PREVALENCE OF BRUCELLA ANTIBODIES IN MIGRATORY FULANI CATTLE HERDS IN KADUNA STATE, NIGERIA

BY

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DECLARATION

I declare that the work in the thesis entitled " *The Prevalence of Brucella Antibodies in Migratory Fulani Cattle Herds in Kaduna State*" has been performed by me in the Department of Veterinary Public Health and Preventive Medicine, under the supervision of Professors I. Ajogi, O.O.J. Bale and J.U. Umoh.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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CERTIFICATION

This thesis entitled "*Prevalence of Brucella Antibodies in Migratory Fulani Cattle Herds in Kaduna State*" by Mbuk, Elsie Uduak meets the regulations governing the award of degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To my adorable children,

OTITO RUBY AND OSA-HAMBILI ALMA ONWUHAFUA.

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ABSTRACT

The prevalence of *Brucella* antibodies in migratory cattle in Kaduna State was determined using the Milk Ring Test (MRT), Rose Bengal Plate Test (RBPT) and Microtitre Serum Agglutination Test (MSAT). A total of 2,799 serum samples from 93 Fulani cattle herds in various Local Government Areas of the state were tested. The data obtained were analysed using the Chi-square to see the association of age and sex specific reactors. The level of statistical significance was assessed at $p< 0.05$.

Thirty-one (1.4%) of the 2,149 milk samples collected tested positive for *Brucella*. Out of 2,799 serum samples collected, 28 (1.0%) tested positive in MSAT and 198 (7.1%) in RBPT.

Twenty-four of 2,439 (0.86%) females and 4 out of 360 (0.14%) males tested positive to MSAT. There was significant difference ($p<0.0001$) in number of reactors between age groups and sex. The rate of infection was highest in females of age 2-3years who had 0.67% (19/2799) cases with up to 1:20 titre (40 iu) and above. There was association between sex and brucellosis (Odds ratio (OR) = 0.9). The 95% confidence interval (CI) was $-14724 \leq x \leq 14.33$.

Twenty-seven sera were positive in both MSAT and RBPT, of which one had titre of 1:80 (160 iu) in MSAT. Two sera were MSAT positive only, while, one hundred and

sixty-seven sera were positive for RBPT only. Thirty-one milk samples were positive in MRT.

Zango Kataf LGA had the highest individual animal prevalence (IAP) of 0.4% (11/2799) and the highest herd prevalence (HP) of 5.4% (5/93). There were differences in infection rate between LGAs, with the highest prevalence seen in Zango Kataf 11 (0.4%) followed by Birnin Gwari 4 (0.14%), Chikun 2 (0.07%), Kaura 2 (0.07%), Jaba 2 (0.07%), Jemma'a 2 (0.07%), Kaduna South 2 (0.07%), Kachia 1 (0.04%), Igabi 1 (0.04%), Lere 1 (0.04%); while samples collected from Kaduna North, Zaria, Giwa, Soba and Kudan LGAs were all negative to the test.

Questionnaires assessment of the 93 herds sampled showed 57 (61.3%) respondants had knowledge of brucellosis and its modes of transmission while 34 (36.6%) boil milk before consumption.

This report provides current information and confirms the involvement of *Brucella* in some of the cases of abortion in animals frequently reported from the field. The socio-economic, public health and control measures of brucellosis are indicated in this study. More researches should be carried out to isolate and characterize *Brucella* isolates obtained from hygroma fluid, aborted fetuses and other sources for possible vaccine production.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

1. National Agency for Food and Drug Control (NAFDAC)
2. Rose Bengal Plate Test (RBPT)
3. Microtitre Serum Agglutination Test (MSAT)
4. Milk Ring Test (MRT)
5. Local Government Area (LGA)
6. Degree Centigrade (^{o}C)
7. International Unit (iu)
8. Micron (µ)
9. Statistical Analysis System (SAS, 2004) Cary New York, USA. Version 9.0
10. Individual Animal Prevalence (IAP)
11. Herd Prevalence (HP)
12. Percentage (%)
13. Restriction fragment-length polymorphism (RFLP)

CHAPTER ONE

1.1 STATEMENT OF THE PROBLEM

Brucellosis is a contagious disease of animals that also affects humans. This highly contagious zoonotic disease is characterized by recurrent abortions and infertility in a variety of animal species and undulant fever in man (Halle and Ajogi, 1997).

Brucellosis is caused by brucellae which are intracellular, aerobic, Gram-negative coccobacilli that produce urease and catalyses nitrite to nitrate, most cocci measuring 0.5µm to small bacilli 1 by 0.5µm long (Eze, 1977a). They have a lipopolysaccharide coat that is much less pyrogenic than other Gram-negative organisms (Stevens, 1995), which accounts for the presence of high fever in brucellosis. Clinically, there is neutropenia, lymphocytosis and monocytosis (Hope, 1998).

Occupationally, *Brucella* can gain entry into humans (cattlemen, animal handlers, veterinarians, slaughterhouse workers) through breaks in the skin, or unbroken skin, mucous membranes, conjunctiva, respiratory and gastrointestinal tracts (Eze, 1977a; Bale, 1991).

Although brucellosis can be found worldwide, it is more common in countries that do not have good standardized and effective public health and domestic health programs.

In developed countries, data on the prevalence of brucellosis and the success or failure to control or eradicate the disease are now only of historical value since eradication programs employed in these countries (Denmark, Finland, Norway, Britain, USA, Sweden, Switzerland, Cuba, New Zealand and Israel) have successfully eliminated *Brucella abortus* infection from cattle, unlike in Nigeria where existing records indicate that brucellosis in Nigerian livestock has existed for a long time, and is still existing despite efforts by the government to improve the livestock industry (Bale and Kumi-Diaka, 1981; Ocholi *et al*., 2004).

Brucellosis is commonly transmitted to susceptible animals by direct contact with infected animals. Aborted fetuses, placentae, membranes or fluids, and other vaginal discharges after an infected animal has aborted or calved are all highly contaminated with infectious organisms (Morgan, 1970). Cows may lick those materials or the genital area of other cows or ingest those carrying organisms and contaminating food or water. The infection is transmitted from one herd to

another when an infected or exposed animal is bought by a herd owner as replacement cattle, also, when an affected herd mingles with brucellosis-free herds.

Milk from cow is the major raw material for many yoghurt and fresh milk producing companies in Kaduna State. Local customs encourage the consumption of unpasteurized dairy produce such as raw milk, yoghurt and cheese (mai-shanu) as part of the diet in most parts of Northern Nigeria. Under such conditions public health hazards due to brucellosis definitely exists (Ocholi *et al*., 2004).

Human infection is mostly by consumption of raw or unpasteurized milk, but there have been reports of infection through the consumption of cheese and meat products like sausages and bacon, (WHO, 1995; Taliani *et al* ., 2004) however, bacterial load is said to be low in meat products. It is however not uncommon for some Fulanis to sell infected animals for slaughter thereby increasing the burden of the disease in man (Eze, 1977a).

Recently, there have been complaints of abortion, stillbirths, birth of weak calves, hygroma, e.t.c. by the Fulanis to the National Commission for Normadic Education, Kaduna State. However, the Fulanis recognise abortion but do not

consider it infectious and abortion due to trypanosomosis infection has apparently been confused with brucellosis abortion (Eze, 1977a). It therefore became necessary that the disease in Kaduna State be further investigated.

According to Rodostits and Blood, (2000) man can also get infected through contact with aborted materials and through aerosol in the laboratory. Sexual transmission of the infection in man has not been convincingly documented (WHO, 1995). There is the possibility of misdiagnosing human infection due to *Brucella abortus* for typhoid in our hospitals (Baba et al., 1998).

1.2 IMPORTANCE OF THE PROBLEM

Reports from various areas of the country reveal that animal brucellosis is widely distributed in Nigeria (Esuruoso, 1974; Chukwu, 1987; Halle and Ajogi, 1997; Farouk, 2006; Bertu, 2008). Prevalence patterns of low, medium and high have been described in specific areas of the country and this also varies between herds in the same area (Nuru and Dennis, 1975; Ogundipe *et al*., 1994; Ocholi *et al*., 2004).

Earnshaw and O'Brien in 1928, reported the first contagious abortion outbreak in stockfarms in Nigeria and since then, there have been other reports of brucellosis as a zoonosis acquired through humans, cattle, camel, poultry, horses, sheep and goats by various investigators (Esuruoso, 1979a; Falade, 1981; Bale and Kumi-Diaka, 1981; Brisibe *et al.,* 1993; Ogundipe *et al*., 1994; Baba *et al*., 1998; Ocholi *et al*., 2004; Farouk, 2006; Bertu, 2008).

The disease has a relatively long and variable incubation period (1-8 weeks). Many infections are asymptomatic with abortion and infertility being the common manifestations (Rodostits and Blood, 2000). Brucellosis is a herd problem and causes lowered productivity with devastating effect on milk and animal protein industry (Nuru, 1975) with resultant economic losses (Ocholi *et al.*, 1993).

Because of predilection for joints and the chronic nature of the disease, it can result in relatively long term disability among animals and people that work with animals (butchers, veterinarians, e.t.c) leading to decrease in productivity (Nuru, 1975).

Prevention through vaccination is cheaper than chemotherapy, but when infection has occurred, patients (animal and human) respond favourably to appropriate antibiotic therapy like Doxycycline and Rifampin (Topley and Wilson, 1998). Test and slaughter is usually recommended if the Government will pay compensations to affected farmers (Eze, 1977a).

The economic loss from brucellosis in developed countries arises from the slaughter of infected cattle herds, payment of compensation to farmers, cost of replacement animals, etc., while in Nigeria, it is from the actual abortion of calves (Nuru, 1974) and resulting decreased milk yield (Esuruoso, 1979a), birth of weak calves that die soon

after birth, cost of treatment of retained placentae, impaired fertility and sometimes arthritis or bursitis. The actual financial loss is really difficult to estimate, as this depends on the type of cattle farming, herd size, and whether it is an intensive or extensive cattle farm (Alausa, 1979; Alausa, 1983). Esuruoso (1979b), estimated the annual loss due to bovine brucellosis in Nigeria as N140.8m. Furthermore, financial loss from human brucellosis can be substantial.

The prevalence rates evaluated from testing slaughtered animals were about 6% in Western Nigeria (Ogundipe *et al.,* 1994) and about 7% in Northern Nigeria (Ocholi, 1990). Reports, however, put prevalence rates as high as 20% - 60% in Southern Nigeria and 0-20% in Northern Nigeria (Chukwu, 1987; Ocholi *et al*., 1993; Farouk, 2006; Bertu, 2008).

There have been reports of high rates of infertility and abortion among nomadic and settled herds in Western Nigeria (Alausa, 1983; Ogundipe *et al*., 1994). Also infection

rate in cows was significantly higher than in bulls. A slightly higher infection rate was recorded for pregnant cows than for non-pregnant cows (Acha and Szyfres, 1980; Oyedipe *et al*., 1981; Nayamanda, 1994; Farouk, 2006).

1.3 JUSTIFICATION FOR THE STUDY

Brucellosis has been reported in various parts of Nigeria affecting our domestic animals and also humans. The disease is important as a major cause of economic losses in the livestock industry. It is also a zoonosis of great public health

significance (WHO, 1986a). Losses in livestock are in terms of abortion, infertility, low conception rate and low survival rate of neonates (Osori, 1976; Oyedipe *et al*., 1981; Halle and Ajogi, 1997).

