (SAT) are used routinely for survey work in Nigeria (Eze, 1977; Ocholi

et al., 1993) and competitive Enzyme Linked ImmunoSorbent Assay (COMPELISA) (Junaidu *et al*., 2006; 2008).

1.4 JUSTIFICATION

Cattle are a major source of meat supply in Taraba State with an estimated cattle population of 4.8 million (MOA, 2008). Brucellosis is a major cause of economic loss in the livestock and a serious zoonosis of great public health importance (Cadmus *et al.*2006). It has been reported that brucellosis is significantly higher among people whose occupations constantly bring them in close contact with animals (Alausa and Osoba, 1975; Refai, 2002). There is paucity of information on bovine brucellosis in Taraba State.

It is increasingly evident that brucellosis is enzootic and represents one of the major setbacks to livestock in Nigeria (Ate *et al*., 2007), and as suggested by Ocholi *et al*. (2004) there is increasing trend in the prevalence of the disease. This calls, therefore, for the need to have the prevalence of bovine brucellosis documented in Taraba State for planning and control measures.

1.5 AIMS AND OBJECTIVES OF THE STUDY

The major aim of this study was to determine the seroprevalence of bovine brucellosis in Taraba State.

The objectives were to:

i. Determine the Prevalence of bovine brucellosis in Taraba State by Rose Bengal Plate Test and Indirect ELISA.

ii. Screen milk to determine prevalence of bovine brucellosis from lactating Cows in the state using Milk Ring Test (MRT)

iii. To relate the prevalence to age, sex and management practice.

iv. Assess with the help of questionnaires on the awareness of the disease among the high risk groups in the state.

1.6 RESEARCH NULL HYPOTHESES

1. Cattle in Taraba State do not habour brucellosis.

2. There is no significant difference between prevalence rates in female and male cattle.

3. There is no significant relationship between age and sex prevalence rate of bovine brucellosis.

4. There is no significant difference between the uses of RBPT and i-ELISA in detecting *Brucella* antibody.

CHAPTER 2

LITERATURE REVIEW

2.1 HISTORICAL NOTES ON BRUCELLOSIS

Brucellosis is an infectious disease occurring in many species of animals and man attributable to bacterial organism of the genus Brucella. Humans become infected by ingestion of animal food products (Kubuafor *et al*., 2000), direct contact with infected animals, or inhalation of infectious aerosol (Kose *et al*., 2006). The disease now called brucellosis, under the name "Mediterranean fever", first came to the attention of British medical officers in Malta during the Crimean war in the 1850s *Brucella abortus*. The causal relationship between organism and disease was first established by Dr. David Bruce in 1887 (Wilkinson, 1993). In 1897, Danish Veterinarian Bernhard Bang isolated as the agent, and the additional name "Bang's disease" was assigned. In modern usage, "Bang's disease" is often shortened to just "**Bangs**" when ranchers discuss the disease or vaccine. Maltese doctor and archaeologist Sir http://en.wikipedia.org/wiki/Themistocles_ZammitThemistocles Zammit identified unpasteurized milk as the major source of the pathogen in 1905, and it has since become known as Malta fever. In cattle this disease is also known as contagious abortion and infectious abortion.

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 "Mediterranean fever" and "Malta fever". *Brucella suis* was isolated in USA from an aborted porcine fetus (Traum, 1914). Evans

(1918) showed that these organism were related morphologically, biochemically and serologically and they were included in the genus *Brucella* (Meyer and Shaw, 1920). Three more species of Brucella has been described; Buddle and Boyes (1953) who isolated *Brucella ovis*, the cause of epididymitis and abortion in sheep in New Zealand and Australia; Stoener and Lackman (1957) isolated *Brucella neotonae* from the desert wood rat (Neotona lepida) in Utah, USA and *Brucella canis* which causes canine epizootic abortion and epididymitis, isolated from Beagle dogs in the USA by Carmichael and Kenny (1968). In 1989, Saudi Arabian neurologists discovered neurobrucellosis**,** a neurological involvement in brucellosis (Malhotra, 2004; Al-Sous *et al*., 2004) in humans.

Species infecting domestic livestock are *B. melitensis* (goats and sheep), *B. suis* (pigs,), *B. abortus* (cattle and bison), *B. ovis* (sheep), and *B. canis* (dogs). *B. abortus* also infects bison and elk in North America and *B. suis* is endemic in caribou. *Brucella* species have also been isolated from several marine mammal species such as pinnipeds and cetaceans (Foster *et al.*, 2002).

2.2 AETIOLOGY

Bovine brucellosis is commonly caused by the biotypes of *Brucella abortus*. In countries where *B. melitensis* occur in sheep and goat, cattle may contract *B. melitensis* from them (Nielsen *et al*., 1988). Experimentally, cattle can also be infected with *B. suis* (Stableforth, 1959). Nine different biotypes of *B. abortus* have so far been recognized to cause brucellosis in cattle (Nielsen and Duncan 1990). Out of which, biotypes 1, 2, 3 and 4 have been isolated in Nigeria (Eze, 1987; Bale and Kumi Diaka, 1981; Ocholi, *et al*., 2004, 2005)

2.3 SURVIVAL OF *BRUCELLA* 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, 1985). Thus, when pH, temperature and light conditions are favourable: i.e pH>4, high humidity, low temperature and absence of direct sunlight, *Brucella* may retain infectivity for several months in water at -4^0C, aborted foetuses and fetal membranes, the organism can survive in faeces, slurry, or liquid manure 85-103 days in the winter, 120-210 days in spring, and 30-180 days in summer and 50-120 days in autuum (Nicoletti, 1980). Wool in warehouse 4 months, hay several day-months, on buildings, equipment and cloths. *Brucellae* are able to withstand drying particularly in 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 20^0C and 40% relative humidity (Alton, *et al*., 1996). Survival is prolonged at low temperatures, especially below 0^0C (Alton, 1985; WHO, 1986; Nicoletti, 1980). The survival of *Brucella* in milk is related to a variety of factors including the type and age of product, humidity level, temperature, changes in pH, moisture content, biological action of other bacteria and condition of storage (Plommet *et al*., 1988).

Contaminated equipment can be sterilized by autoclaving at (121^0C). Chemical can be used to destroy *Brucella* in contaminated premises. Xylene (1ml/litre) and calcium cyanamide (20kg/m^3) have been found to be effective in liquid manure after 2-4 weeks. A 1hour treatment with 2.5% sodium hypochlorite, 2-3% caustic soda, 20% freshly slaked lime suspension or 20%formaldehyde solution will suffice to destroy *Brucellae* on contaminated surfaces.

2.4 DISTRIBUTION

Though its distribution is worldwide; brucellosis is more common in countries with poorly standardized animal and public health programme (Capasso, 2002). The routes of infection are multiple i.e., food-borne, occupational or recreational, linked to travel and even to bioterrorism. New *Brucella* strains or species may emerge and existing *Brucella* species adapt to changing social, cultural, travel and agricultural environment (Godfroid *et al.,* 2005).

Bovine brucellosis has occurred mostly to parts of the world where extensive cattle herding are practiced (Carpenter and Hubbert, 1963). Brucellosis is endemic disease in Nigeria and has been reported sporadically since 1921 (Ocholi *et al.,* 1993). In animals the presence and transmission of *Brucella* is moderated by an inter-relationship of factors including climate, type of production system (Normadic/transhumance or sedentary; extensive or intensive); herd size, livestock breeds and the age of the animal (Akakpo *et al*., 1987).

Fewer than 200 total cases in humans per year (0.04 cases per 100,000 populations) are reported in the USA. The incidence is much higher in other regions such as the Middle East; countries bordering the Mediterranean Sea; and China, India, Mexico, Peru; for example, 33 cases per 100,000 population in Jordan (1987) and 88 cases per 100,000 population in Kuwait (1985), (Mousa *et al.*, 1987). According to the office of International Epizootics (OIE, 1987; 2000), Burkina Faso, Cameroon, Mali and Ivory Coast were the only West African Countries cited as either reporting or suspecting the presence of bovine *Brucella*. While some areas may have high incidence of acute infections, the low incidence

reported in other known brucellosis endemic areas may suggest low levels of surveillance and reporting.

2.5 TRANSMISSION

The bacteria are shed from an infected animal at or around the time of calving or abortion. Once exposed, the likelihood of an animal becoming infected is variable, depending on age, pregnancy status, and other intrinsic factors of the animal, as well as the amount of bacteria to which the animal was exposed (Radostits *et al.,* 2000). Transmission of *B. abortus* may likely to occur via the oral route, because cattle tend to lick aborted fetuses and the genital discharge of an aborting cow (Cunningham, 1977). Also, via utero (Fensterbank, 1978) or when calves were fed on colostrums or milk from infected dams (Bercovich *et al*., 1998). WHO (1997) incriminated direct exposure to dogs carrying pieces of placenta or aborted fetuses from one place to another, while indirect exposure could be mediated by wildlife, birds and water ways (contaminated with urine, uterine discharge, or slurry from aborting cattle).

Brucellosis in bulls does not always result in infertility but semen quality may be affected. Bulls that remain fertile and functionally active will shed *Brucella* organism with the semen during the acute phase of the disease (Kubuafor *et al*., 2000) but the shedding may cease or become intermittent. Unlike artificial insemination, bulls used in natural service may fail to spread the infection, as the infected semen is not deposited in the uterus (Ray, 1988). Infection may also occur via the conjunctivae, mucosa of the respiratory and gastrointestinal tract (Brinley –Morga and Corbel, 1990). Also transmission can occur through the teat from infected milk of another cow (Arthur *et al*., 1982)

2.6 SUSCEPTIBILITY

The disease is of adult where pregnant female and non pregnant animals that has not been previously exposed or immunized are highly susceptible (Morgan, 1970).Susceptibility of cattle to *B. abortus* infection is influenced by the age, sex and reproduction status of the individual animal, sexually mature pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex due to maternal immunity (Crawford *et al*., 1990). Natural exposures to field strains occur primarily at the time of parturition of infected cows. The greater the number of infected cows that abort or calved, the greater the exposure risk to the other cattle in the herd (Crawford *et al*.1990). Bulls may acquire the infection at early stage and may retain infection into adult life (Lambert *et al.*, 1964a). *B. abortus* affects cattle mainly, and occasionally sheep, swine, dogs and horses (Morgan, 1967). Bison, deer, elk, camel reindeer, water buffalo, rabbit and mice have been reported to be susceptible to infection (Avong, 2000). Man can also be infected by brucellosis through drinking unpasteurized or un-boiled milk or by exposure to infected discharges or by accidental injection of *B. abortus* strain 19 vaccines and laboratory hazard (Crawford *et al*., 1990).