Literature has shown that, it is about twenty years ago that work was done on migratory bovine brucellosis in Kaduna State (Ocholi, 1990). There is, therefore, the need to update information on the disease in the state in order to create awareness and develop necessary interventions.

1.4 OBJECTIVES OF THE STUDY

The objectives of this study are to:

1. Determine the prevalence rate of brucellosis among migratory Fulani cattle herds in 15 Local Government Areas of Kaduna State using the Rose Bengal Plate Test (RBPT), Microtitre Serum Agglutination Test (MSAT) and Milk Ring Test (MRT).

2. To determine sources of *Brucella* transmission to local livestock populations and humans.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HISTORY OF BRUCELLOSIS

The description of brucellosis dates back to the days of Hippocrates, although the organism was first isolated by British Army physician David Bruce in 1887 with the discovery of the bacterial species subsequently named *Brucella melitensis*. A type of febrile illness characterized by regular remissions or intermissions had been recognized in the Mediterranean region for centuries. Many names have been applied to it, often relating to the course and localities in which it was particularly prevalent; Malta fever, Crimean fever, Mediterranean fever, Gibraltar fever or Rock fever and Undulant fever are probably the best known (Biscop, 1994).

The cause of the disease was obscure until Bruce reported numerous small coccal organisms in stained sections of spleen from five fatally infected soldiers, he named the organism *Micrococcus melitensis*. Eventually the connection was made between *M. melitensis* and the organism (*Brucella abortus*) described by the Danish veterinarian Bang Bernard (1897) as the cause of contagious abortion in cattle. The genus *Brucella* was proposed in recognition of Bruce's discovery.

The disease caused by *Brucella abortus* is known as Brucellosis, Bang's disease, contagious abortion or infectious abortion. It affects many animal species on every continent and is a zoonosis of economic importance, as well as a public health hazard.

Brucellosis is primarily a reproductive disease characterized by abortion (Falade, 1974), retained placenta and impaired fertility in the principal animal host. *Brucella abortus* is to a certain extent distinguishable from other *brucellae* by biochemical reactions and by serological means. The serological differences are related to the amounts of A and M antigens that a *Brucella* strain possesses (Eze, 1977a).

An increasingly complex pattern of strains have subsequently emerged in which specific members of the genus *Brucella* have been linked to a particular primary animal host. The potential range of interactions with humans has increased with each new species, because each *Brucella* species has distinctive epidemiological features.

2.2 THE BIOLOGY OF *BRUCELLAE*

Taxonomic Tree

Kingdom - Proteobacteria [01 PROT]

Class - Rhodospirilli [21RHOD]

Order - Rhizobiales [31RHIZ]

Family - Brucellaceae [41BRUC]

Genus - *Brucella* [51BRUC]

According to Corbel *et al*., (1978), the organism responsible for brucellosis is a small, Gram-negative, coccobacillus that is non-motile, non-encapsulated and non-spore-forming. In infected tissue it normally occurs singly or in small groups. The colonies are coccobacilli and 1µm by 0.5µm in diameter. Smooth colonies appear translucent, honey-coloured and bluish-green or unstained with crystal violet. Rough colonies appear granular, reddish-yellow and deep violet-red when

stained with crystal violet. Mucoid colonies are stringy and appear transparent grayish and light bluish-red when stained with crystal violet.

Several commercial firms produce media suitable for the isolation of *Brucella*. On primary isolation some species require the addition of 5-10% carbon dioxide and take

3-5days incubation at 37^0C for visible colonies to appear. *Brucella abortus* produces

hydrogen sulphide (H_2S). It usually grows in the presence of basic fuchsin, but not always in the presence of thionin, it is catalase and oxidase positive. It is lysed by brucellaphages Weybridge (Wb), Firenze (Fz), Tbilisi (Tb) and Berkeley (Bk) (Cruickshank *et al*., 1975; Corbel *et al*., 1978).

Each *Brucella* has a specific oxidative metabolic pattern that can be used to determine the ability of an isolate to utilize oxygen on various amino acid and carbohydrate substrates (Ray, 1979).

The genus itself is highly homogenous with all members showing >90% homology in DNA-DNA pairing studies, thus classifying *Brucella* as a mono-specific genus (Verger and Grayon, 1985).

Restriction fragment-length polymorphism (RFLP) has been used to distinguish between species within the genus *Brucella* and has supported host preference as a basis for classifying the *brucellae.*

Polymerase Chain Reaction (PCR) of selected sequences followed by restriction analysis has provided further evidence of polymorphisms in a number of genes including the omp 2, dnak, htr and ery (Young, 1995).

The genus *Brucella* is divided into six main species based on animal source or features of infection; *B. abortus, B. melitensis, B. suis, B canis, B. ovis* and *B. neotomae*. Out of these six, four have moderate-to-significant degree of human pathogenicity. The old nomenclature (Table 2.1) has been retained with the former specie names being used as the nomen species. Within these, biovars 1,2,3, and 4 of *B. abortus* have been isolated in Nigerian cattle and horses (Eze, 1978; Bale and Kumi-Diaka, 1981; Oladosu *et al*., 1984; Ocholi *et al*., 2004).

Table 2.1: Recognised species and biotypes with host range and degree of human pathogenicity

Species	Biotype(s)	Host(s)	Degree of Pathogenicity in the host(s)
B. abortus	1, 2, 3, 4, 5, 6, 7, 8, 9	Cattle, Dogs, Horses Sheep and Man	Moderate
B. suis	1, 2, 3	Pigs, Cattle, Dogs Hares and Man	High

B. melitensis	1, 2, 3	Sheep, Goats, Cattle and Man	Highest
B. ovis		Sheep	Moderate
B. canis		Dogs and Man	
B. neotomae		Desert wood rat	

Sources : Alton *et al*., 1975; Corbel *et al*., 1978

Brucellosis is a zoonosis, and with few exceptions infections in humans result from direct or indirect contact with animal sources. Rare instances of person-to-person transmission have been recorded, either in circumstances implicating sexual contact or breast-feeding or by the transfer of tissue, including blood and bone marrow (Henry *et al*., 1998; Mantur *et al.,* 2004). Another more frequent

source of infection is laboratory-acquired brucellosis, caused by exposure to *B. melitensis, B. abortus, B. suis* and *B. canis* as a result of spillage or inhalation of aerosols generated during the manipulation of cultures (Rodostits and Blood, 2000).

Study has suggested that in the past, brucellosis was acquired in the laboratory more frequent than any other bacterial disease (Topley and Wilson, 1998). This last method of transmission is remarkably efficient given the relatively low concentration of organisms (as few as 10-100 bacteria) needed to establish infection in humans and has brought renewed attention to this old disease, which is now touted as a potential biological warfare agent (Franz *et al*., 1997; White and Eitzen, 2000).

Given the ease of aerosol transmission of *Brucella* species, researchers attempted to develop it into a biological weapon beginning in 1942. In 1954, it became the first organism weaponized by the old United States Offensive Biological Weapons Program.

Field testing on animals soon followed. By 1955, The United States was producing *Brucella suis*-filled cluster bombs for the United States Air Force at the Pine Bluff Arsenal in Arkansas (Hoover and Friedlander, 1997).

Most cases of infection caused by *B. abortus, B. melitensis, B. suis* and *B. canis* arise from occupational or domestic contact with infected animals or with an environment contaminated by their discharges. Farmers and their families, abattoir workers, butchers and veterinarians are particularly at risk. Infected animals that have recently aborted or calved present the greatest hazard. Infection may occur through ingestion or inhalation, or contamination of the conjunctiva or skin with discharges. The main source of infection of the general population is dairy products prepared from infected milk (Falade, 1974; Topley and Wilson, 1998).

2.3 HOST RANGE

Serological studies in Nigeria have demonstrated evidence of brucellosis in virtually all domesticated animals and human (Falade, 1974; Halle and Ajogi,

1997; Baba *et al*., 1998; Ocholi *et al*., 2004). The disease is endemic with prevalence rates varying from low in nomadic herds in Northern Nigeria (Ocholi,1990; Farouk, 2006) to moderate in some government and semi-settled herds to high in animals reared in ranches (Halle and Ajogi, 1997), watering and treatment centers, and in the wet (Nayamanda, 1994; Bertu, 2008), humid Southern parts of Nigeria (Ogundipe *et al*., 1994).

Brucellae have definite host preferences. Secondary hosts play small part if any in the maintenance or spread of a particular *Brucella* species. *Brucella abortus* mainly infects cattle and is the main cause of contagious abortion in cattle (Manthei and Carter, 1950; Dekeijzer, 1981; Crawford *et al*., 1990). However, sheep do not easily become infected with *B. abortus* (Collier and Molello, 1964; Allsup, 1974; Brisibe *et al*., 1993) but they may become carriers and excrete *brucellae* for up to 40 months once they have acquired the infection (Luchsinger and Anderson, 1967). The low prevalence of naturally acquired *B. abortus* infections reported in goats makes this animal species irrelevant as a host for *B. abortus* (Mathur, 1967; Bale, 1980). But in Northern Nigeria, the herding of cattle, sheep and goats together provides conditions which greatly increase the ease

with which infections are transmitted from one animal group to another (Ocholi *et al*., 1993).

Isolation of *B. abortus* from swine (Ray, 1979), horses (Robertson *et al*., 1973), and camels (Al-Khalaf and El-Khaladi, 1989) in areas with enzootic brucellosis clearly indicates that they may acquire infection with *B. abortus* but are not host of *B. abortus*

since they do not intermingle with cattle. Farm dogs with naturally acquired *B. abortus* infections play an important role in the epidemiology of cattle brucellosis (Chary, 1970; Prior, 1976; Forbes, 1990).

Feral animals like buffalo, swine, deer, fox, hare and rodents are susceptible to *brucellae* but are not host (Cook *et al*., 1966; Moore and Schnurrenberger, 1981; Schnurrenberger *et al*., 1985) and data compiled during brucellosis control and eradication campaigns in the United States of America suggest that the infection disappears from wildlife when it is eradicated from domestic animals (Ray, 1979).

Bale and Nuru (1982) first reported the presence of this disease in Nigerian fowls. The significance of fowl as a reservoir of *Brucella* is unclear (Anczykowski, 1972)

but, Adesiyun and Abdu (1984) found 2.0% of slaughtered Nigeria local chickens affected in Zaria.

However, experimentally induced and naturally occurring *Brucella* infection in flies, arthropods and other parasites suggest that they may be susceptible to the infection (Britov and Mel'nikova, 1979).

2.4 TRANSMISSION OF *BRUCELLA ABORTUS*

The oral route of transmission is very likely because cattle tend to lick aborted fetuses and the genital discharge of an aborting cow (Cunningham, 1977). Exposure to *Brucella* organisms is also likely to occur *in-utero* (Fensterbank, 1978, Bale and Nuru, 2001) or when calves born to healthy dams are fed on colostrums or milk from infected dams (Bercovich and Taaijke, 1990; Bale and Nuru, 2001).

It has been established that brucellosis in bulls does not always result in infertility, although semen quality may be affected. Bulls that remain fertile and functionally active will shed *Brucella* organisms with the semen during the acute phase of the disease (Bale and Nuru, 2001). Shedding, however, may cease or become

intermittent (McCaughey and Purcell, 1973). In contrast to artificial insemination, bulls used in natural service may fail to spread the infection, as the infected semen is not deposited in the uterus (Morgan, 1970; Ray, 1979).

While indirect exposure to *Brucella* organisms could be mediated by wildlife, birds and waterways (contaminated with urine, uterine discharge, or slurry from aborting cattle) it seems that only dogs carry pieces of placenta or aborted fetuses from one place to another (Forbes, 1990) causing direct exposure.

Contamination of a cowshed or pasture takes place when infected cattle abort or have full-term parturition. Although it is generally accepted that *B. abortus* is not excreted for any considerable time before abortion occurs, excretion in the vaginal discharge of infected cattle may occur as early as 39 days after exposure (Philippon *et al*., 1970). A massive excretion of *brucellae* starts after abortion and may continue for 15 days. Once the foetal membranes are expelled the uterine discharge diminishes and the number of *Brucella* organisms excreted decreases rapidly (Nicoletti, 1980). Although the infectious material from the genital tract usually clears after 2-3 months, some infected cattle become carries of *Brucella*

and excrete it intermittently for many years (Philippon *et al*., 1970; Herr *et al*., 1990).

2.5 SURVIVAL OF *BRUCELLA* IN THE ENVIRONMENT

Brucellae are sensitive to direct sunlight, disinfectant and pasteurization. In dry conditions they survive only if embedded in protein. *Brucellae* can survive in tap water for several months and several years in frozen tissues or medium (Davies and Casey, 1973).

Brucellae can survive up to 60 days in damp soil, and up to 144 days at 20^0C and 40% relative humidity (Wary, 1975).

Also, *brucellae* can survive 30 days in urine, 75 days in aborted fetuses and more than 200 days in uterine exudates. In bedding contaminated with infected faecal material *Brucella* will be destroyed at 56-61^0C within 4.5 hours (King, 1957). However, there are conflicting reports as to its survival in liquid manure. According to one study *B. abortus* can survive at least 8 months at 12^0C (Plommet,

1972) whereas another study indicates that *brucellae* could not be recovered from slurry after 3 months (Rankin and Taylor, 1969). Yet another study indicates that the survival of *Brucella* is subject to seasonal influences. It has been found that *Brucella* can survive in faeces, slurry or liquid manure 85-103 days in the winter, 120-210 days in spring, 30-180 days in summer, and 50-120 days in autumn (Kerimov, 1983).

Although *B. abortus* is relatively resistant in the environment and may survive for a considerable time, the environment is not considered to be an important source of infection (Wray, 1975).

2.6 RESISTANCE TO INFECTION

Age, sex, stage of pregnancy and natural resistance to *Brucella* may influence the progression of infection. Heifers born to infected dams usually test seronegative for *Brucella* for a long period (Bercovich *et al*., 1990). Because the stage of pregnancy at the time of infection determines the incubation period, abortions in cattle caused by *B. abortus* seldom occur before the fourth or fifth month of

pregnancy (Thomsen, 1950; Henry *et al.,* 1998). Pregnant females are more likely to become infected than non-pregnant cattle or males because, a gravid uterus sustains growth of the organism (Crawford *et al*., 1990). The course and incidence of the disease is also influenced by natural resistance to infection with *Brucella* (Hellmann *et al.,* 1984).

2.7 PATHOGENESIS AND PATHOLOGY

Brucellae are facultative intracellular bacteria that can survive within host cells causing a chronic infectious disease that may persist throughout the life of an animal. According to Enright (1990), it seems that the initiation of *Brucella* infection depends on exposure dose (10-100 CFU), virulence of the organism and natural resistance of the animal to *Brucella.*

Resistance to infection is based on the host's ability to prevent the establishment of a mucosal infection by the destruction of the invading organism. 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 defence by the bacteria, results in a local infection and the escape of *brucellae* from the lymph nodes into the blood. During the bacteraemic phase (may last 2-8 weeks) the bones, joints, eye and brain can be infected, but the bacteria are frequently isolated from supramammary lymph nodes, mammary lymph nodes, milk, iliac lymph nodes, spleen and uterus (Blood and Henderson, 1968).

The tropism of *Brucella* in the male or female reproductive tract was thought to be by erythritol (Ocholi, 1990; Halle and Ajogi, 1997), 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 (Manthei and Deyoe, 1970). In the acute stage of infection, abortion occurs at four or five months into pregnancy and cattle usually abort only once. Abortion and retention of the placenta, late abortions or calving of infected full-term calves is common in herds with endemic brucellosis. Excretion of *Brucella* after parturition may persist for months or years

and may re-occur after any consecutive normal parturition. Infected cattle excrete *brucellae* in the colostrums or milk (Manthei and Deyoe, 1970; Ray, 1979). In bulls the predilection sites for infection are the reproductive organs and the associated lymph nodes. During the acute phase of infection the semen contains large numbers of *Brucella* but as the infection becomes more chronic the number of *brucellae* excreted decreases and excretion may cease altogether or it may continue to be excreted for years or just intermittent. Usually, orchitis, epididymitis and infection of the accessory sex glands also occur (Jubb and Kennedy, 1963; Kocak *et al*., 2004).

Abortion and expulsion of the foetus was thought to be the results of a placentitis caused by *Brucella*. Proliferation of *Brucella* in the uterus induces necrosis and destruction of the foetal and maternal placental membranes resulting in death and

then expulsion of the foetus. The pathologic changes in the caruncles and cotyledons prevent normal separation and expulsion of the placenta (Jubb and Kennedy, 1963). Although placentitis impairs the normal function of the placenta, *Brucella* endotoxins may also play a role in inducing abortion (Anderson *et al*., 1986). *Brucella abortus* may induce production of high concentration of cortisol

that decrease progesterone production and increases oestrogen production. Decreases in progesterone level and increases in oestrogen levels induce a premature parturition (Enright *et al.,* 1984).

2.8 DIAGNOSIS

Diagnosis of human brucellosis in Nigerian hospitals is almost non-existent (Eze, 1977a; Nayamanda, 1994; Baba *et al*., 1998).

Several cases of human brucellosis had been reported in different parts of Nigeria (Collard, 1967; Falade, 1974; Nayamanda; 1994; Baba *et al*., 1998) and there is also the possibility of misdiagnosis of *B. abortus* infections among cases suspected of typhoid fever (Baba *et al*., 1998). Since according to Nayamanda (1994) the proliferation and dissemination of the organism during the septicaemic phase of brucellosis, resembles typhoid fever, other studies revealed that the variable symptoms, the paucity of physical signs and the occurrence of subclinical and atypical infections and numerous differential diagnosis make the clinical diagnosis of both brucellosis and typhoid difficult (Levine *et al.,* 1978; Morgan and

Corbel, 1990; Hook, 1990). Consequently, Morgan and Corbel (1990) postulated that cases of pyrexia of unknown origin (PUO) and any acute or chronic inflammatory disease presenting diagnosis difficult should be considered for brucellosis especially among patients whose occupation could predispose to *Brucella* infection. Therefore, it is possible that many cases tested or probably treated for typhoid among patient with PUO could have been *Brucella* infections.

In nomadic and semi-nomadic cattle, abortion due to trypanosomosis infection has apparently been confused with brucellosis abortion (Anon, 1963) but it is rather fortunate that the incidence of *Brucella* abortion in these groups is low (Eze, 1977a) since the control and eradication of brucellosis from these herds have never been considered. Although the reason might be because most of the available disease survey figures originate from government herds (Eze, 1977a) and abattoirs (Nayamanda, 1994; Ogundipe *et al*., 1994; Ajogi, 1997a) where screening tests can be easily carried out with very scanty data from nomadic and semi-nomadic herds.

2.8.1 Clinical Signs

Demonstration of characteristic clumps of *Brucella* organisms in stained smears of hygroma fluid, chorionic epithelium, or the use of fluorescent antibody techniques to examine foetal stomach content and uterine, or vaginal exudates may provide a tentative diagnosis (Corbel, 1973).

2.8.2 Bacteriological Examination

Bacteriological examination of lochia of aborting cattle is the method of choice for diagnosing early infections (Erasmus, 1986) but this procedure is laborious, time consuming, costly and cannot routinely be used as a diagnostic procedure in developing countries. The probability of successful recovery of *B. abortus* is greatly reduced when the sample material is heavily contaminated although negative culture results do not exclude infection (Bale and Kumi-Diaka, 1981; Ocholi, 1990).

a) **Gram Stain:** *Brucella* species are small Gram-negative rods that often appear coccobacillary.

b) **Modified Ziehl-Neelsen:** Some of the *Brucella* species are partially acid-fast in that they are not decolourised by 0.5% acetic acid in the modified Ziehl-Neelsen stain. In this stain the carbol fuchsin is retained and the *brucellae* appear as red-stained coccobacilli.

c) **Culture**: No growth occurs after incubation of Blood Agar or Serum Dextrose Agar (SDA) plates for 24 hours at 37^0C in 5-10% CO_2. After incubation of Blood Agar for 48 hours at 37^oC in 5-10% CO_2 colonies are 1mm, non-haemolytic and have a metallic sheen. After 48 hours incubation on SDA *Brucella* species are slightly larger than on blood agar and are cream coloured.

Some *Brucella* species will grow without increased CO_2 concentration whilst other species have requirement of 5-10% CO_2 for growth. *Brucella* species will not grow anaerobically.

In addition, the *brucellae* are non-motile, catalase-positive, oxidase-positive, hydrolyse urea, reduce nitrate and are indole negative. Using

known positive antisera a rapid slide-agglutination test can be used to presumptively identify *Brucella* species.

d) **Molecular Identification:** Polymorphisms in the dnak gene have been used to differentiate *B. melitensis* from the other *Brucella* species. Primers that amplify the entire dnak gene were used for nucleic acid amplification by polymerase Chain Reaction (PCR). The resulting PCR product was digested with EcoRV.

B. melitensis has one EcoRV site whilst all the other *Brucella* species have two EcoRV sites (Cloeckaert *et al*., 1996).

In the study of the antigenic character and differentiation between the smooth strains of *B. melitensis, B. abortus* and *B. suis,* they were found to contain the same A and M antigens but different quantitative relations. Eze, (1977a), described a further antigen Z in *B. ovis* which is also present in the rough phases of *B. melitensis* and *B. abortus*.

B. canis has R antigen (Joni, 2003).

2.8.3 Serological Tests

Samples of any body fluid or of tissue collected surgically or at necropsy, may be cultured. Blood is the material most commonly examined, but bone marrow may more likely give positive results. Blood cultures are best performed on samples collected

during a febrile episode, preferably while the temperature is rising. The isolation rate is improved if several samples are taken over 24 hours (Topley and Wilson, 1998).

Body fluids such as serum, uterine discharge, vaginal mucus, milk, or semen plasma from suspected cattle may contain different quantities of antibodies of the M, G_1, G_2, and A types directed against *Brucella* (Beh, 1974). Since infected cattle may or may not produce all antibody types in detectable quantities, several tests are used to detect brucellosis.