2.7 PATHOGENESIS

Brucella abortus is a facultative intracellular gram-negative bacterium that causes abortion and temporary infertility in cattle (Enright *et al*., 1984; Gorham *et al*., 1986; Meador and Deyoe, 1989). It is also a zoonotic pathogen causing fever, weakness, endocarditis, arthritis, osteomyelitis, and meningitis in humans (Young, 1995). In cattle, the infection tends to be chronic with tropism for the reproductive system of pregnant cows. Abortion is the most significant clinical sign, but an infected cow may be completely

asymptomatic. Transmission of the disease occurs mainly after abortion or parturition of infected cows via contaminated fetus, fetal membranes, and uterine secretions (Crowford *et al*., 1990; Samartino and Enright, 1993). During the early stages of infection, *B. abortus* is found mostly in lymph nodes. The infection may progress to bacteremia and colonization of the uterus, where the organism replicates preferentially within trophoblasts in the rough endoplasmic reticulum (Detilleux *et al*., 1990a; Detilleux *et al*., 1990b; Meador and Deyoe, 1989). As a result, the cow develops placentitis, fetal death, and abortion, particularly during the last third of the gestation (Anderson *et al*., 1986a).

B. abortus grows primarily in the extracotyledonary trophoblasts and then spreads to the cotyledonary (placental) trophoblasts (Anderson *et al*., 1986b). Therefore, proliferation of *B. abortus* within trophoblastic cells is a key event in the mechanism of abortion. Trophoblasts favour bacterial growth by producing erythritol and progesterone, which stimulate in vitro growth of *B. abortus* (Essenberg *et a*l., 2002; Smith *et al*., 1962). In acute infection of pregnant cows, more than 85% of the bacteria are located in the placenta and allantoic fluid (Smith *et al*., 1961). In spite of the importance of intracellular multiplication of *B. abortus* within trophoblastic cells and consequent placentitis, which are key events in the pathogenesis of *B. abortus*-induced abortion, information regarding the interaction between *B. abortus* and bovine trophoblast is extremely scarce.

In human the *Brucella* may enter the body through digestive tract, lungs or mucosal layers and intact skin. Then it may spread through blood and the lymphatic system to any other organ where it infects the tissues and causes localized infection (Lapaque *et al*., 2005). The organism is able to escape phagocytic killing through inhibiting the phagosome-lysosome fusion and reproducing inside macrophages (Young, 2005). After a variable

incubation period ranging from less than one week to several months, non-specific systemic symptoms such as fever, headache, malaise, night sweats and arthralgia followed, resembling flu like disease. During the early stage of the disease, patients are frequently bacterimic that has a continuous pattern, making circulating *Brucella* easily detectable by blood culture. Once in the blood stream, the organism is seeded to multiple organs/systems, especially those rich in reticuloendothelial tissue, such as liver, spleen, skeletal and hematopoietic system (Greenfield *et al*., 2002).

Invading *Brucella* usually localize in the lymph nodes draining the invasion site, resulting in hyperplasia of lymphoid and reticuloendothelial tissue, and the infiltration of inflammatory cells, survival of the first line of defense by the bacteria, result in a local infection and escape of *Brucellae* from the lymph nodes into the blood (Enright, 1992).

During the bacteraemic phase (which may last 2-8 weeks) the bones joint, eyes and brain can be infected, but the bacteria are most frequently isolated from suprammary lymph nodes, mammary lymph nodes, milk iliac lymph nodes, spleen and uterus (Enright *et al*., 1984). The tropism of Brucella to the male or female reproductive tract was thought to be erythritol, which stimulates the growth of the organism, but *Brucella* has also been in the reproductive tract of animal with no detectable level of erythritol (Enright, 1992).

In the acute stage of infection, abortion occurs at four or five month into pregnancy and cattle usually abort only once. Abortion and retention of the placenta, late abortion or both of infected calves is common in herds with endemic brucellosis. Excretion of Brucella after parturition may persist for months or years and may re-occur after much consecutive normal parturition. Infected cattle excrete Brucellae in the colostrums or milk although it cannot always be detected (Manthei and Deyoe, 1970).

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

The predilection sites for infection in bull are the reproductive organs and the associated lymph nodes. During the acute phase of infection, the semen contain large number of Brucellae but as the infection become more chronic the number of Brucellae excreted decrease and excretion may cease or occur as orchitis, epididymitis and infection of the accessory sex glands (Jubb and Kennedy, 1963).

2.8 CLINICAL FINDINGS

"Abortion storm" occurs in immunologically depressed pregnant cows often calls attention to bovine brucellosis infection in the herds (Seifert, 1996) especially in non-vaccinated pregnant cattle abortion after the 5^{th} month of pregnancy is documented (Rodostits *et.al.*, 2000). In subsequent pregnancies, the fetuses are usually carried to full-term, although second or even third abortions may occur in the same cow (Corbel, 1985). Abortion do occurs during the last trimester of pregnancy and is followed by retention of fetal membranes (Anon, 2001). In certain herds abortion is uncommon but retained placenta constitute the only evidence of brucellosis. In addition decreased milk production and, occasionally hygroma of the knee or other localization of infection may be seen (Duran-Ferrer, 1998). Orchitis, epididymitis, seminal vesiculities and later infertility are the major signs seen in males while hygroma in particular on the carpal joint is observed in

both sexes (Fensterbank, 1978). The inflammatory changes in the infected mammary gland reduce milk production (Alton, 1990).

2.9 PATHOLOGY

Brucella- infected animals generally develop granulomatous inflammatory lesions which frequently are found in lymphoid tissues and organs such as reproductive organs, udder and supramammary lymph nodes and sometimes joints and synovial membranes. The following could be observed: necrotizing placentitis, palpable testicular alterations, necrotizing orchitis with subsequent granuloma, necrotizing seminal vesiculitis and prostitis (Festerbank, 1987). In the bull, lesions are confined to the genital organ. There may be evidence of acute or chronic orchitis, epididymitis and seminal vesiculities may contain multiple granulomas with infiltration of lymphoid, plasma and epitheloid cells surrounded by giant cells and some granulomas may be calcified (Lambert *et al*., 1960).

Acute mastitis with palpable nodules and the productin of clotted and watery milk. Some aborted foetuses may have an excess blood-stained fluid in the body cavities, with enlarged spleen and liver. The fetuses may be macerated (Seifert, 1996). Infected foetal membranes show changes affecting part or all of the membrane and the necrotic cotyledons loses their blood-red appearance becoming thickened and dull-grey in colour (Radostits *et al.,* 2000).

Microscopically, the necrotic testicular lesions in bull contained focal areas of mineralization, large numbers of neutrophils, and abundant degenerate cellular debris. These areas were surrounded by infiltrates of macrophages, lymphocytes, and plasma cells and were separated from remaining parenchyma by dense, collagenous connective tissue.

Binucleated or multinucleated giant cells were present in the inflammatory cell infiltrate, often adjacent to mineralized foci. Many macrophages contained few to numerous small, gram negative coccobaccili distributed diffusely in the cytoplasm or clustered in cytoplasmic vacuoles (Jack, *et al*., 1997). Organisms were also present in swollen degenerate cells in the centers of some necrotic areas. Remaining testicular parenchyma had diffuse interstitial fibrosis, focal interstitial accumulations of large and small mononuclear inflammatory cells, and aspermatogenic seminiferous tubules. Testicular tunics had numerous focal accumulations of mononuclear inflammatory cells (predominately lymphocytes and plasma cells), usually adjacent to blood vessels, and rare small pyogranulomas with central necrosis and mineralization. Intact bacilli were seen in some macrophages but were always within large cytoplasmic vacuoles. In some foci, large macrophages had intracytoplasmic aggregates of calcified coccobacilli that were embedded in an amorphous, finely granularmatrix (Jack, *et al*., 1997)

2.10 IMMUNITY

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

2.10.1 Natural Immunity

Although abortion appears to be the result of an acute disease, brucellosis is really a chronic disease. Since lymph tissue is usually predominantly infected, most animal show natural resistance to the disease. Brucella abortion is known to persist for a long period after initial infection (Marchant and Packer, 1975). Lambert *et al*., (1960) have shown that

the organism persisted for as long as four years after exposure. Age resistance to the disease has also been demonstrated. Young calves can be infected but may get rid of the infection, or it become dormant to re-activate when the animal reaches sexual maturity (Rodostist and Blood, 1997). The sexually mature animals are easily infected and the infection is more lasting (Marchant and Packer, 1975).

2.10.2 Acquired Immunity

Contact with the disease confers varying degrees of immunity. Some infected cattle remain disease throughout their lives; others recover completely in few month but may either merely become tolerant or highly resistance to subsequent re-infection (Merchant and Packer, 1975). The duration of the disease and the amount of immunity acquired depends on the character of the particular strain with which the animal is infected (Merchant and Packer, 1975). The use of a vaccine prepared from avirulent strain, *B abortus* strain 19, develop by Buck (1930) in immunizing the calves before they reach the age of maturity confers immunity of reasonable duration of 3years (Gilman, 1963).

2.10.3 Cellular Immune Response in Host Animal and Man

Following phagcytosis 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 entry into the body, the *Brucella* organisms encounter the cellular defense of the host, in particular neutrophils (Cunning *et al.*, 1985), Natural killer (NK) cells (Yabu *et al*., 1991) macrophages (Jiang *et al*., 1999) and T-lymphocytes (Smiths,1990) studies have shown that protective immunity against *Brucella* infection is due mainly to cell mediated immunity (CMI) mediated by t-cells of

both CD4+, CD8+ and $\gamma\delta$ subsets (Schurig *et al*., 2002;Ko and Splitter,2003). The critical functions of the CMI are the production of cytokines. The cytokines regarded as key players in brucellosis are interleukin1 (IL-1) IL-2, IL-12, IFN-y and tumour necrosis factor (TNF-aα) and IL-6(Ko and Splitter, 2003). IL-12 is a key cytokine produced by B cells and macrophages and leads Th1 immune responses in the host that will ultimately induce the secretion of IFN-R from T- cells (Zhan and Cheers, 1995; 1998). The major role of T-cells in Brucella immunity is secretion of 1FNr for the activation of bacteria function in macrophages and cytotoxic T-lymphocyte activity as well as IgG2a and IgG3 antibody isotype switching. Functionally, this can be classified in 3 mechanisms. First IFNr produced by CD4+, CD8+, and T-cell activate the bactericidal function in macrophages to hamper the intracellular of Brucella secondary, cytotoxicity of CD8+ and T cells kills the infected macrophages. Thirdly, Th 1-type antibody isotype such as IgG2a and IgG3 opsonize the pathogen to facilitate phagocytosis (Ko and Splitter, 2003).