2.8.3.1 **Milk Ring Test (MRT):** This is cheap, easy, simple and quick to perform (Eze, 1977b). It detects lacteal anti-*Brucella* IgM and IgA bound to milk fat globules. It could test false positive when milk that contains colostrum, milk at the end of the lactation period (drying off

period), vaccinated animals with S19, milk from cows suffering from a hormonal disorder or milk from cows with mastitis are tested (Bercovich and Moerman, 1979). Milk that contains low concentrations of lacteal IgM and IgA or which is lacking the fat-clustering factors tests false-negative (Keer *et al.,* 1959; Tanwani and Pathak, 1971). Since lacteal antibodies rapidly decline after abortion or parturition, the reliability of the MRT, using 1ml milk, to detect *Brucella* antibodies in individual cattle or in tank milk is strongly reduced (Hill, 1966). Not every *Brucella* infected animal has udder infections and this can lead to a false negative reaction, i.e. negative MRT but positive blood serology (Morgan, 1970; Bercovich and Lagendijk, 1978).

2.8.3.2 **Serum Agglutination Test (SAT):** Routinely used for survey work in Nigeria (Eze, 1977a). This measures agglutinating antibodies of the IgM, IgG_1, IgG_2, and IgA types (Pietz, 1970; Levieux, 1974; Alton, 1987). It is relatively simple and easy to perform but it requires basic laboratory equipment. It can be used to detect acute infections; as

antibodies of the IgM type usually appear first after infection and are more reactive in the SAT than antibodies of the IgG_1 and IgG_2 types (Beh, 1974; Levieux, 1974). The result of SAT is affected by vaccination of animals and as such the result of the test should be based on the vaccination status of herd or flock (Corbel *et al.* 1984).

2.8.3.3 **Complement Fixation Test (CFT):** This detects specific antibodies of the IgM and IgG_1 type that fix complement (Hill, 1963a; Levieux, 1974). It is laborious and requires highly trained personnel as well as suitable laboratory facilities. Although its specificity is very important for the control and eradication of brucellosis it may give false negative when antibodies of the IgG_2 type hinder complement fixation (MacMillan, 1990). It helps to differentiate antibody reactions following recent vaccination and infection and in establishing the *Brucella* status of chronically infected animals.

2.8.3.4 **Rose Bengal Plate Test (RBPT):** This is a quick qualitative test of macroscopic agglutination performed with only one dilution. The test detects specific antibodies of the IgG_1 and IgM (Levieux, 1974). The test may yield false negative results in infected cattle that give positive results with the CFT (Rose and Roepke, 1957). Residual antibodies after *B*. *abortus* Strain 19 vaccination (where animals are vaccinated) are believed to cause these false positives (Nicoletti, 1967). Although the pH (+3.6) of the antigen enhances the specificity of the test, the temperature of the antigen and the ambient temperature at which the reaction takes place may influence the sensitivity and specificity of the Rose Bengal test (Falade, 1980; Duguid *et al*., 1984; MacMillan, 1990).

2.8.3.5 **Anti-globulin (Coombs) Test:** This detects (Incomplete *Brucella*) antibodies of the IgA and IgG_2 type and is used to confirm SAT results (Hill, 1963b). It is important to perform when SAT is positive and CFT results are negative or inconclusive (Kiss, 1971). The limitation of Coombs test results is that, they are indicative for infection only

when it titres are at least two times the titres of the SAT (Hill, 1963b) but, not all infected cattle show this ratio. It is advisable for use where brucellosis incidence is chronic and vaccinations have not yet been performed.

2.8.3.6 **2-Mercaptoethanol (2-ME):** It serves to separate IgG from IgM. Usually used to differentiate between infected and vaccinated cattle as well as detecting the carrier animal (Rossi and Cantini, 1969).

2.8.3.7 **Rivanol Test (RT):** It serves to separate IgG from IgM. Usually used to differentiate between infected and vaccinated cattle as well as detecting the carrier animal (Rossi and Cantini, 1969).

2.8.3.8 **Enzyme Linked Immuno-Sorbent Assay (ELISA):** This is a simple test (Sutherland *et al*., 1986), specific and sensitive as the MRT and SAT in detecting *Brucella* antibodies in milk and serum (Ocholi, 1990). It is used for screening and confirmation, and the results usually agree

with CFT results (Ruppanner *et al*., 1980; Stemshorn and Nielsen, 1980). However, depending on the traces of colostrum in the milk, or presence of low concentrations of lacteal immunoglobulin, the ELISA may give false positive or false negative (Bercovich and Taaijke, 1990; Kerkhofs *et al*., 1990). It seems that the ELISA is less sensitive than the CFT, as some infected cattle that test positive with the CFT may test negative with the ELISA (Sutherland, 1984; Cargill *et al*., 1985). ELISA is very costly when only a few samples are tested; therefore, it is unsuitable for testing individual animals but it is the ideal test for screening suspected herds.

Since the reliability of serological tests to detect brucellosis depends on antibodies that may or may not be present at the time of examination, inevitably some infected animals may elude detection.

2.8.3.9 **Skin Test :** The skin-delayed-type-hypersensitivity (SDTH) test is independent of circulating antibodies and should be added to the serological tests to improve detection of brucellosis. The SDTH test confirms serologic results, confirms brucellosis in cattle with

ambivalent serologic test results and detects latent carriers of *Brucella*. Furthermore, the SDTH test does not sensitize cattle for several consecutive SDTH tests (Bercovich, 1999). Therefore, the SDTH test should be the test of choice in developing countries, as cattle in those countries are usually not tagged so that serological test results could be related to the individual animal. Where the animals are tagged a combined use of the SAT and SDTH tests increase the reliability of brucellosis diagnosis (Bercovich, 1999).

2.9 DISTRIBUTION OF BOVINE BRUCELLOSIS

Bovine brucellosis is a zoonotic disease of worldwide distribution. The disease primarily affects domestic animals and the latter are the main source of infection to human beings. The disease is of great economic importance and it is a major public health hazard.

Animal brucellosis has been shown to occur in Nigeria and has been reported increasingly since 1928 (Earnshaw and O'Brien). Reports from various areas of the

country reveal that animal brucellosis is widely distributed in Nigeria (Eze, 1977a; Ocholi, 1990; Halle and Ajogi, 1997; Ocholi *et al*., 2004; Farouk,2006; Bertu, 2008).

There is a pattern of low, medium and high prevalences in specific areas of the country and prevalence also varies between herds in the same area (Nuru and Dennis, 1975). The prevalence of this disease varies considerably between herds, areas and countries (Blood and Henderson, 1968; Roberts, 1971) and also depends on the system of management (Nuru and Dennis, 1975).

Frequency of brucellosis varies across nations but obviously is higher in more agrarian societies and in places where handling of animal products and dairy products is less stringent. The highest incidence is observed in the Middle East, Mediterranean Basin (Portugal, Spain, Southern France, Italy, Greece, Turkey, North Africa), South and Central America, Eastern Europe, Asia, Africa, the Caribbean. These countries do not have good standardized and effective public health and domestic animal health programs. Unpasteurized cheeses, sometimes called 'village cheeses', from these areas may present risk for tourists (Dimitrov et al., 2004; Taliani *et al*., 2004).

In the United States, frequency is related to the number of infected animals. Infected animals are rare in the United States and pasteurization of milk has eliminated that potential reservoir, thus infection generally occurs by occupational exposure (Cattlemen, Veterinarians, Slaughterhouse workers) (Topley and Wilson, 1998). The incidence is approximately 200 per year or 0.04 per 100,000. Human patients in the United States are found in Texas, California, Virginia and Florida (Dimitrov *et al*., 2004).

2.10 PREVENTION AND CONTROL OF BRUCELLOSIS

Prolong treatment of infected cattle with high dosage of antibiotics is not recommended due to the appearance of antibiotics in the human food chain and it interferes with the production of milk products. Moreover, as *brucellae* are facultative intracellular bacteria, relapses after treatment usually occur. Therefore, efforts should be directed at prevention or eradication of the disease (Dimitrov *et al*., 2004; Farouk, 2006).

The primary method of control is cell-mediated immunity rather than antibodies, as there is no successful treatment although some immunity to re-infection is provided by serum immunoglobulins. Initially, immunoglobulin M (IgM) levels

rise, followed by immunoglobulin G (IgG) titers. IgM may remain in the serum in low levels for several months, whereas IgG eventually declines. Persistently elevated IgG titers or second rises in IgG usually indicate chronic disease, carrier state, inapparent state or relapsed infection (Stevens, 1995).

There is no successful vaccination protocol in Nigeria. Legislation is needed to effectively control and eradicate brucellosis. Currently, there is no government policy on the control of brucellosis in Nigeria (Ajogi, 1997b). In the United States, there are

various combinations of diagnostic tests used to detect cattle infected with *B. abortus* in tagged animals. Untagged animals are repeatedly (every 5-6 weeks) tested with the SDTH test, as the test does not sensitize cattle for subsequent SDTH tests (Bercovich *et al*., 1992). Suspect herds must be tested at regular interval until all animals test negative. Animals that test positive are removed from the herd.

In areas with endemic brucellosis like in Nigeria, only vaccination will reduce the number of infected animals and eventually permit disease control. The live *B. abortus* Strain19 vaccine produced at National Veterinary Research Institute

(NVRI), Vom is government approved for use in Nigeria but the whole cell killed adjuvant *B. abortus* 45/20 vaccine (Duphavac) is imported and used unofficially by privately owned farms (Ajogi, 1997b). In the last decade a new *B. abortus* strain RB51 vaccine was introduced in the United States (Plommet, 1991).

Vaccination with 40-120 x 10^9 CFU (classical dose) of living *Brucella abortus* Strain 19 (S19) gives a fair to good protection but it also has some disadvantages (Plommet, 1991). It may cause abortion in pregnant cattle (Ajogi, 1997b) and/or induce an antibody response that confuses the serological diagnosis of brucellosis for 12-36 months. It is excreted in the milk and may induce brucellosis in humans. To diminish these undesirable effects of vaccination with S19, two vaccination procedures have been suggested. In one procedure calves are vaccinated once with 3-10 x 10^9 CFU (reduced dose) at an age of 4-8 months and for second time with 3-10 x 10^9 CFU as adults. The second procedure suggests a conjunctival vaccination of calves with two drops of vaccine containing 4-10 x 10^9 CFU at an age of 4-10 months and a second conjuctival vaccination with the same dose six months later (Montaraz and Winter, 1986).

In many countries of the world, control of brucellosis in cattle is principally based on the use of live *Brucella abortus* S19 vaccine (WHO, 1986b). This vaccine considerably increases the resistance of animals to infection and reduces the number of *Brucella* induced abortions. However, the use of this vaccine presents serious problems in the control and eradication of brucellosis.

Though the vaccine induces protection against infection, it has several serious limitations; it induces antibodies in vaccinated cattle that interfere with the diagnosis of field infection, it causes abortion in cattle vaccinated as adults, and it is pathogenic for human beings (Crawford *et al.,* 1990). The problems associated with the use of S19 vaccine have renewed interest in the search of *Brucella* isolates that can be used as vaccine candidate in replacement for this vaccine currently in use (Cheville *et al*., 1993).

Brucella abortus strain RB51 used for vaccination was selected by growth of *B. abortus* strain 2308 in the presence of rifampicin (Cheville *et al*., 1996). The protective effect of this vaccine in cattle is similar to that of S19. Compared with S19 *B. abortus* RB51 vaccine causes less abortion (Cheville *et al*., 1996) and does not induce production of agglutinating antibodies of the IgM type, although specific IgG is produced (Stevens and Olsen, 1996).

Depending on the doses of RB51 used it may cause placentitis that leads to pre-term expulsion of the foetus. The vaccine has been approved for use in the United States of America to allow additional data on field use under controlled conditions (Tobias *et al.,* 1992; Stevens *et al*., 1994).

RB51 and Strain Rev1 *B. melitensis* are the currently licensed vaccines in cattle and small ruminants in the United States and are the preferred alternative by the agriculture community because they do not induce antibodies detectable on standard test and are 70% effective (Stevens and Olsen, 1996). RB51 virtually eliminates adverse

postvaccinal reaction in cattle, such as abortions and localized inflammation at injection site. Most importantly, unlike S19, strain RB51 does not stimulate the same type of antibody that can be confused on standard diagnostic tests with those antibodies produced by the infection (Stevens, 1995).

The use of *Brucella abortus* Strain 45/20 adjuvant vaccine (Duphavac) is less common than S19 because in comparison to S19 it does not give lasting immunity. The vaccine does not induce detectable agglutinating antibodies and is not harmful but it gives a marked skin reaction on the injection site. Two initial

vaccinations at specific intervals and an annual booster are needed for good protection (Plommet, 1991).

All current vaccines are parenterally delivered which is logistically difficult, economically expensive, ecologically invasive, and severely limits management flexibility. Alternatives are needed to reach large segments of cattle population affected by brucellosis.

The disease may be avoided by employing good sanitation and management practices. Replacement animals should be tested when purchased and retested after a 30 to 60 days period during which they are kept separate from the remainder of the herd. This practice allows detection of animals that were in the incubation period of the disease prior to purchase. For best results, female calves should be vaccinated when they are 6 months old. At the time of vaccination, a tattoo is applied in the ear; that tattoo identifies the animal as an 'Official vaccinate'. The tattoo identifies the year in which vaccination was administered (Plommet, 1991).

2.11 ZOONOSES AND FOOD SAFETY

Brucellosis synonyms in man include Malta fever or undulant fever (Ansorg *et al*., 1983). Symptoms of acute brucellosis caused by *Brucella abortus* are 'flu-like' and highly nonspecific and may include fever, sweats, headaches, back pains and physical weakness, severe infections of the central nervous systems or lining of the heart may occur (Yinnon and Morali, 1993; Rodostits and Blood, 2000). Brucellosis can also cause long lasting or chronic symptoms that include recurrent fevers, joint pain and fatigue.

This potential for long-lasting infection that can disable workers in either military or civilian circles makes *Brucella* species an appealing choice for a biological weapon (Franz *et al*., 1997). Chronic brucellosis is an insidious disease with vague symptoms that might be confused with other diseases affecting various organ systems (Yinnon

and Morali, 1993). Human usually acquire brucellosis by consumption of raw milk or milk products. Brucellosis is also recognized as an occupational hazard for farmers, veterinarians, and workers in the meat industry within areas with enzootic *B. abortus*.

Farmers and workers in the meat industry may contract brucellosis percutaneous, conjuctival or by nasal mucous membrane infection (Schnurrenberger *et al*., 1975; Eze, 1977a).

Veterinarians may become infected when handling aborted fetuses or apparently healthy calves born to infected cows and by performing gynaecological and obstetric manipulations, or rectal examination of infected cattle (Schnurrenberger *et al*., 1975; Dekeijzer, 1981; Peelman and Dekeyser, 1987). Because cattle and small ruminants are the major source of human infection, programmes to eradicate human brucellosis have to be aimed at these animal species, since there is no vaccine available for humans.

Where a Veterinarian is accidentally jabbed with the animal vaccine, doxycycline and rifampin should be used for S19 and Rev-1, or doxycycline alone for RB51 for 3weeks. The same recommendations hold true for spraying vaccine in the eyes or open wounds on the skin (Henry *et al*., 1998)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 LOCATION OF SAMPLED HERDS

Kaduna State is one of the states in North Central geopolitical zone of Nigeria, it is located on Latitude (DMS) 10 19' 60N and Longitude (DMS) 7^0 45' 0E. The capital city, Kaduna, was the capital of the then northern region of Nigeria (Figure 3.1).

Migratory cattle herds located in 15 Local Government Areas (LGAs) of Kaduna State were chosen for this study. These are: Birnin Gwari, Chikun, Kaduna North, Kachia, Lere, Kaduna South, Zango Kataf, Kaura, Jemma'a, Jaba, Giwa, Zaria, Soba, Kudan and Igabi.

Birnin Gwari, Giwa and Kudan LGAs are located at the extreme northern part of the State and are bordered in the North West by Niger and Zamfara States while on the North East by Katsina and Kano States.

Kachia, Chikun and Lere LGAs are located in the centre of the State. They share boundaries with Niger State on the West and Bauchi State on the East.

Jaba, Jemma'a and Kaura are located on the Southern end and the lower part of the State. They are bordered by the Federal Capital Territory, Abuja on the West and Plateau State on the East.

The cattle population in the State as at 1990 was 1,006, 624 (Personnal information). There has been no recent survey since then but the State Ministry of Agriculture estimates annual increase of 1.5% therefore there should be about 1.5million cattle now; and the White Fulani (*Bos indicus*) breed makes up about 90% of this cattle population. Extensive husbandry system, where the pastoralists move around with their animals in search of green fields is widely practiced.

3.2 SAMPLE SIZE

Sample size (n) = $\frac{Z^2 P q}{\alpha^2}$

Where, n = minimal sample size

p = prevalence of a disease (0.07)

q = 1-p (0.93)

α = allowable error (0.05)

Z = 1.96

(Snedecor and Cochran, 1980)

The calculated sample size was 1000 but a total of 2799 samples were collected to give a better result and less error.

Fig. 3.1 Map of Kaduna State showing the 15 Local Government Areas sampled.

- Birnin Gwari
- Giwa
- Soba
- Igabi
- Chikun
- Kachia
- Kaura
- Jaba
- Zango Kataf
- Lere
- Kaduna North & South
- Jema'a
- Kudan
- Zaria
- LGAs not sampled

3.3 CRITERIA USED FOR HERD SELECTION

The criteria used for herd selection include: Herds where there were complaints of symptoms of abortion, birth of weak calves, still births, infertility, orchitis, hygroma, e.t.c. Ten per cent of cattle were randomly selected and sampled in every herd visited, animals showing any of these symptoms were sampled.

3.4 THE ANIMALS/FARMS

Physical examination of the animals on the farms visited were carried out to check for hygroma, orchitis and weakness in calves. Sanitary/hygienic levels of operation, general health status of the herd were recorded. Then 10% of the animals were randomly picked and sampled.

Ageing of the animals was done by using a combination of eruption of permanent incisors (Akerejola and Ojo, 1976).

3.5 ADMINISTRATION OF QUESTIONNAIRES

In every herd visited, a questionnaire was administered to herd owner in order to assess their knowledge on human and bovine brucellosis. General information on herd, such as the identity of the herds, herd composition, symptoms and signs of brucellosis such as abortion, still-births, weak calves, infertility, orchitis, hygroma, addition of replacement animals, milk yield, state of milk pasteurization, etc were asked (Appendix 1).

3.6 SAMPLE COLLECTION

3.6.1 Milk

Herds were visited at 7:30 a.m. before the animals were released for grazing. The cows were properly restrained and the udder cleansed with mild soap (LUX ^{R}) and rinsed with warm water then toweled dry. The udder was massaged thoroughly and the fore milk stripped off. About 5mls of fresh milk was then expressed from 4 quarters of lactating cows in labeled sterile bottles and transported in ice box at 4^{0}C to the laboratory, where they were refrigerated.

3.6.2 Sera

Age and sex were recorded and 10% of animals were sampled from each herd. After proper restraint of the animal, about 5mls blood was aseptically collected via the jugular vein of individual cattle using 10ml syringe. The blood was transferred into clean labeled sample bottles without anticoagulant and allowed to stand for one hour for sera to separate from the cellular components. The clotted blood samples were centrifuged at 3,000G for 10-15minutes. The sera were transferred into labeled sample bottles and stored at -20^{0}C (freezer) and used within one week of collection.

All the antigens used in this work were obtained from the Central Veterinary Laboratories Agency, NewHaw, Surrey, Weybridge, United Kingdom.

3.7 LABORATORY ANALYSIS OF THE SAMPLES

3.7.1 Milk Ring Test (MRT) On Milk Samples

Test milk sample and antigen from refrigerator was left on the bench for one hour. The milk was gently mixed in the sample tube to ensure homogeneity

of the cream. Using a pipette vertically above the test tube a drop (0.03 ml) of the *Brucella* MRT antigen was added to 1.0 ml of test milk sample in a labeled narrow test tube (Khan tube 12 x 75mm/6ml volume), the contents were gently mixed within one minute of adding the antigen then incubated at 37^0C for one hour.

A positive case meant that the antigen (Ag)/antibody (Ab) complex attached to fat globules and floated on top as bluish ring cream line as the cream rises (Agglutination) with a white milk column. While a blue milk column and a whitish ring cream line meant negative reaction (Cruickshank *et al*., 1975).

3.7.2 Serologic Testing

Thawed serum samples were measured for antibody to *Brucella abortus* by Rose Bengal Plate Test (RBPT) and Microtitre Serum Agglutination Test (MSAT).

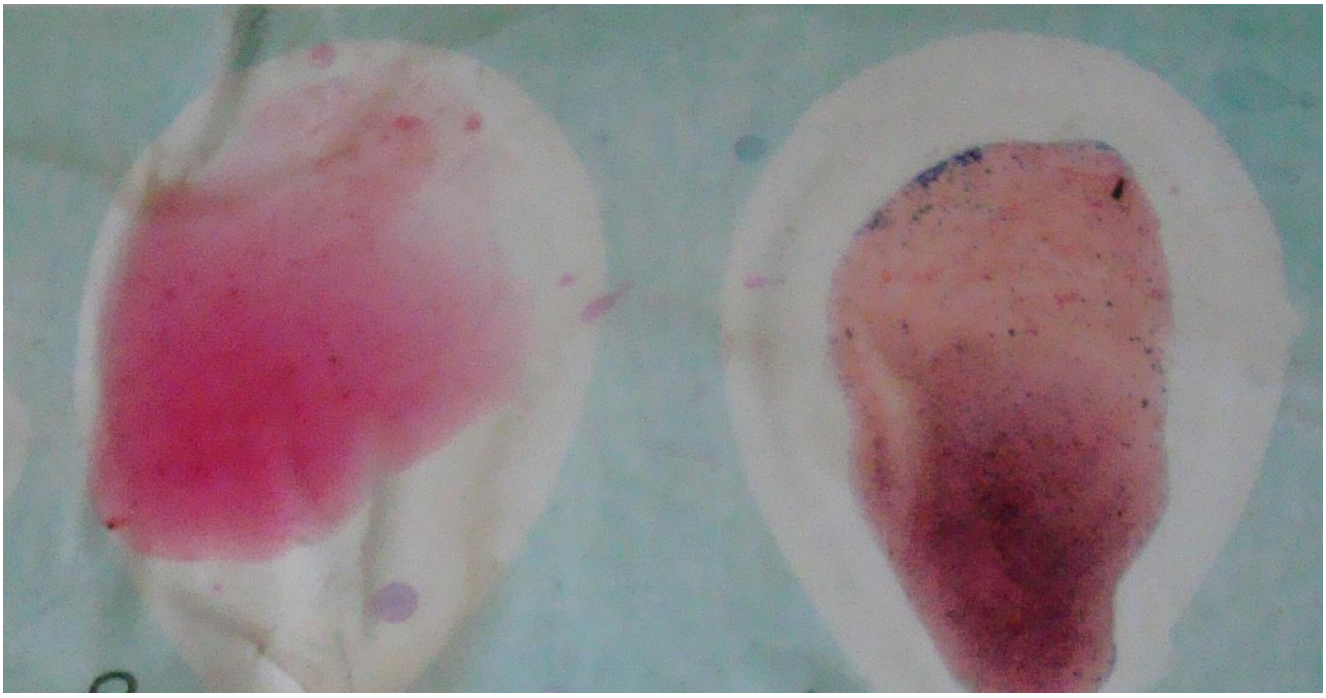
a) Rose Bengal Plate Test (RBPT)

This was done in batches of 25 samples. The stored sera were removed from the freezer and thawed at room temperature. Using a rubber bulb attached to a capillary tube, a drop (0.03 ml) of undiluted serum was placed on a glass plate by

squeezing the rubber bulb. An equal volume of the Rose Bengal *Brucella abortus* stain antigen was placed on the glass plate test area, adjacent to the serum. A separate broad end, clean tooth pick was used to mix the antigen suspension with serum.