2.10.4 Immunoglobulin Production in Brucellosis

Humoral immune response is also elicited in *Brucella* infection (Wilson and Miles, 1975). The patterns of immunoglobulin production after vaccination and natural infection have been studied in both animals and man (Wilson and Miles, 1975). The pattern of production is the same in both vaccinated and naturally infected hosts; antibody isotypes produced in response to *B. abortus* infection in cattle include IgM, IgG1, IgG2 and IgA (Rose and Amerault, 1964; Rose and Roepke, 1964;Rice and boyes 1971;Beh, 1973, 1974, 1975). The proportional distribution usually differs in chronically infected and vaccinated cattle (Beh and Lascelles, 1973). In both naturally infected and vaccinated cattle, it is often

impossible to pinpoint with accuracy the time onset of the appearance of immunoglobulin molecules (Beh, 1974).

The pattern show that in some cases in the very early antibody response, IgM may be the main antibody class found in the serum (Rose and Amerault, 1964, Rose and Roepke, 1964; Rose *et al*., 1964) while in other cases the IgG isotypes prevail (Beh 1974) the former finding however, has occurred very infrequently from experience (Nielsen, *etal*., 1988). All the four isotypes of antibody are present in milk (Beh, 1973) although IgG1 and IgA predominate. In naturally infected cattle IgG1 is usually present in serum in greater amount than IgM and IgM2 and these antibody isotype are present in higher proportion in naturally infected than in vaccinated cattle (Beh, 1974).

2.11 DIAGNOSIS OF BOVINE BRUCELLOSIS

The existence of the disease may be suspected from herd history indicating occurrence of one or several abortions at about the seventh month of pregnancy. Differential diagnosis of abortion may be difficult clinically and require laboratory assistance for confirmation (Morgan, 1971)

2.11.1 Direct Smear

Microscopic examination of smears made from fetal membranes, milk and semen stained by modified kosier staining, modified Ziehl-Neelsen (Staemp *et al*., 1950) and fluorescent antibody techniques (Janney and Berman, 1962) may be diagnostic for brucellosis.

2.11.2 Isolation

The only unequivocal method for the diagnosis of brucellosis is based on the isolation of *Brucella* bacteria (Alton *et al*., 1988). The spleen and lymph nodes are the most reliable samples for isolation purpose in necropsied animal (Marin*et al*., 1996a).Microscopic examination of smear from vaginal swabs, placentas or aborted foetuses stained with the Stamp modification of the Ziehl-Neelsen method is recommended (Alton *et al.,* 1988).Various culture media or selective media with antibiotics are incubated in an atmosphere of 10% CO_2 at 37^0C for 3-9 days (Kudzas and Horse,1953).Selective media are needed for isolation purpose to avoid the growth of contaminants (Corbel *et al*., 1985) the Farrell's selective medium, developed for the isolation of *B. abortus* from milk is recommended (Alton *et al.,* 1988). The sensitivity of bacteriological diagnosis is significantly increased by the simultaneous use of both the Farrell's and modified Thayer-Martin's media (Marin et al., 1996b). Cultural identification is by metabolic requirement and characteristics, colonial morphology biochemical characteristics, dye sensitivity, antigenic characteristics staining reactions and sensitivity to Brucella phage (Merchant and Packer, 1975).

2.11.3 Animal Inoculation

Guinea pigs are more susceptible to Brucella than any other laboratory animal; however, mice are 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, *B. abortus* is recovered from spleen by culturing on to appropriate media (Alton *et al*., 1988).

2.11.4 Serological Diagnoses

The serological diagnosis of brucellosis in cattle is frequently difficult to achieve because the serological response in cattle infected with *B. abortus* is influenced by several factors. These including the long and variable incubation period during which serological tests result are negative, cattle vaccination status, type of exposure variable responses of vaccinated and infected cattle at the time of exposure in infection (Anon, 1971). Bercovich (1998) advocates the use of more than one diagnostic test in order to determine the status of an individual animal. The commonly used conventional tests for the diagnosis of brucellosis are the Rose-Bengal Plate Test (RBPT), serum Agglutination test (SAT), complement fixation test (CFT) and Milk Ring Test (MRT) (Van Aert *et al.*, 1984). Other conventional methods include Heat inactivation test anti globlobulin (Coombs) test, 2-Mercapteothanol test, Rivanol test and Enzyme linked immunosorbent assay test.

2.11.4.1 *Rose Bengal plate test (RBPT)*

The RBPT was developed more than 30 years ago for the diagnosis of bovine brucellosis and was extensively used (Trap and Gaumont, 1975; Fensterbank and Maquere, 1978; Farina, 1985; Macmillan, 1990; Alton, 1990). The acid plate agglutination test employing a suspension of *B. abortus* cells stained with Rose Bengal dye and buffered at pH 3.65 was developed for rapid screening of cattle for brucellosis under range conditions but has a setback of picking non-specific reactors. To buttress its effectiveness, Rose and Roeke (1957) introduce the plate agglutination test in which the antigen was buffered at pH

4.0 capable of differentiating specific *Brucella* agglutinins from non-specific reactors. It was observed that at this pH agglutination of *B. abortus* cells by non-specific agglutinins of bovine serum was inhibited whereas the activity of specific *Brucella* antibodies was unaffected. Its value as a supplement test for screening field samples of sera for *Brucella* specific agglutinins was subsequently confirmed (Lambert and Amerault, 1962 a, b). However, it has some limitation for use, since the test is rather too sensitive in vaccinated herd and might give false/positive reaction (Morgan *et al.*, 1969).

2.11.4.2 *Serum agglutination test (SAT)*

The SAT antigen was established by Welch and Mickel (1933). It is the most widely used serological test for the diagnosis of brucellosis and for conducting eradication programs. 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 concentration of the cell in the antigen. 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).

2.11.4.3 *Complement fixation test (CFT)*

The CFT is the most widely used test for the serological confirmation of brucellosis in cattle(Morgan *et al*., 1969). There is agreement for its effectiveness for serological diagnosis in sheep and goats (Farina, 1985; Macmillan, 1990; Alton, 1990).The technique

was introduced by Alton *et al*, (1975). It is used to distinguish between serological reaction due to vaccination with living attenuated vaccines and those of natural infection (Morgan, 1967) because in natural infection, the antibodies produced are very competent at fixing complement at 0-4^{0}C (Alton, 1975). The drawback of CFT is its complexity, variability of reagent, prozones, anticomplementary activity of sera, difficulty to perform with hemolysed sera, subjectivity of the interpretation of low titres and failure to detect 1gG, an antibody isotype which might result in false negative reaction (Nielsen *et al*., 1988).

2.11.4.4 *Enzymes linked immunosorbent assay* (*ELISA*)

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

The majority of EIAs in used in brucellosis diagnosis are the indirect ELISAs (iELISA). ELISAs are methods that involve the immobilization of one of the active components on the solid phase, and iELISAs are those in which the antigen is bound to a solid phase, usually a polystyrene microtiter plate so that antibody, if present in a sample, bind 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).

Another method is the competitive ELISA (cELISA) (Gorrell *et al*., 1984; Rylatt *et al*., 1985; Sutherland *et al*., 1986; Macmillan *et al*., 1990; Nielsen *et al*., 1991 Marin *et al*., 1999; Nielsen et al., 2000). In this test, Brucella antigen is immobilized as in the iELISA. 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 occur if there is no antibody in the serum sample which is bound preferentially.

These assays give objective results and are extremely sensitive reagents present in less health hazards, and they are stable and have long shelf-lives. Moreover, the estimation of the results can either be visual or be made with rather simple spectrophotometer (ELISA Reader) of the type found in most laboratories and are suitable for large application (Voller*et al.*, 1976; Nielsen*et al.*, 1987).

2.11.4.5 *Milk ring test (MRT)*

Fleischaner developed the Brucella Milk Ring Test (MRT) in 1937 for detecting the presence of Brucella antibodies in milk using stained Brucella antigen. The test has found widespread use in indicating infected or potentially infected herd as well as ensuring Brucella free herds milk that contain colostrums milk at the end of the lactation period and milk from cows suffering a hormonal disorder or mastitis may test false positive (Kerkhofs *et al*., 1990).

However, this test has limitation for usage: positive reactions to the MRT in the absence of infection can be produced by vaccination of adult animal with strain -19. Reactions to the test reappear during the drying- off period in pregnant animal. Furthermore, the presence of mastitis also causes reaction to be produced to the MRT because of the passage of antibodies from the blood stream. The test is also affected by fat globules and time of sampling (Morgan, 1967).

2.11.4.6 *Other conventional methods*

Other conventional tests for the diagnosis of Brucella–infected cattle are available though not commonly used. Morgan (1967) reviews such tests which include their development, test procedure and limitations for their usage. These include Heat in-activation test (HIT), 2- Mercapto- ethanol test (2-MET), the Rivanol (Acridine) test, incomplete antibody agglutination test, coombs test, fluorescent antibody techniques, surface fixation test precipitation (gel diffusion) test, milk ring serial dilution test, whole milk plate agglutination test, Whey tube agglutination test, Whey plate agglutination test and Brucellosis Skin Test (BST).

2.12 IMMUNOASSAY

Immunoassay techniques was developed to overcome the limitations of the conventional methods which has the capacity of rapidly and accurately detecting humoral antibodies to brucellosis by primary binding assay such as flouroimmunoassay (FIA), Radioimmunoassay (RIA) and enzyme immunoassay (EIA) techniques.