The glass plate was manually rocked slowly for 4 minutes and examined under light in the wet form to find out the test serum with clump or presence of agglutinins indicating presence of antibodies to *Brucella* (Morgan, 1967; Alton *et al*., 1988).

Where the clumps of agglutinated cells were separated by clear liquid, it was recorded as agglutination/positive (+) and negative (-), where there was no sign of agglutination taken place (Plate 3.1).



1 2

Plate 3.1 Rose Bengal Plate Test (RBPT).

1. Negative reaction – No agglutination
2. Positive reaction – Presence of agglutination

b) Microtitre Serum Agglutination Test (MSAT)

The serum samples were further subjected to Microtitre Serum Agglutination Test (MSAT) using the standardized antigen. The 96 wells of microtitre plate was divided into 3 columns of test wells vertically, with each plate holding 24 tests. Eighty microlitre of normal saline was pipetted into the first well of each test and 50 µl into the remaining three wells.

Twenty microlitre of the test serum was pipetted into the first well and mixed, then 50 µl of the mixture transferred to each succeeding well. Fifty microlitre of mixture was discarded from the last well. Each well finally contained 50 µl of diluted serum at 1:10, 1:20, 1:40 and 1:80. 50 µl of antigen at working strength of 1:10 was added into each well after staining with safranin to serve as indicator of the reaction. The microtitre plate was covered with sealing tape and mixed thoroughly for 30 seconds, then incubated at 37^0C for 20 hours.

Known positive control sera of known agglutination titer and known negative control were incorporated into each test and result read by examining the bottom of the wells reflected in a concave mirror placed above the plate.

Sedimentation in the form of compact buttons with clear edges and having an intense red colour was read as negative (-) and a diffused veil which is evenly distributed as positive (+) (Alton *et al*., 1975). Titers of 1:20 or 40 iu and above were regarded as diagnostic (Eze, 1977a). MSAT was used as a confirmative test.

3.8 STATISTICAL ANALYSIS OF DATA

The Statistical Analysis System (SAS, 2004) version 9.0 was used to analyse the data obtained. The test statistic used was Chi-square (Snedecor and Cochran, 1980) to see the association of age and sex specific reactors. The level of statistical significance was assessed at $p < 0.05$.

Based on MSAT result, two prevalence rates were calculated:

$$\text{Herd Prevalence (HP)} = \frac{\text{Number of herds with at least one positive reactor}}{\text{Number of herds sampled}} \times 100$$

$$\text{Individual Animal Prevalence (IAP)} = \frac{\text{Number of individual positive reactors}}{\text{Number of serum samples tested}} \times 100$$

The rate of confirmation of positive RBPT by MSAT was calculated as :

$$\frac{\text{Number of MSAT reactors}}{\text{Number of positive RBPT samples}}$$

Using the results of MRT, herd prevalence rates of *Brucella* contaminated milk was calculated as described above.

CHAPTER FOUR

4.0 RESULTS

4.1 Questionnaire Analysis

A total of 93 herds were visited in the 15 LGAs of Kaduna State covered. It was found that all the herds visited move to the southern part of the country during the dry season when grasses were depleted and return during the rainy season.

Thirty-three out of 93 respondents (35.5%) reported that they had at one time vaccinated their animals against brucellosis.

Also, 8% (176/2152) cases of abortion were reported while still births and birth of weak calves were 2% (35/2152) and 5% (97/2152) respectively.

A total of 2,799 bovine sera samples were collected from the following local government areas: Birnin Gwari (199), Chikun (178), Kaduna North (157), Kachia (176), Lere (184), Kaduna South (168), Zango Kataf (205), Kaura (201), Jemma'a

(201), Jaba (192), Giwa (193), Zaria (193), Soba (181), Kudan (188) and Igabi (183) (Table 4.1).

TABLE 4.1 Number of herds and animals visited per LGA

LGA	No. of herds	No. of Animals in herds	No. of animals sampled
Birnin Gwari	7	2,000	199
Chikun	7	1,800	178
Kaduna North	8	1,500	157
Kachia	7	1,800	176
Lere	5	2,000	184
Kaduna South	6	2,000	168
Zango Kataf	6	2,000	205
Kaura	6	2,000	201
Jemma'a	6	2,000	201
Jaba	6	2,000	192
Giwa	7	2,000	193
Zaria	7	2,000	193
Soba	5	2,000	181
Kudan	6	2,000	188
Igabi	5	1,800	183

Total	**93**	**28,900**	**2,799**

Three per cent (70/2457) of animals with hygroma were observed. There was no significant difference between sex and hygroma (X^2 = 0.06).

There were reports of infertility and orchitis but the herdsmen sold off unproductive animals. The herds intermingle and animals were replaced.

Questionnaires assessment of the 93 herds sampled showed 61.3% (57/93) of owners had knowledge of brucellosis and its modes of transmission while 36.6% (34/93) of owners boil milk before consumption.

4.2 Milk Ring Test (MRT)

4.2.1 Prevalence rates

Table 4.2 shows the results of the MRT. A total of 31 milk samples out of 2,149 tested positive to *Brucella* by MRT. The highest MRT positive reactor of 9 (0.42%) was from Zango Kataf. The others were Jemma'a 5 (0.23%), Birnin Gwari 4 (0.18%), Chikun 4 (0.18%), Kaduna North 3 (0.14%), Kaduna South 2 (0.09%), Kaura 2 (0.09%) , Kachia 1 (0.05%), Lere 1 (0.05%), the six Local Government Areas with 0.0% were Jaba, Giwa, Zaria, Soba, Kudan and Igabi.

TABLE 4.2 Investigation of bovine milk for evidence of *Brucella* antibodies in Kaduna State using Milk Ring Test (MRT)

Source of milk	**Total No. Examined**	**No. +ve**	**% +ve**
Birnin Gwari	156	4	0.18
Chikun	140	4	0.18
Kaduna North	113	3	0.14
Kachia	129	1	0.05
Lere	135	1	0.05
Kaduna South	126	2	0.09
Zango Kataf	150	9	0.42
Kaura	161	2	0.09
Jemma'a	151	5	0.23

Jaba	152	0	0.0
Giwa	151	0	0.0
Zaria	150	0	0.0
Soba	141	0	0.0
Kudan	150	0	0.0
Igabi	144	0	0.0
Total	**2,149**	**31**	**1.4**

The herd prevalence rate (HP) of brucellosis was 18.3% (17/93) for MRT while the individual animal prevalence rate (IAP) was 1.4% (31/2,149) from the 15 Local Government Areas sampled.

4.3 Rose Bengal Plate Test (RBPT)

4.3.1 Prevalence rates

The prevalence rate varied from one area to another but in all 198 (7.1%) sera samples tested were positive. *Brucella* antibodies in sampled cattle were detected in all the fifteen Local Government Areas of Kaduna State tested by RBPT but

Zango Kataf had the highest prevalence rate of 1.11%, followed by Jemma'a (1.04%), Kaura (0.68%), Jaba (0.61%), Soba (0.57%), Igabi (0.57%), Kudan (0.5%), Birnin Gwari (0.39%), Kaduna South (0.29%), Zaria (0.32%), Giwa (0.32%), Chikun (0.21%), Kachia (0.21%), Kaduna North (0.14%) and Lere (0.11%) (Table 4.3).

TABLE 4.3 Prevalence of Bovine Brucellosis in Kaduna State As Detected By RBPT

LGA	Sampled No.	No. +ve	% +ve
Birnin Gwari	199	11	0.39
Chikun	178	6	0.21
Kaduna North	157	4	0.14
Kachia	176	6	0.21
Lere	184	3	0.11
Kaduna South	168	8	0.29
Zango Kataf	205	31	1.11
Kaura	201	19	0.68
Jemma'a	201	29	1.04
Jaba	192	17	0.61
Giwa	193	9	0.32
Zaria	193	9	0.32
Soba	181	16	0.57
Kudan	188	14	0.5
Igabi	183	16	0.57
Total	**2,799**	**198**	**7.1**

4.4 Prevalence Rate of Brucellosis As Obtained By Microtitre Serum Agglutination Test (MSAT)

The prevalence rate was also highest in Zango Kataf when tested by MSAT (0.4%), followed by Birnin Gwari (0.14%), Chikun (0.07%), Kaura (0.07%), Jaba (0.07%), Jemma'a (0.07%), Kaduna South (0.07%), Kachia (0.04%), Igabi (0.04%), Lere (0.04%).

While animals tested from Kaduna North, Giwa, Kudan, Soba and Zaria tested negative (0.0%) (Table 4.4). The herd prevalence rate was 18.3% (17/93) while individual animal prevalence rate of test samples that were positive at dilution of 1:20 and above was 1.0% (28/2799).

TABLE 4.4 Prevalence of Bovine Brucellosis in Kaduna State As Detected By MSAT

LGA	Sample No.	1:10	1:20	1:40	1:80	% +ve
Birnin Gwari	199	4	4	1	0	0.14
Chikun	178	2	2	1	0	0.07
Kaduna North	157	3	0	0	0	0.0
Kachia	176	5	1	0	0	0.04
Lere	184	1	1	0	0	0.04
Kaduna South	168	5	2	0	0	0.07
Zango Kataf	205	18	11	1	1	0.4
Kaura	201	14	2	0	0	0.07
Jemma'a	201	16	2	0	0	0.07
Jaba	192	12	2	0	0	0.07
Giwa	193	6	0	0	0	0.0
Zaria	193	2	0	0	0	0.0
Soba	181	10	0	0	0	0.0
Kudan	188	4	0	0	0	0.0

Igabi	183	7	1	0	0	0.04
Total	**2,799**	**108**	**28**	**3**	**1**	**1.0**

4.5 Age and Sex-Specific Brucellosis Prevalence Rates in Kaduna State

On table 4.5, out of 2,149 milk samples tested with MRT 31 (1.4%) were positive. Out of these, animals of age 1-2years had 1 (0.05%), animals of age 2-3years had 25 (1.16%) and animals above 3 years of age had 5 (0.23%) positive milk samples.