2.12.1 Fluoro Immunoassay Technique

Fluoroimmunoassay (FIA)has been extensively used (Nicolleti and Tanya, 1993; Greenlee *et al*., 1994). The technique has much in common with the cELISA in that antigen is immobilized onto polystyeren, but here is inform of sub-micron particles held in the suspension. Serum is incubated with anti-brucella conjugate in the presence of the antigen coated particles, and they compete for binding. Here the antibody is labeled with a fluorescent probe. The unreacted reagents are separated by concentration through the membrane at the bottom of each well.

2.12.2 Radio Immunoassay Technique

The technique involve the attachment of isotypes to the antibodies or antigens. It is highly sensitive and allowed precise quantification and is well suitable for large brucellosis herd screening (Chapel *et al*., 1982; Chapel and Hayes, 1983).The limitations were:(1) isotype label may rapidly decay with short shelf life complex (2) sophisticated equipment is necessary for their assessment (3) it must be handled by highly trained personnel (4) centralized and specialized laboratories is required because of the medical hazards involved in their usage (Voller, *et al*., 1976).

2.13 MOLECULAR DETECTION AND TYPING

While culturing is a specific method, the time required for culturing field specimens can be long and tissues or fluids that are only contaminated with non-viable or a low number of Brucella may not be detected (Anon, 2001). Therefore, the identification of a stable DNA-specific marker is considered a high priority for diagnostic, epidemiological and taxonomic purpose.

Detection by Polymerase Chain Reaction (PCR) for routine diagnosis was investigated (Fekete *et al.,* 1990, 1992). However, since brucellae share a high degree of DNA homology (> 90% for all species), on the basis of a DNA – DNA hybridization which did not yield the necessary clues to explain the phenotypic differences among extant *Brucella* species and biovars. Brucella has been proposed as a monospecific genus (Verger *et al*., 1987).

Several molecular techniques have been employed to find DNA polymorphisms which would enable the molecular typing of *Brucella species* and their different biovars.

These include enterobacterial repetitive intergenic consensus (ERIC) sequence–PCR, NAD repetitive intergenic palindromic (REP) (Mercier *et al.*,1996, Tcherneva *et al.*, 2000) randomly amplified polymorphic DNA–PCR or arbitrary primed – PCR (Fekete *et al.*, 1992; Tcherneva *et al.*, 2000). Several investigators reported a good sensitivity of PCR for detecting Brucella DNA on pure culture (Bailey *et al.*, 1992; Romero *et al*, 1995; Da Costa *et al.*, 1996). Others have shown that PCR could be potentially useful tool when used in combinations with labeled probes to differentiate some Brucella species and biovars (Grimont *et al.*, 1992; Bricker and Halling 1994, 1995; Cloeckaert *et al.*, 1995; Ouahrani – Bettache *et al.*, 1996; Fitch *et al.*, 1990; 1996).

Among these, detection of restriction fragment length polymorphism is considered a reproducible and easily performed method within a laboratory. However, laboratory-laboratory reproducibility has been problematic and this makes the universal application of the methods unlikely (Bricker *et al.*, 2003). Alternatively, pulsed –field gel electrophoresis (PFGE) of total genomic DNA digested with restrictive 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* (Jansen *et al*., 1995; 1999), but a common problem is incomplete protease digestion by RE of the DNA caused by incomplete cell lysis or incomplete protease digestion (Faber, 1996).

Major Outer 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 RELP profiles of two closely related genes OMP2a and OMP 2b, encoding and potentially expressing the *Brucella* spp. major OMP of 36 kDa (Fitch *et al.*1990) and for 25 kDa (Wergifossa *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.,* 1998). However, as a tool for the epidemiology of brucellosis in livestock, this technique is limited by the low rate of natural sequence divergence within the locus of the biovar level (Bricker *et al.,* 2003). Consequently, *B. canis* remained indistinguishable from *B. suis* biovar 3 and 4 (Cloeckaert *et al.*, 1995).

The publishing of complete genome sequence of *B. melitensis* (Del Vecchio *et al.,* 2002).*B. canis* (Paulsen *et al.,* 2002) and the complete of the genomic sequencing of *B. abortus* (Sachnez, *et al.,* 2001) have made is possible to search the regions of DNA sequence variability that might be useful as markers for diagnostic identification and differentiation. This led to the development of a technique known as "HOOF" prints for Hyoer-variable Octametric Oliggonucleotide Finger-Prints (Bricker *et al.,* 2003). The micro satellite fingerprinting exploits the occurrence of variable number tandem repeats (VNTRs). The technique is highly discriminatory among *Brucella species*, among previously characterized Brucella Strains, and among unrelated field isolates that could not be differentiated by classical methods.

2.14 TREATMENT

Brucellae are facultative intracellular bacteria, relapses after treatment usually appear (FAO, 2004). However, a practical and effective treatment regimen could be used to treat valuable breeding animals and would benefit producers in countries which cannot afford to control the disease by the test and slaughter policy (Eaglesome and Garcia, 1992). Reports of unsuccessful attempts to treat bovine brucellosis involved the use of multiple

injections of combination of a long acting tetracycline and streptomycin (Milward *et al.*, 1984). The inadequacy of antibiotics regiments in controlling *B. abortus* may be due to the failure of antibiotic to penetrate cell membrane barriers effectively. Treatment in domestic animals with high dose of antibiotic is not indicated, because the antibiotics appear in the human food chain and it interferes with the production of milk products.

Treatment for human brucellosis is indicated and consists of antibiotics or combinations of antibiotics. Tetracycline seems to be most potent antibiotic, when combined with streptomycin (Wilson and Miles, 1975). The optimum antibiotic therapy for human brucellosis is still disputed despite extensive studies over the past 15 years (Corbel, 1997). The treatment recommended by the World Health Organization (WHO) for acute brucellosis in adults in a combination of rifampicin and doxycycline daily for a minimum of 6 weeks (WHO, 1986). Infections with complications, such as meningoencephalitis and treatment in children may require therapy of rifampicin and trimethoprim /sulphomethazolin (Lubani *et al.,* 1989) while endocarditis may be treated with rifampicin, streptomycin and doxycycline for 6 weeks (Chan and Hardiman, 1993). A triple therapy of doxycycline, together with rifampin and cotrimoxazole has been used successfully to treat neurobrucellosis (McLean *et al*., 1992)

2.15 CONTROL

Bovine brucellosis causes a heavy economic loss to the livestock industry worldwide. A successful control measure would require the designing campaign compatible with the socio-cultural, economic and political factors of the cattle owners in addition to the biologic characteristics of the disease agent, the transmission and the host responses (Rikin,

1988a).A uniform program for the eradication and control of bovine brucellosis is not possible as it depends on the prevalence of brucellosis and the husbandry practices. Thus, Anon (1971) recommended guidelines for a systematic approach to the control program which include surveillance, testing and vaccination procedures.

2.16 SOCIO-ECONOMIC IMPORTANCE OF BOVINE BRUCELLOSIS

The significance of brucellosis in man and livestock has been extensively investigated (Boycott, 1964; Ajogi, 1997). Brucellosis like other abortion disease of cattle causes economic loss through reproductive wastages as high abortion rate at first conception, low conception rate, low calving rate, low calf-survival rate, reduced fertility, lower milk yield and slow growth rate of calves and young cattle (Osori, 1976; Oyedipe *et al*., 1981) temporary infertility from chronic metritis, death from acute metritis, sterility, and increased cost of animal replacement as well as lowered sale value of infected cows (Bothwell, 1962).

Brucellosis is of particular importance among the zoonosis not only because of the heavy economic losses and reduction in animal protein supplies to which it is responsible, 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 remain a matter of concern in many parts of the world (Ajogi *et al*., 1998). Annual loss through bovine brucellosis in Nigeria is estimated to be about 224 million US dollars (Esuruoso, 1979). 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 who meets periodically to discuss and review current problems in human and animal population (Anon, 1971)

2.17 THE SITUATION OF BOVINE BRUCELLOSIS IN NIGERIA

The earliest reference to the occurrence of bovine brucellosis in Nigeria is contained in the Annual Report of the Veterinary Department for the year 1927 when ten cases of contagious abortion were reported. Earshaw and O'Brien (1929) reported cases knee joint hygroma in indigenous cattle. Hall (1934) diagnosed contagious bovine abortion at a government cattle ranch, where subsequently sero-agglutination test revealed a positive reactor rate of about 15% and at the same time field reports showed a low incidence of the disease in nomadic cattle.

There was no reference to animal brucellosis in the Veterinary Department Reports from 1934 to 1944, but from 1945 onward, brucellosis had been more or less regularly reported from Government Livestock Centre in the Northern Nigeria, where the disease had become enzootic, on account of domestication and overcrowding. Incidence of the disease in the nomadic and semi nomadic herds which comprises of over 90% of the cattle population mostly in the hands of the Fulani herdsmen were not determined (Banerjee and Bhatty, 1970). Despite the adoption of 'Test and Slaughter'' policy in the late 1940's, the disease in most government herds were said to be on the increase as the Annual Report of the Veterinary Department of Nigeria for the years, 1946, 1947, 1948 indicated reactor rates of 9.2% (2,272 cattle), 30% (983 cattle) and 11.8% (1,735 cattle) respectively (Mettam, 1946, 1947, 1948).

The control by *B. abortus* strain 19 vaccination recommended in 1946 was enforced with the production of the lyophilized *B. abortus* S.19 vaccine (Birkett, 1950), and this combined with culling of reactors proved effective in the disease control as the situation in the livestock farm had shown steady improvement. Although, isolated cases of Brucellosis were still being reported, the overall incidence had fallen to less than 5% (Banerjee and Bhatty, 1970).

Bovine brucellosis had long been recognized in cattle of nomadic and semi-nomadic Fulani herds as indicated in the Annual Report of the Veterinary Department of Nigeria for the year 1934 (Anon, 1934). Wilson (1955) reported an abortion rate of 20–24% in the local herds in Adamawa Province but the exact etiology of the abortion storms remained obscure. However, subsequent serological test carried out in the affected herds' revealed significant number of positive reactors' in Bauchi, Mambilla Plateau andMaiduguri (MacGregor, 1960, 1963, 1964).

For the past three decades, observations on nomadic herds of cattle in Northern state of Nigeria, and government owned settled cattle herds in different past of the country revealed widespread of bovine brucellosis in Nigeria, with a variable infection rates (Esuruoso, 1965; Banejee and Bhatty, 1970; Esuruoso and Hill, 1971; Esuruoso and Van Blake, 1972; Esuruoso, 1974a, Nuru and Dennis, 1975; Chukwu, 1987b; Ajogi, 1997; Junaidu *et al.,* 2006, 2008). Esuruoso(1979) investigated as high as 60% infection rate among breeding cows and heifers in western states of Nigeria. Also epidemiological investigations reveals prevalence rate of 50% and abortion rate of 42% of bovine brucellosis in one of the farm estate in the western Nigeria (Esuruoso, 1974b).

In Eastern Nigeria, Chukwu (1987a) reported a prevalence of 8.15% for bovine brucellosis among slaughter cattle in Enugu and Nsukka. In the Northern States of Nigeria, the infection rates in various herds have been generally low; 0 – 20% of animals examined showed serological evidence of infection. Extensive serological survey by Banerjee and Bhatty (1970) and Nuru and Dennis (1975) revealed prevalence of 8.06% and 7.1% respectively in cattle herds. A prevalence of 0.4% was recorded by Nuru (1975) using slaughter cattle in North and Central State. Among the reasons that were given for the difference in the infection rates between cattle in the two areas of the country were: (1) that the cattle in nomadic herds in the north were constantly moving and are therefore not likely to accumulate infection or spread it to each other as effectively as settled herd in the west and (2) that nomadic in the intense Savannah heat of the North probably impose a natural limit on the rate of *B. abortus* infection in cattle (Alausa, 1983).

The status of bovine brucellosis in Nigeria including the epidemiology (Alausa, 1983; Ocholi, 1990; Ajogi, 1997), prevalence, significance and areas of research needs (Nuru and Schnurrenberger, 1975; Eze, 1987) have been reviewed to bring the disease into proper perspective. Proposals for a practical approach to the intensive and extensive husbandry system (Rikin, 1988a) and the benefit-cost analysis for a brucellosis control programme in Nigeria (Rikin, 1988b) have been presented. However, there is no established control programme in the country. Although efforts to determine the prevalence rate of bovine brucellosis in various parts of the country have been attempted, accurate figures are not available (Rikin, 1988a). However, more works were done by (Junaidu *et al*., 2006; Ate, *et al*., 2007; Junaidu *et al*.,2008).

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY AREA

This research was conducted in Taraba State which occupies an estimated land area of about 54,428 sq.km. The state lies between latitudes $6^{o}25'N$ and $9^{o}30'N$ and between longitudes $9^{o}30'E$ and $11^{o}45'E$. Taraba State is located in the North-Eastern corner of Nigeria. It is bordered on the west by Gombe and Plateau States, and by Adamawa to the Northeast. It also shares its southwestern boundary with Benue State. An international boundary on the east separates Taraba State from the Republic of Cameroun (GSN, 1994). Based on the 2006 National Population census, Taraba state has a human population of 2,300,736 (NPC, 2006) and cattle population of about 4,805,820 (MOA, 2008). Taraba state consists of undulating landscape dotted with a few mountainous features. The state lies largely within the tropical zone and has a vegetation of low forest in the southern part and grassland in the northern part. The Mambilla Plateau with an altitude of 1,800 meters (6000ft) above sea level has a temperate climate all year round (GSN, 1994) (Fig 3.1) .



Scale: 1.38 inch = 1Km

Fig. 3.1 Map of Taraba State Showing the Study L.G.As

L.G.As ⟶

Roads ——————

Rivers ∿∿∿

3.2 SAMPLE SIZE

Sample size was calculated using Z^2pq/L^2 (Mahajan, 1997)where

Z = 1.96,

P= prevalence from previous studies, q =Complimentary probability, 1 - p, L = level of significance (5%) as described by Thrusfield (2002). The sample size of 540 using prevalence rate of 10.8% (Shehu, *et al*., 1999) was used.

$$\text{Sample size} = \frac{(1.96)^2 \times 0.108 \times (1 - 0.108)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.108 \times 0.892}{0.0025}$$

$$= \frac{0.3701}{0.0025}$$

$$= 148$$

The sample size was increase to 540 in order to reduced sampling error.

3.3 SAMPLING TEHNIQUES.

Sampling was done by cluster sampling in which the herd identified is considered as a cluster. Identified herd in this study means a settled herd with 20 or more cattle.

Three Local Government Areas each from the 3 Senatorial Districts of the state were randomly selected by balloting without replacement. In each Local Government Area, 10 herds (clusters) were also randomly selected.

Each of the cluster was stratified into male and female cattle of breeding age and, systematic random sampling of 6 cattle was implored with ratio of 1 male: 3 females. This is because cows outnumbered service bulls in herds by ratio of 3:1.

3.4 SAMPLE COLLECTION

Five milliliters of blood samples were collected from the jugular vein with a 10ml syringe (18G Needle) from well restrained identified settled cattle herds. The blood samples were transferred into properly labeled sterile screw-capped universal bottle and kept on slanting position for one hour to clot. The serum were decanted into a sterile plain sample bottles containing well labeled sample number, herd location, age and sex. It was maintained in cool flask to laboratory and Centrifuge at 112g for 10 minutes and stored in Bijou bottles at -20^{0}C for serological tests. Thus, the serum is subjected to Rose Bengal Plate Test (RBPT) and i-ELISA

3.5 LABORATORY ANALYSIS FOR THE FIELD SAMPLES

3.5.1 Source of the Antigens

All Antigens and the Negative, Positive reference (control) for all tests were obtained from Veterinary Laboratory Agency (VLA) Weybridge, United Kingdom.

3.5.2 Rose Bengal Plate Test

The sera collected were screened by Rose Bengal Plate Test (RBPT). This was carried out using Standard Rose Bengal Plate Test antigen obtained from Central Veterinary Laboratory, Weybridge U.K, as described by Morgan (1967) and Alton *et al*. (1975) as follows:

a) One drop (0.03 ml) of the serum was placed on a test area of a clean glass plate.

b) One drop (30µl) of the Rose Bengal *Brucella abortus stained* antigen was then placed adjacent to the test serum.

c) Using tooth pick, the serum and the antigen were mixed gently.

d) The glass plate was hand rocked slowly for 4 minutes (rotation period).

e) The result after 4 minutes rotation period was interpreted as follows:

Positive (+) =any degree of distinct pink agglutination.

Negative (-) = No agglutination,

Doubtful (±) =Doubtful to be repeated.

3.5.3 Indirect Enzyme Linked Immunosorbent Assay

The assay used was indirect Enzyme Linked Immunosorbent Assay (I-ELISA) for the bovine brucellosis diagnosis.

The I-ELISA procedure was done according to manufacturer's manual (2009).

Coated Plate: A 96 well plate coated with *B. abortus* antigen prepared from smooth lipopolysaccharide for indirect test.

Diluting buffer: The diluting buffer was prepared by adding 5 tablets of phosphate buffer saline (PBS), 0.5 ml of phenol red indicator, 25µl of tween 20 into 500ml of distilled water. The pH was kept between 7.2 and 7.6; phenol red will turn yellow below pH of 7.2 and violet above pH of 7.6. The preparation was stored at 4^0C.

Washing solution: The washing solution was prepared by adding Na_2HP^{04} and 1ml of tween 20 into 10 liters of distilled water and stored at room temperature.

Substrate buffer: This was prepared by dissolving 1 tablet of the substrate buffer into 120ml of distilled water. Test pH is between 3.9 and 4.4

Chromogen: This was prepared by dissolving 2 ABTS tablets in 1ml of sterile distilled water.

Stopping solution: This was prepared by diluting the contents of the ampoule of sodium azide with 500ml of distilled water.

Conjugate: this was reconstituted by adding the contents of the ampoule kit (HRPO monoclonal antibody) into 11ml of diluting buffer.

Controls: The positive and negative control sera in the kit were reconstituted with 1ml of distilled water each. They were then allowed to stand until an even suspension was obtained and then stored at 4^0C.

I-ELISA Procedure

The microtitre plate has 96 wells coated with *B. abortus* antigen; twelve wells arranged horizontally (1-12 wells) while vertically, eight wells were arranged (A-H).

The plate was prepared by adding 100 µl of the diluting buffer to each well, followed by 20µl of negative control sera into wells A11, A12, B11, B12, C11 and C12 while 20µl of positive control sera were placed into wells F11, F12, G11, G12, H11 and H12. While, D11, D12, E11, and E12 served as conjugate controls. Twenty microliter (20µl) of each of the test sera were then added to the remaining wells giving a serum dilution of 1/6. One hundred microliter (100µl)of the prepared conjugate was immediately dispensed into each well. The microtiter plate was shaken with hand for 2 minutes, in order

to mix the serum and conjugate solution. The microtitre plate was then covered with lid and left at room temperature for 30 minutes. The microtitre plate was again shaken for 30 seconds, followed by 10 seconds hand shaking at 10 minutes interval for an hour. This will be done carefully to keep the liquid within their respective wells. The contents of the plate was then discarded and rinsed 5 times with washing solution and then dried by tapping on an absorbent tissue paper. The substrate chromagen solution was immediately prepared and mixed thoroughly; 100µl of the solution was then added to each well. The plate was left at room temperature for 10 minutes; the reaction can be slowed by adding 100µl of the stopping solution to each well (Manufacturers guidelines).

INTERPRETATION

Negative (-) = Very weak or no colour development in wells indicates absence of antibodies in the serum.

Positive (+) = A strong colour development (green) in wells indicates presence of Brucella antibodies.

3.5.4 Milk Ring Test (MRT)

Fresh milk were collected at 7.00 am in the morning from different herds and tested for *Brucella* antibodies using milk ring antigen, 24 hours after collection. The test was carried out as described by Hamilton and Hardy (1950) and the results interpreted (Cruickshank *et al*., 1982).

All sampled lactating cows from the herds surveyed were restrained and hand milk by the help of the herdsmen into a cleaned test-tube. The milk was then transported in ice park box to the laboratory for analysis.