Table 4.5 Distribution of Brucellosis in the population of cows sampled by age (MRT)

Female	**Total**	**No. +ve**	**% +ve**
1-2years	502	1	0.05
2-3years	1,159	25	1.16
>3years	488	5	0.23

Total	**2,149**	**31**	**1.4**

Tables 4.6 and 4.7, show variation in prevalence of bovine brucellosis by animal type. Higher prevalence of infection in both males and females of ages 2-3years were observed in all the fifteen Local Government Areas sampled by both RBPT and MSAT respectively. The differences were statistically significant ($p< 0.0001$).

The RBPT results revealed that of the 1,159 sera of females of age 2-3years examined, 109 (3.89%) were positive compared to 23 (0.82%) out of 195 males of same age. The prevalence was highest for this age group.

Among, females above 3years old 41 (1.46%) out of 488 sera tested positive compared to males of the same age where 3 (0.11%) out of 48 sera were positive. The sera of 21 (0.75%) of the females of age 1-2years old were positive while the

sera of the males of same age group were negative. Females below 1year of age had only 1 (0.04%) positive serum (Table 4.6).

TABLE 4.6 Distribution of Brucellosis in the Population of Cattle Sampled by Age and Sex (RBPT)

Age and Sex	**Total**	**No. +ve**	**% +ve**
	FEMALE		
<1year	285	1	0.04
1-2years	507	21	0.75
2-3years	1,159	109	3.89
>3years	488	41	1.46
	MALE		
<1year	57	0	0.0
1-2years	60	0	0.0

2-3years	195	23	0.82
>3years	48	3	0.11
Total	**2,799**	**198**	**7.1**

Similarly, the MSAT result shows that 19 (0.67%) out of 1,159 females of age 2-3years tested positive and 4 (0.14%) out of 195 males of the same age also tested positive.

For the females above 3years old, 3 (0.11%) out of 488, females of 1-2years had one (0.04%) and less than 1year old females had 1 (0.04%) positive. While the males of the same age groups were negative to MSAT at 40iu (1/20), (Table 4.7). However, Odds ratio showed that the variable (Brucellosis) is protective (OR =0.9) and the 95% confidence interval was $-14.24 \leq x \leq 14.33$.

Numerically, females of age 2-3years old which have attained sexual maturity seem more at risk 19 (0.67%) than the males 4 (0.14%) of same age judging from the samples examined (Tables 4.6 and 4.7). However the differences between the

prevalence rates in males and females were statistically significant ($p<0.0001$) in all the 15 Local Government Areas studied.

4.6 Summary of the Three Serological Tests

Twenty-seven sera were positive in both MSAT and RBPT, of these one had titre of 1:80 (160 iu) in MSAT. Two sera were MSAT positive only, while, one hundred and sixty-seven sera were positive for RBPT only. There were 31 positive milk samples.

The rate of confirmation of positive RBPT by MSAT was 14.1% (28/198).

Table 4.7 Distribution of Brucellosis in the Population of Cattle Sampled by Age and Sex (MSAT)

Age and Sex	**Total**	**No. +ve**	**% +ve**
	FEMALE		
<1year	285	1	0.04
1-2years	507	1	0.04
2-3years	1,159	19	0.67
>3years	488	3	0.11

	MALE		
<1year	57	0	0.0
1-2years	60	0	0.0
2-3years	195	4	0.14
>3years	48	0	0.0
Total	**2,799**	**28**	**1.0**

CHAPTER FIVE

5.0 DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

The overall prevalence of *Brucella* antibodies in Migratory cattle herds in the 15 Local Government Areas of Kaduna State sampled in this study as determined by RBPT is 7.1%, MSAT is 1.0% and MRT is 1.4%.

In this investigation, RBPT gave the highest positive result. This was followed by MRT then MSAT. This however shows that no single test can detect cases of brucellosis conclusively as observed by Olascoaga, 1976.

Rose Bengal Plate Test is a highly sensitive screening test but heterospecific as infections due to organisms like *Salmonella*, *Pasteurella spp, Yersinia enterocolitica* type IX*, Vibrio cholera* or other members of the *Brucellaceae* family could give false positive results to *Brucella* antigen although antibody titers in these cases are usually low (Olascoaga, 1976). Heterospecificity is a problem in RBPT because it is carried out

in only one dilution but the MSAT uses serum diluted to determine antibody titer therefore eliminates the problem in active infection.

The detection of higher antibody titres by RBPT (7.1%) than the MSAT (1.0%) is in agreement with previous studies (Nicoletti, 1967; Morgan, 1967; Bale, 1980) and show RBPT to be more sensitive than the MSAT. Similarly, the observation made by Okewole *et al*., (1988) in goats and sheep that some sera which tested negative with RBPT were found to be positive with MSAT is valid in this study with cattle.

The MSAT result obtained here 1.0% (28/2799) is less than the 7.1% (57/805) and 3.6% (12/337) reported by Nuru and Dennis (1975) in both local herds of Northern Nigeria and Government herds of Northern Nigeria respectively. But higher than Nuru's (1974) result of 0.4% (7/1659) for Trade cattle of North Central (now Kaduna State). This could be because most farmers can recognize the symptoms of brucellosis and the S19 vaccine is presently produced and made available to the veterinarians by the NVRI, Vom for vaccination of cattle .

This figure (1.0%) is less than 3.8% reported by Bale and Kumi-Diaka (1981) in Northern Nigeria and 1.5% reported by Ajogi (1997a) from slaughtered cattle in Northern Nigeria and also less than 6.6% (50/762) reported by Ocholi (1990) from field samples in various parts of Kaduna State. This shows that, the prevalence of brucellosis is on the decrease because the farmers have improved on the sanitary conditions of their farms with proper disporsal of aborted materials.

Numerically, it would appear the prevalence of brucellosis is higher in Southern part of the state compared to that of the Northern part this could be due to more rainfall and greener pasture in the Southern part which is conducive for survival of *Brucella* in the environment compared to the drier Northern part (Ajogi, 1997b).

In this study, brucellosis occurred in both sexes though there was significant difference ($p<0.0001$) in the prevalence rate. This agrees with Chukwu's report of 1987 where he got 5.94% for males and 8.15% for females in Eastern Nigeria.

The prevalence rate of infection was highest among adult animals of age 2-3years in both sexes. Ocholi's (1990) study showed it was highest for >3years age groups. These animals are of breeding age therefore the presence of brucellosis infection will result in reproductive wastages like abortion, neonatal mortality, infertility or sterility.

The prevalence rate for animals <1year of age was nil for males and 0.04% for females who probably got infected through suckling of diseased cows or antibodies found may be maternal antibodies. Calves which become infected through ingestion of infected milk could shed *Brucella* organism in their faeces thereby contaminating the grazing

grounds (Arthur *et al*., 1982) and could become diseased if they reach sexual maturity, thus serving as source of infection for other animals.

The higher prevalence rates observed in Zango Kataf and the surrounding Local Government Areas (Jemma'a, Kaura and Jaba) could be attributed to the congregation of animals throughout the year in these areas due to availability of feed all year round. Many herds graze around and use the same water points thereby coming in contact with one another which could enhance the spread of the infection. The herds from these LGAs had no vaccination history.

Studies have shown that there could be infection but without excretion of the organism in the milk at the time of examination, as *Brucella* is known to be excreted intermittently (Alton, 1960). The stage of lactation of most of the cows could not be determined precisely as, a number of *Brucella* reactors are usually dry either because of abortion or death of weak calves (Eze, 1977a). The MRT result of 1.4% from this study is of particular zoonotic and public health importance. In most parts of the Northern states of Nigeria, local customs encourages the consumption of raw bovine milk. Under such conditions public health hazards due to brucellosis definitely exists (Ocholi *et al*., 2004).

This study is in agreement with Nuru's findings of 1974, that the prevalence rate of brucellosis is related to the husbandry system. The animals, in this study, were

from migratory herds managed extensively by the Fulanis. It is thought that under extensive husbandry conditions, the incidence of brucellosis should be insignificant (Banerjee and Bhatty, 1970) but contrary to this, conditions favourable for dissemination and persistence of brucellosis in these herds exist.

(I) Proper sanitary measures are lacking in the event of an abortion due to *Brucella abortus* within a herd. The aborted fetus, fetal membrane and persistent vaginal discharges rich in *Brucella* organism play an important role in the dissemination of the infection through contamination of grazing pastures and water points (Banerjee and Bhatty, 1970).

(ii) The cows which have aborted or infected cows calving at term are not isolated, thereby disseminating the infection throughout the herd (Nuru and Dennis, 1975).

(iii) During the dry season grazing, there is high concentration of animals around water points, therefore dissemination of the infection is high. Animals are also kept in close contact in the night kraal (Nuru, 1974).

The result from this investigation suggests a moderate prevalence of bovine brucellosis among migratory Fulani cattle herds. If the current effort of the government to settle the Fulanis is successful then the disease will be on the increase in the absence of any meaningful control measures since, bovine brucellosis is a disease of congregation.

Esuruoso (1979b), estimated the annual loss due to bovine brucellosis in Nigeria at N140.8m, with the prevalence rate of 1.0% from this study in Kaduna state, the economic losses in the cattle industry could be high.

With the developing dairy industry in Nigeria, human brucellosis should not be overlooked since the presence of brucellosis in animals is an indicator of its probable presence in man especially in those working with cattle or their by-products or consuming unprocessed milk and milk products (Nuru and Dennis, 1975). Nuru, 1974

reported that over 95% of raw cow milk in the Northern states of Nigeria is untreated and sold in the market daily.

5.2 CONCLUSIONS

In this study it has been shown that the testing for brucellosis with respect to:

1. MSAT of 2,799 cattle gave a prevalence rate of 1.0%
2. RBPT of 2,799 cattle gave a prevalence rate of 7.1%
3. MRT of 2,149 cows gave a prevalence rate of 1.4%

The questionnaires survey of the migratory cattle herds showed 8% of abortion, 2% still births, 5% birth of weak calves and 3% hygroma cases. Also, 61.3% of the 93 respondants had knowledge of brucellosis while 36.6% of them boil milk before consumption.

5.3 RECOMMENDATIONS

Presently, there are no existing control policy established by the government on this disease despite the potential economic and public health hazard posed by it. Using the data obtained in this study, it is suggested that:

1. Compulsory vaccination of all cattle (calfhood and adulthood) be instituted with identification like tattoo put on them.

2. Proper screening and vaccination of all newly purchased calves be done before addition to herds.
3. If government is willing to pay compensations, test-and-slaughter in areas with very low prevalence should be adopted.

Other measures like movement restrictions especially from neighbouring countries, quarantine, hygienic standards and education of government, herdsmen and community on brucellosis, if co-ordinated with full co-operation of cattle owners, local and state government could lead to effective control (Chukwu, 1987).

There should be proper disinfection of the infected premises and proper disposal of the aborted fetuses and placentas in cases of abortion.

All livestock farmers must be enlightened about disease reporting especially cases of abortions in order for investigation of abortions due to *Brucella* and other infectious agents be done. The farmers must be encouraged to utilize the diagnostic services provided at the government veterinary clinics, Universities or Institutes.

This report provides current information and confirms the involvement of *Brucella* in some of the cases of abortion in animals frequently reported from the field. Human patients suffering from pyrexia of unknown origin (PUO) or typhoid should be screened for *Brucella* infections. Also cattle that have aborted due to trypanosomosis should be screened for *Brucella* infections.

More researches should be carried out to isolate and characterize *Brucella* isolates obtained from hygroma fluid, aborted fetuses and other sources for possible vaccine production.