MILK RING TEST PROCEDURE

1. 1 ml of milk was pipette into narrow test tubes
2. One drop (0.03 ml) of milk ring antigen (Hematoxylin stain) was added.
3. It was then mixed gently but properly by hand rotation.
4. The sample was then incubated at 37^0C for one hour.
5. The colour of cream ring and colour of milk column was read and interpreted as indicated in the Table 3.1

Table 3.1 interpretation of Milk Ring Test based on Colour Changes

Colour of Cream Ring	Colour of Milk Column	MRT Interpretation
Definitely blue	White	+3
Definitely blue	Slightly blue	+2
Definitely blue	Definitely blue	+
Slightly bluer than same or same colour as the milk column	Definitely blue	±
Incomplete blue ring	Definitely blue	Trace
White	Definitely blue	_

3.6 STRUCTURED QUESTIONNAIRE

A structured questionnaire was design and administered to all the 90 herds visited and the 9 butchers (Sarkin Pawa) making a total of 99 questionnaires which was used to assess their knowledge of brucellosis. Information on herd, such as herd composition,

retained placenta, stillbirth, milk pasteurization and symptoms in cattle and man were obtained (Appendix I).

3.7 STATISTICAL ANALYSIS OF DATA

All the data obtained from the various investigations were statistically analyzed using SPSS Version 13 for Chi-square test and Win EPISCOPE Version 2 for Odds ratio and Confidence interval of odds ratio, to observe the association of age and sex specific reactors. Herd prevalence, Individual and L.G.A. animals' prevalence rates were calculated as indicated bellow.

$$\text{Herd prevalence} = \frac{\text{Number of herds with atleast one positive reactor}}{\text{Number of herds sampled.}}$$

$$\text{Local gov"t prevalence} = \frac{\text{Total number of herd with positive reactors}}{\text{Total number of L.G.A sampled herds}}$$

$$\text{Individual prevalence} = \frac{\text{No of individual positive reactors}}{\text{No of serum samples tested}}$$

The rate of confirmation of positive RBPT by I-ELISA was calculated by (number of I-ELISA reactors/number of positive RBPT samples).

Using result of MRT, herd prevalence rates for *Brucella* contaminated milk were determinedby.

$$\text{HPR} = \frac{\text{Number positive to MRT}}{\text{Total number of herds}}$$

$$\text{IPR} = \frac{\text{Individual positive reactors}}{\text{Number of milk samples tested}}$$

CHAPTER 4

RESULT

4.1 STUDY POPULATION

The Rose Bengal Plate Test (RBPT) was used as screening test and Indirect Enzyme Linked Immunosorbent. Nine out of 16 L.G.A, were studied for the evidence of brucellosis. A total of 90 herds from 9 local government areas Gashaka, Sardauna, Bali, Jalingo, Karimlamido, Zing, Donga, Takum and Ibi were used in the screening survey from 90 herds comprising of 2,475 cattle. Where 540 total samples were obtained consisting of 162 milk and sera from lactating cows, 81 dry cows,73 heifers, 119 bulls and 105 calves (Table 4.1) .

Table 4.1 Structure of the population of the cattle herds sampled in Taraba State

LGA/Towns	No.of herds	No. of animals in each herd	Milk samples	Categories	Of animal	Sampled	Total
				Cows and heifers	Bulls	Calves	
Gashaka	**10**	**Gashaka**	**23**	**39**	**13**	**8**	**60**
Serti	5	180	15	20	6	4	30
Goje	2	32	3	9	3	-	12
Karamti	3	76	5	10	4	4	18
Bali	**10**	**Bali**	**29**	**43**	**9**	**8**	**60**
Bali	4	152	16	17	3	5	25
Gazabu 1	3	80	6	14	3	2	19
Gazabu 2	3	54	7	12	3	1	16
Sardauna	**10**	**Sardauna**	**20**	**30**	**19**	**11**	**60**
Gembu	5	143	13	14	9	4	27
Nguroje	3	83	4	9	7	4	20
M/ndaga	2	58	3	7	3	3	13
Jalingo	**10**	**Jalingo**	**23**	**44**	**9**	**7**	**60**
Jalingo	7	195	17	28	6	7	41
L.I.B.C.	1	33	2	5	1	-	6
Sabon-gari	2	43	4	11	2	-	13
K/lamido	**10**	**K/lamido**	**18**	**35**	**11**	**14**	**60**
Karim	7	201	13	24	8	9	41
Bandawa	3	70	5	11	3	5	19
Zing	**10**	**Zing**	**11**	**32**	**12**	**16**	**60**
Zing	8	220	8	24	9	11	44
Pantisawa	2	59	3	8	3	5	16
Donga	**10**	**Donga**	**11**	**26**	**19**	**15**	**60**
Donga	7	184	7	18	12	11	41
Mararaba	3	68	4	8	7	4	19
Takum	**10**	**Takum**	**13**	**32**	**13**	**15**	**60**
Takum	8	229	10	25	10	12	47
Kashinbila	2	42	3	7	3	3	13
Ibi	**10**	**Ibi**	**14**	**35**	**14**	**11**	**60**
Ibi	8	237	11	**27**	**10**	**9**	46
Tunga	2	43	3	**8**	**4**	**2**	14
Total	**90**	**2475**	**162**	**316**	**119**	**105**	**540**

4.2 PREVALENCE OF BOVINE BRUCELLOSIS BY RBPT AND CONFIRMATION BY I-ELISA IN THE STUDY AREAS

All blood samples (sera) collected were screened using Rose Bengal Plate Test. Out of 540 sera tested, 80 (14.80%) reacted positive and were subjected to Indirect Enzyme Linked Immunoabsorbent Assay Test for confirmation. Sixty nine of 540 (12.80%) of the positive sera in RBPT were confirmed by I-ELISA (Table 4.1). Percentage prevalence rate in each LGAs by i-ELISA are shown (Figure 4.2).

The infection rate of brucellosis varied among the sampled LGAs. Brucella antibodies in cattle was detected in all the Local Government Areas of the State involved in the survey but Takum has the highest prevalence of 20.0% followed by Sardauna (18.3%), Gashaka (16.7%), Ibi (15.0%), Jalingo (13.3%), Bali and Donga (10.0%) each, K/lamido (6.7%) and zing (5.0%).

Highest individual animal prevalence (IAP) was obtained in kashinbilla (4.8%) followed by Tunga and Sabongari (4.7%) respectively, Bali (4.0%). While highest herd prevalence rate was obtained in Serti (120%), followed by Karamti,Nguroje, Mayondaga and Sabongari (100%) each and others ranges from 80-33.3% (Appendix II)

Table 4.2 Prevalence of brucellosis by RBPT and I-ELISA in the study areas

L.G.A	No of cattle in the area	Cattle Sampled	RBPT Positive (%)	IELISA Positive (%)
Gashaka	288	60	10 (16.7)	10 (16.7)
Bali	286	60	7 (11.7)	6 (10.0)
Sardauna	284	60	12 (20.0)	11 (18.3)
Jalingo	264	60	9 (15.0)	8 (13.3)
k/Lamido	271	60	7 (11.7)	4 (6.7)
Zing	279	60	5 (8.3)	3 (5.0)
Donga	252	60	6 (10.0)	6 (10.0)
Takum	271	60	13 (21.7)	12 (20.0)
Ibi	280	60	11 (18.3)	9 (15.0)
Total	**2475**	**540**	**80 (14.8)**	**69 (12.8)**

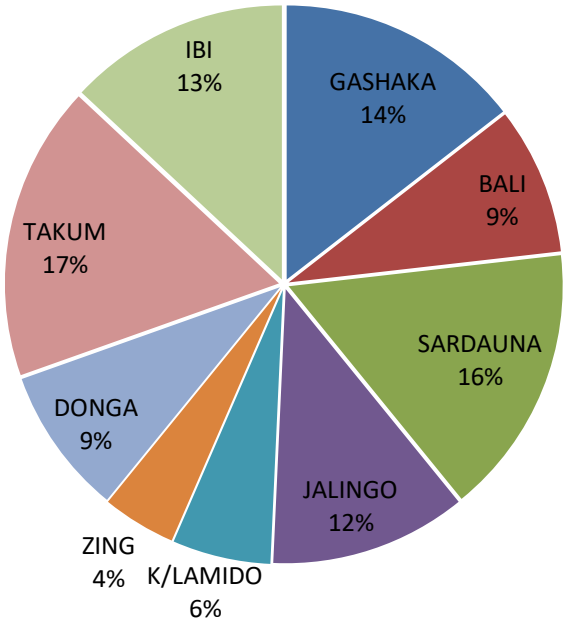


Fig 4.1 IELISA readings of *Brucella* antibodies in the nine Local government of Taraba state

4.3 CONFIRMATION RATE IN I-ELISA FOR RBPT POSITIVE SAMPLES IN EACH LGA

Gashaka and Donga have 10 samples positive for RBPT each and were confirmed 100% positive by IELISA. Takum 12/13 (92.3%), Sardauna 11/12 (91.7%), Ibi 9/10 (90%), Jalingo 8/9 (88.9%), K/Lamido (4/6) 66.7%, and Zing (3/5) 60%. All giving total confirmatory rate of (69/80) 86% (Table 4.3)

Table 4.3 Confirmation rate in IELISA for RBPT positive samples in each LGA

L.G.A	Total No. of sampled animals	RBPT Positive (%)	IELISA Positive (%)	Confirmation rate (%)
Gashaka	60	10 (16.7)	10 (16.7)	100
Bali	60	7 (11.7)	6 (10.0)	85.7
Sardauna	60	12 (20.0)	11 (18.3)	91.7
Jalingo	60	9 (15.0)	8 (13.3)	88.9
L/lamido	60	6 (10.0)	4 (6.7)	66.7
Zing	60	5 (8.3)	3 (5.0)	60
Donga	60	6 (10.0)	6 (10.0)	100
Takum	60	13 (21.7)	12(20.0)	92.3
Ibi	60	10 (16.7)	9 (15.05)	90
Total	**540**	**80 (14.8)**	**69 (12.8)**	**86.3**

4.4 SEX-BASED PREVALENCE RATE OF BOVINE BRUCELLOSIS IN THE STUDY AREAS

The sex-based prevalence rate of the population sampled showed 27 (16.5%) out of 164 male cattle were positive to brucellosis and 42 (11.2%) out of 376 females were also positive. The infection rate was higher in males than in females. The result obtained were statistically significant ($X^2 = 4.51$, df = 1, $P < 0.05$). Comparing the prevalence rates in both sexes, male and female, an odds ratio of 1.56 was obtained and a Confidence interval of odds ratio, CI = 0.93 – 2.64, were also obtained, $p < 0.05$ (Table 4.4)

Table 4.4 Sex-based prevalence rate of bovine brucellosis in the study areas

Sample type	Sex	Total No. of animals	No samples tested	ELISA Positive (%)	ELISA Negative (%)	X^2 P<0.05	Odds Ratio
Serum	Male	515	164	27(16.5)	137(83.5)		1.56(0.93-2.64)
	Female	1960	376	42(11.2)	334(88.8)	4.51	
Total		**2475**	**540**	**69(12.8)**	**471(87.2)**		

4.5 AGE-BASED PREVALENCE RATE

There were differences in prevalence rates among the different age groups of cattle screened. Cattle older than 3 years record a prevalence of 24.9% (43/540) while a prevalence of 0.08% (22/540) was obtained among cattle between 1-3 years and those of 0.3 year to one year also had a prevalence of 0.04% (4/540) (Table 4.5)

An odds ratio for age groups 0.3 year to 3 years gave a value of 0.23 and confidence interval of (CI:0.14- 0.39). In the number of reactors by age, the infection rate was higher among cattle over 3 years of age than those between 0.3 year and 3 years, (X^2 =33.32, df = 1, $p < 0.05$) as presented in Table 4.6

Table 4.5 Age-based prevalence rate using IELISA

Age	Number positive	Number Negative	Total	I-Elisa Positive (%)
One year or less	4	101	105	0.04
1-3 years	22	240	262	0.08
3years and above	43	130	173	24.9
Total	**69**	**471**	**540**	**12.80**

Table 4.6 Age- based prevalence rate (0.3 year – 3 years and > 3 years) using IELISA

Age Class	**ELISA Positive (%)**	**ELISA Negative (%)**	**Total**	**X^2 (P<0.05)**	**Odds Ratio**
0.3year-3yrs	26(7.1)	341(92.9)	367		0.23(0.14-0.39)
>3yrs	43(24.9)	130(75.1)	173	33.32	
Total	**69(12.80)**	**471(87.20)**	**540**		

4.7 PREVALENCE OF *BRUCELLA* ANTIBODIES IN FRESH MILK USING MILK RING TEST (MRT).

The herds' prevalence of Brucella agglutinins was detected in the milk from the study areas and the herd's prevalence rate differs between Local Government Areas as follows; 3/10(30%) prevalence rate at Gashaka, while Sardauna, Jalingo, Takum and Ibi had 20% (2/10) each, Bali, K/lamido and Donga had 10% (1/10).

The highest individual animal prevalence rate was obtained at Takum 2/13(15.4%), followed by Ibi 2/14(14.3%), Gashaka 3/23(13.0%) and K/Lamido 1/11(11.1%); while others ranged between 3.4 to 9.1%. The overall herd prevalence for the milk tested was 16.7% (15/90) while the overall individual animal prevalence rate was 9.9% (16/162) as shown in Table 4.7

Table 4.7 Prevalence of *Brucella* antibodies in fresh milk per L.G.A In Taraba State

L.G.A	No. of herds sampled	Population of animals sampled jn each area	Milk collected from sampled animals	Herds positive with MRT	Herd prevalence (%)	Individual +ve reactors	Individual animal prevalence (%)
Gashaka	10	60	23	3	30.0	3	13.0
Bali	10	60	29	1	10.0	1	3.4
Sardauna	10	60	20	2	20.0	2	10.0
Jalingo	10	60	23	2	20.0	2	8.7
k/lamido	10	60	18	1	10.0	2	11.1
Zing	10	60	11	1	10.0	1	9.1
Donga	10	60	11	1	10.0	1	9.1
Takum	10	60	13	2	20.0	2	15.4
Ibi	10	60	14	2	20.0	2	14.3
	90	**540**	**162**	**15**	**16.7**	**16**	**9.9**

4.8 QUESTIONNAIRE ANALYSIS

A total of 99 structured questionnaires were administered to 90 herdsmen and 9 butchers from the 9 Local Government Areas (Appendix III).

4.8.1 Awareness of Symptoms:

It was 22.2% of 99 herdsmen and butchers were aware of the disease with Gashaka and Jalingo having 50% each respectively, followed by Sardauna and K/lamido (30%) each, Bali (20%), Ib I (10%) and Buchers (33.3%).

4.8.2 Vaccination of Cattle with *Brucella* Vaccine:

None of the herdsmen and butchers (100%) in all the 9 local government areas do vaccinate their cattle against brucellosis.

4.8.3 Methods of Milking:

The survey indicated 100% hand milking by the herdsmen in all the 9 local government areas.

4.8.4 Type of Milk Consumed:

Ninety one percent of the respondents (82 herdsmen and 9 butchers) consumed boiled milk while 9% consumed raw milk. The highest rate of raw milk consumption was observe in Takum 6/10 (60%) followed by Zing 2/10 (20%) and Sardauna 1/10 (10%).

4.8.5 Retained Placenta:

In Ibi (30%) of the respondents observed cases of retained placenta. Gashaka and Sardauna (20%) respectively, followed by Zing and Donga (10%) each.

4.8.6 Profuse Night Sweating:

The survey revealed 32 (11.3%) of the population of 283 experiences profuse night sweating in humans.

CHAPTER 5

DISCUSSION

All serum samples screened for the prevalence of brucellosis were subjected to Rose- Bengal PlatAssay (iELISA) as confirmatory test, recommended as standard procedures by OIE (2001). In this study, 13.8% of the positive samples in RBPT were not positive in IELISA. This could be explained that the Rose Bengal test can detect antibodies of other microorganism in the serum other than Brucella, thereby producing false positive result (Nakavuma, 1994). In the case of IELISA, it is a highly specific test than the RBPT (Nielsen *et al*., 1992).

The overall prevalence of *Brucella* antibodies in cattle herds in Taraba State was considered to be 12.8%. This is percentage of samples that were positive of both tests (RBPT and IELISA) used in this study. This figure is higher than the prevalence of 10.8% for the Bauchi State obtained by Shehu *et al*. (1999) which is in the same geographical zone with Taraba State. The result of this present study is higher than the result obtained in previous serological survey for brucellosis in Four Northern States of Nigeria by Ajogi (1997) who reported 3.1%. This could be due to porosity of Nigeria-Cameroon border whereby the cattle from Cameroon Republic crossing into Taraba State for dry season grazing. Also lack of instituting control measures by the government makes the disease to be on the increase among cattle in the State.

There were differences in prevalence rates between individual animals and herd basis observed in this study. The highest individual animal prevalence (IAP) was obtained at Takum, Ibi, Sardauna and Gashaka respectively. Takum and Ibi were located on the

southern part of the state; these are areas where pastoralists settle with their cattle because of good pasture and water. Varying cattle herds might be meeting at drinking point and possibly grazing on the same pasture which favours spread of the infection from one animal to another and herd to herd (Asmare *et al*., 2010). Secondly, Gashaka and Sardauna are situated in the central part of the state that has temperate climatic condition which favours the survival of *Brucella* organisms in the environment and that can increase the rate of infection (Joint FAO/WHO, 1986). In the other locations and towns in this study, an individual animal prevalence ranging between 1.4-4.0% was obtained. Karim Lamido and Zing (Taraba North), has prolong dry period which do not favour longer survival of *Brucella*, hence, the low infection observed in those areas.

Brucellosis is a herd disease problem rather than individual animal problem. In the case of overall herd prevalence rate, (HP), Serti was the highest followed by Karamti, Nguroje, M/ndaga, Sabongari each followed by Bali and the least was Karimlamido and Donga. An explanation to this could be the nature of husbandry system, which allows different herds with different status of infection drinking water from same watering points which favours spread of infection from herd to herd. Hence, clustering could result from common-source outbreaks or time-space clustering of factors that increase the risk of infection (Chomel *et al* 1994; Fosgate *et al.,* 2002). Equally, due to porous boundary with Cameroon Republic, the cattle cross the Nigerian-Cameroon boundary to mingle with cattle in Taraba State at grazing points during dry seasons which increases rate of infection. The cattle in Gashaka grazed in an enclaved area of the Gashaka- Gumti National Parks which intermingle with wild ungulate (Antelopes, deers) that are potential reservoir of *B. abortus* (Moore and Schnurrenberger, 1981).

It is evident from this survey that brucellosis occurs in both sexes (male and female) with significant difference ($p < 0.05$). The prevalence rate was higher in females with 7.8% than in males with 5.0%. Brucellosis is essentially a disease of the sexually mature animals, the predilection site being the reproductive tract, especially the gravid uterus. Allantoic factors including, erythritol, possibly steroid hormones and other substances stimulate the growth of most of the *Brucellae* (Radolf, 1994). The tropism of *Brucella* to the male or female reproductive tract was thought to be by erythritol, which stimulates the growth of the organism, but *Brucella* has also been found in the reproductive tract of animals with no detectable levels of erythritol (Anonymous, 2007). Erythritol, a sugar alcohol synthesized in the ungulate placenta and stimulates the growth of virulent strains of *Br. abortus*, has been credited with the preferential localization of this bacterium within the placenta of ruminants (Smith *et al*., 1962). Erythritol, a polyhydric acid found in higher concentration in the placenta and foetal fluids of females than in seminal vesicles and testis of males (Keppie *et al.*, 1965) could probably be responsible for the observed disparity in infection rates between the sexes. There are controversial reports regarding the prevalence of brucellosis in relation to sex of animals, as some of the research workers reported significantly higher prevalence in females than in males (Hussein *et al.*, 2005), whereas Muma *et al. (*2006) reports indicated that *Brucella* antibody titers are not associated with sex.

The chi square test show significant association between the ages while the 0.23 odds ratio indicated that the disease is less likely to occur in the age group of 4 months -3 years. It appears that the high prevalence of brucellosis among older cows more than 3 years as compared to younger cows might be related to maturity with the advancing age.

Thereby, the organism may have propagated to remain either as latent infection or it may cause clinical manifestation of the disease (Kazi *et al.,* 2005). The infection increases with age and most diseased animals carry the infection throughout their lives (Oloffs, 1996, 1998). Ahmed and Munir (1995) also reported low prevalence in young stock than in adult cattle. So, calves have a reduced risk of infection at early age due to maternal immunity, hence the low prevalence among them (Rhodostits *et al*., 1997).

The prevalence of *Brucella* agglutinins in fresh milk in this study showed that Gashaka has the highest herd prevalence rate followed by Sardauna, Jalingo, Takum and Bali, K/lamido, Zing and Donga respectively. All of the positive results from the milk were I-ELISA positive. Fifteen out of 162 (16.7%) were positive with I-ELISA (serum) and MRT (milk). The highest prevalence obtained from all the local government areas could be that, all the lactating cows were shedding the *Brucellae* at the time of sampling.

As questionnaire revealed in this study, 22.2% of the people questioned claimed to be aware of brucellosis with hygroma (bakalle) as the only sign known to them. However, hygroma affects productivity due to pains from the joints during mating(Arthur *et al*., 1982). Gashaka and Jalingo local government areas has 50% awareness respectively and was said to be through symposium and lectures organized by the veterinary divisional offices.

All the herdsmen (100%) do not vaccinate their cattle against bovine brucellosis even amongs the enlightened herdsmen; instead they culled the affected animals. This promotes the spread of the disease in cattle and humans in the state, as prevention of

human brucellosis is dependent on control of the disease in domestic livestock mainly by test and slaughter and mass vaccination (Nicoletti 2001).

The quantity of milk produced for commercial purpose is not large, traditionally; milking was done by bare hands usually by women and children. This method of milking is risky because the Brucella organism can enter through intact skin and abrasions (Mantur and Amarnath, 2008). The disease is spread from herd to herd through milking.

Herd owners around Gashaka, Bali, Jalingo, K/lamido, Donga, and Ibi, boil milk before consumption, while herd owners at Takum, Sardauna and Zing do souring which constitute a public health risks for consumers of milk in the areas, as souring inhibits Brucella growth only but does not kill the Brucella organisms.

It has been shown that 10% of the respondents observed retained placenta in their cattle. Retained placenta is an indicator of brucellosis. The most common clinical signs of cattle infected with *Brucella abortus* are high incidences of abortions, arthritic joints and retained after-birth. There are two main causes for spontaneous abortion with retained placenta in animals. The first is due to erythritol, which can promote infections in the fetus and placenta. Second is due to the lack of anti-Brucella activity in the amniotic fluid (Anonymous, 2009). Chahota *et al* (2003) have reported a severe outbreak of brucellosis in anorganized dairy farm leading to abortions, retained placentaand still birth in cows.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

A prevalence rate of 12.8% in cattle herds and 9.9% from milk was obtained in Taraba State in this survey. There was high agreement between the two tests with i-ELISA more specific and less sensitive than RBPT. The sex-based prevalence rate was higher in male than in female and also, higher in older cattle than in young cattle based on age ground. All the herd owners' interviewed in this study area are unaware of the zoonotic nature of brucellosis. However, they know brucellosis as (bakalle) meaning hygroma with no zoonotic implication. None of the herds' owner vaccinates cattle against bovine brucellosis and they milk with bare hands which predisposes them to brucellosis. Herd owners in Sardauna and Zing do souring instead of boiling their milk.

6.2 CONCLUSION

This study appears to be the first to document the prevalence of bovine brucellosis in Taraba State. Brucella antibodies were detected in milk samples from all the LGAs surveyed, this is of public health importance. There was poor awareness of the disease among the stakeholders which accounts for zero percent vaccination against Brucellosis in all the 90 herds study. More attention should be paid towards implementing a proper control program for bovine brucellosis and more efforts should be directed towards improving the animal health delivery system in the State by vaccination, isolation, cattle trade routes, test and slaughter and compensation etc, as its large in size and share borders with other states and Republic of Cameroon.

6.3 RECOMMENDATIONS:

i. A further serological survey on brucellosis in Taraba State should be carried out in areas that were not involved in this study; this will help in providing a more comprehensive prevalence rate.

ii. Serological survey involving risk groups such as veterinarians, cattle herdsmen, milk sellers and butchers etc, should be carried out, in order to asses the occurrence of the disease in humans.

iii. It is also recommended that isolation and characterization of the *Brucellae* should be done in order to produce potent vaccine for the National control policy.

iv. Mass sensitization/ awareness campaign among stakeholders should be embarked upon, and annual vaccination program against bovine brucellosis in the state.

v. A National Control Policy on this insidious, zoonotic disease of economic importance be instituted.

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Appendix I

Questionnaire on investigation of brucellosis in cattle in Taraba State

I. DEMOGRAPHY OF THE PASTORALIST

1. Location……

2. Family size……..

3. Sex [ ] male [ ] female

   Age: [ ] adult [ ] young

II. Herd structure

4. Herd size……..

5. Number of male cattle……

   Number of female cattle…….

6. Number of adult cattle……

7. Are there other animal species reared alongside cattle?

   [ ] Yes [ ] No

8. If yes give number

   [ ] Sheep [ ] Goats [ ] Dog

III. Herd Health

9. Do you cull your animals?

   [ ] Yes [ ] No

10. Why do you cull your animals?

[ ] Unproductivity [ ] Repeat breeders [ ] Infertility [ ] Abortion

11. How do you milk your animals?

[ ] Hand milking [ ] machine milking

12. Do you wash your hands and the udder before milking?

[ ] Yes [ ] No

If yes, with what? Soap [ ] Sand [ ] Ash [ ] Others [specify]

13. What type of milk do you consume? [ ] boiled milk [ ] consume raw milk

[ ] pasteurized milk

IV. Clinical Manifestation of Brucellosis in Cattle

14. Have any of the following signs occurred in your herd in the past one year?

[ ] Abortion	[ ] Carpal joint swelling (Hygroma)
[ ] Retained placenta	[ ] Orchitis
[ ] Infertility	[ ] Repeat breeding

15. How many pregnant animals have you observed in the herd in the past one year……..

16. Give an average number of abortion in the herd in the past one year ……..

V. Clinical Evidence of Brucellosis in Pastoralist

17. Have you or any member of the family experience any of the following signs?

[ ] Profuse night sweating [ ] weight Loss

[ ] Irregular fever of variable duration and of unknown origin.

[ ] Generalized body aching [ ] Headache

[ ] Lassitude (Body weakness)

18. Did any member of the family suffered from malaria in the last one year?

Yes[ ] No [ ]

If yes, was any treatment given? Yes [ ] No [ ]

If yes, was there any recovery? Yes [ ] No [ ]

19. How many members of the family had the experience?

[ ] Male [ ] Female

VI. Control of Brucellosis in Man and Animals

20. Do you have access to veterinary services?

[ ] Yes [ ] No

If yes, are they accessible? [ ] Yes [ ] No

21. Do you vaccinate your animals against diseases? [ ] Yes [ ] No

22. If yes, give type of vaccine or the disease vaccinated against?

[ ] Brucella vaccine [ ] Black quarter vaccine Others [Specify]

Appendix II L.G.A, herd and Individual animal prevalence based on I-ELISA of cattle herds in Taraba State

L.G.A	Towns	Herds sampled	Herds positive IELISA	Herd prevalence rate (%)	Herd size	Individual positive reactors	Individual animal prevalence (%)	Total No. sampled Cattle	Total positive IELISA	L.G.A. Prevalence rate (%)
Gashaka	Serti	5	5	100.0	180	6	3.3			
	Goje	2	1	50.0	32	1	3.1	60	10	16.7
	Karamti	3	3	100.0	76	3	4.0			
Bali	Bali	4	3	75.0	152	3	2.0			
	Gazabu I	3	2	66.7	80	2	2.5	60	6	10.0
	Gazabu II	2	1	50.0	54	1	1.9			
Sardauna	Gembu	5	4	80.0	143	6	4.2			
	Nguroje	3	3	100.0	83	3	3.6	60	11	18.3
	m/ ndaga	2	2	100.0	58	2	3.5			
Jalingo	Jalingo	7	4	57.1	195	6	3.1	60	8	13.3
	L.I.B.C.	1	0	0	33	0	0			
	Sabongari	2	2	100.0	43	2	4.7			
K/Lamido	Karim	7	3	42.9	201	3	1.5	60	4	6.7
	Bandawa	3	1	33.3	70	1	1.4			
Zing	Zing	8	3	37.5	220	3	1.4	60	3	5.0
	Pantisawa	2	0	0	59	0	0			
Donga	Donga	7	4	57.1	184	5	2.7	60	6	10.0
	Mararaba	3	1	33.3	68	1	1.5			
Takum	Takum	8	7	87.5	229	10	4.4	60	12	20.0
	Kashibila	2	1	50.0	42	2	4.8			
Ibi	Ibi	8	4	50.0	237	7	3.0	60	9	15.0
	Tunga	2	1	50.0	43	2	4.7			
TOTAL		**90**	**55**	**61.11**	**2475**	**69**	**2.8**	**540**	**69**	**12.8**

Appendix III BRUCELLOSIS AWARENESS AMONG HERDSMEN AND BUTCHERS (Questionnaire Analysis)

Location	No. of herds	No. of questionnaires administered	Awareness of symptoms in cattle (hygroma) (%)		Vaccination of cattle with Brucella vaccine (%)		Methods of milking (%)		Types of milk consumed (%)		Retained placenta (%)		Experiencing of Profuse night sweating (%)	
			Aware	Not aware	Yes	No	Hand	Machine	Boiled	Raw	Yes	No	Yes	No
Gashaka	10	10	50	50	0	100	100	0	100	0	20	80	3	36
Bali	10	10	20	80	0	100	100	0	100	0	0	100	2	21
Sardauna	10	10	30	70	0	100	100	0	90	10	20	80	6	24
Jalingo	10	10	50	50	0	100	100	0	100	0	0	100	0	22
k/lamido	10	10	30	70	0	100	100	0	100	0	0	100	0	28
Zing	10	10	0	100	0	100	100	0	80	20	10	90	0	26
Donga	10	10	0	100	0	100	100	0	100	0	10	90	4	28
Takum	10	10	0	100	0	100	100	0	40	60	0	100	7	30
Ibi	10	10	10	90	0	100	100	0	100	0	30	70	6	31
Butchers		9	33.3	66.6					100	0			4	5
Total	**90**	**99**	**22.2**	**77.8**	**0**	**100**	100	0	**91**	**9**	**10**	**90**	**11.3**	**88.7**