REFERENCES

Acha P.N. and Szyfres B. (1980). Zoonoses and communicable diseases common to man and animals. *World Health Organisation Publication,* Washington D.C. 355:15-18.

Adesiyun A.A. and Abdu P.A. (1984). *Brucella abortus* agglutinins in chickens in Nigeria. *Bulletin of Animal Health Production for Africa*. 32:311-331.

Ajogi I. (1997a). Prevalence of Brucellosis in Nigeria: A Chronology of Serological Investigations. *Journal of Medical and Allied Sciences.* 1(1):23-28.

Ajogi I. (1997b). Seroprevalence of Brucellosis in slaughtered cattle in four Northern states of Nigeria. *Tropical Veterinarian.* 15:21-24.

Akerejola O.O. and Ojo S.A. (1976). Anterior teeth development as a means of determining age in Nigerian cattle. Paper presented at the Annual Conference, *Nigerian Society for Animal Production*, Benin City.

Alausa K.O. (1979). The investigation and control of a large scale community outbreak of brucellosis in Nigeria. *Public Health.* 93:1985.

Alausa K.O. (1983). Brucellosis in Nigeria. Epidemiology and practical problems of control. In : *Human ecology and infectious disease*. Academic Press London. 315-332.

Al-Khalaf S. and El-Khaladi A. (1989). Brucellosis of camels in Kuwait. *Comparative Immunology, Microbiology and Infectious Diseases.* 12(1-2): 1-8.

Allsup T.N. (1974). Failure to demonstrate Brucella infection in ewes exposed to natural bovine infection. *Veterinary Record*. 94:183-186.

Alton G.G. (1960). The occurrence of dissociated strains of *Brucella melitensis* in the milk of goats in Malta*. Journal of Comparative Pathology and Therapeutics.* 70: 10-17.

Alton G.G. (1987). Control of *Brucella melitensis* infection in sheep and goats. A review *Tropical Animal Health Production*. 19: 65-74.

Alton G.G., Jones L.M. and Pietz D.E. (1975). Laboratory Techniques in Brucellosis, Second edition. *World Health Organisation monograph series* No 55, WHO, Geneva. 11-59.

Alton G.G., Jones L.M., Angus R.D. and Verger J.M. (1988). Techniques for the brucellosis Laboratory. *Institute National de la Recherche Agronomique.* Paris,
France. 63-129.

Anczykowski F. (1972). Further studies on fowl brucellosis. Studies on *Brucella* variability *in-vitro* and *in-vivo*. *Veterinary Bulletin.* 42:5719.

Anderson T.D., Cheville N.F. and Meador V.P. (1986). Pathogenesis of placentitis in the goat inoculated with *Brucella abortus*. Ultrastructural studies. *Veterinary Pathology.* 23(3):227-239.

Anon (1963). *Annual Report Animal Health Division*. North Province of Nigeria. 11-13.

Ansorg R., Palm G. and Unger U. (1983). Malta fever in a brucellosis free area: analysis of an outbreak in the Gittigen district in 1982. *Zentralblatt fAr Bakteriologie Mikrobiologie und Hygiene.* 255(2/3):299-308.

Arthur G.H., Noakes D.F. and Pearson H. (1982). Brucellosis. *Veterinary Reproduction and Obstetrics*. 5TH Edition. Bailliere Tindall, London. 742-745.

Baba M.M., Moses A.E. and Ajayi B.B. (1998). Serological evidence of *Brucella abortus* infections in patients suspected of typhoid fever. *Nigerian Medical Practitioner.* 35(1/2):9-11.

Bale J.O. (1980). Cultural and Serological study of ovine and caprine brucellosis in Northern Nigeria. *MSc Thesis*. Ahmadu Bello University, Zaria. 1-20.

Bale J.O. (1991). Brucellosis: A threat to livestock production and human health in Nigeria. A paper presented at a *symposium at NAPRI,* Shika, Zaria. 4-5.

Bale J.O. and Kumi-Diaka J. (1981). Serological and bacteriological study of bovine brucellosis from livestock investigation and breeding centers in Nigeria. *British Veterinary Journal.* 132:256-261.

Bale J.O. and Nuru S. (1982). Seriological study of brucellosis in local fowls in Northern Nigeria. *Journal of Animal Production and Research*. 4:161-164.

Bale J.O. and Nuru S. (2001). A case study of brucellosis passive immunity in a *Bos indicus* calf from an infected dam. *Journal of Agriculture and Environment.* 2(2): 193-199.

Banerjee A.K. and Bhatty M.A. (1970). A survey of bovine brucellosis in Northern

Nigeria. (A preliminary Communication). *Bulletin of Epizootic Diseases of Africa*. 18:333.

Bang Bernard (1897). The etiology of epizootic abortion. *Journal of Comparative Pathology and Therapeutics*. 10:125.

Beh K.J. (1974). Quantitative distribution of *Brucella* antibody among immunoglobulin classes in vaccinated and infected cattle. *Research in Veterinary Science.* 17:1-4.

Bercovich Z. (1999). The use of skin delayed type hypersensitivity as an adjunct test to diagnose brucellosis in cattle. *PhD. Thesis*, Utrecht, The Netherlands: ID-Lelydatd, Elink wijk Press. 161-165.

Bercovich Z., Haagsma J. and Laak EAter (1990). Use of delayed-type hypersensitivity test to diagnose brucellosis in calves born to infected dams. *Veterinary Quarterly.* 12(4):231-237.

Bercovich Z., laak EAter and Lipzig JHHvan (1992). Detection of brucellosis in dairy herds after an outbreak of the disease using a delayed-type hypersensitivity test. *Preventive Veterinary Medicine.* 13(4):277-285.

Bercovich Z. and Lagendijk W. (1978). A modified milk ring test for detecting Brucella agglutinins in bulk tank coolers. *Tijdschrift voor Diergeneeskunde.* 103:407-416.

Bercovich Z. and Moerman A. (1979). Non-Specific positive milk ring test(s) in tank milk and Estrumater in the treatment of cattle. *Tijdschrift voor Diergeneeskunde.* 104:713-716.

Bercovich Z. and Taaijke R. (1990). Enzyme immunoassay using mouse monoclonal anti-bovine antibodies for the detection of *Brucella abortus* antibodies in cow milk. *Journal of Veterinary Medicine*. 37(10):753-759.

Bertu W.J. (2008). Sero-epidemiology of brucellosis in small ruminants in Plateau State, Nigeria. *MSc Thesis*, Ahmadu Bello University, Zaria, Nigeria. 17-30.

Biscop G.C. (1994). Bovine Brucellosis In: *Infectious disease of livestock*. Oxford University Press. 2:81-85.

Blood D.C. and Henderson J.A. (1968). Veterinary Medicine, 3rd edition. 461-463. Williams and Wilkins, Baltimore.

Brisibe F., Nawathe D.R. and Bot C.J. (1993). Serological prevalence of brucellosis in
sheep, goats and human beings in Maiduguri metropolis. *Tropical Veterinarian*. 11:27-23.

Brotiv V.A. and Mel'nikova A.l. (1979). Transmission of *Brucella abortus* by *Trichinella spiralis* experiments on guinea-pigs. *Veterinary Bulletin.* 49: 3789.

Cargill C., Lee K. and Clark I. (1985). Use of an enzyme-linked immunosorbent assay in a bovine brucellosis eradication program. *Australian Veterinary Journal.* 62(2):49-52.

Chary J.F.C. (1970). Contribution l'etude de la brucellose du chien. Une enquAte serologique. *PhD. Thesis.* Alfort, Paris, France : *Ecole National Veterinaires.* 53-58.

Cheville N.F., Stevens M.G., Jensen A.E., Tatum F.M. and Halling S.M. (1996). Immune responses and protection against infection and abortion in cattle experimentally vaccinated with mutant strains of *Brucella abortus*. *American Journal of Veterinary Research.* 54(10): 1591-1597.

Chukwu C.C. (1987). Studies on sero-prevalence of bovine brucellosis in Enugu and Nsukka, Nigeria. *Zaria Veterinarian*. 2: 383-387.

Cloeckaert A., Verger J.M., Grayon M. and Grepinet O. (1996). Brucellosis. *Journal of Medical Microbiology* 45: 200-205.

Collard P. (1967). Antibodies against *brucellae* in the sera of healthy persons in various parts of Nigeria. *West African Medical Journal*. 9:172-174.

Collier J.R. and Molello J.A. (1964). Comparative distribution of *Brucella abortus, Brucella melitensis* and *Brucella ovis* in experimentally infected pregnant sheep. *American Journal for Veterinary Research.* 25:930-934.

Cook I., Campbell R.W. and Barrow G. (1966). Brucellosis in North Queensland rodents. *Australian Veterinary Journal*. 42: 5-8.

Corbel M.J. (1973). The direct fluorescent antibody test for detection of *Brucella abortus* in bovine abortion material. *Journal of Hygiene.* 71: 123-129.

Corbel M.J., Gill K.P.W. and Thomas E.L. (1978). Methods for the identification of *Brucella*. *Ministry of Agriculture, Fisheries and Food* . Surrey, Weybridge publication. No. RVC 22.

Corbel M.J., Stuart F.A. and Brewer R.A. (1984). Observations on serological cross-reactions between smooth *Brucella* species and organisms of other genera. *Developments in Biological Standardization.* 56: 341-348.

Crawford R.P., Huber J.D. and Adams B.S. (1990). Epidemiology and surveillance. *Animal brucellosis*. 91: 131-151.

Cruickshank A., Duguid J.P., Marmoin B.P. and Swain R.H.A. (1975). Brucellosis. *Textbook of Medical Microbiology*. 12th edition. 1: 290-390.

Cunningham B. (1977). A difficult disease called brucellosis. In: Crawford RP, Hildago RJ, *Bovine Brucellosis International Symposium*, Texas A&M University Press. 687-711.

Davies G. and Casey A. (1973). The survival of *Brucella abortus* in milk and milk products. *British Veterinary Journal.* 129: 145-353.

Dekeijzer P. (1981). *Brucella* biotypen in Belgi. *Landbouwtijdschrift.* 34: 1513-1520.

Dimitrov T.S., Panigrahi D. and Emara M. (2004). Seroepidemiological and microbiological study of brucellosis in Kuwait. *Medical Principle Practioner*. 13(4): 215-219.

Duguid J.P., Marmion B.P. and Swain R.S.A. (1984). Microbial infections. In : *Medical Microbiology*. 13th Edition. 1: 9-11.

Earnshaw W.V. and O'Brein P.J. (1928). *Annual Report of Veterinary Department*. Nigeria.

Enright F.M. (1990). The pathogenesis and pathobiology of *Brucella* infection in domestic animals. *Animal brucellosis*. 301-320.

Enright F.M., Walker J.V., Jeffers G. and Deyoe B.L. (1984). Cellular and humoral responses of *Brucella abortus*-infected bovine fetuses. *American Journal of Veterinary Research.* 45(3): 424-430.

Erasmus J.A. (1986). Examination of Lochia as an aid to the early diagnosis of bovine brucellosis. *Journal of South African Veterinary Association.* 57(4): 225-226.

Esuruoso G.O. (1974). Bovine brucellosis in two Southern States of Nigeria II. Incidence and implications of infection in range cattle. *Bulletin Epizootic Disease Africa*. 22: 34-35.

Esuruoso G.O. (1979a). Current situation in Nigeria and preliminary evaluation of the problem costs and benefits of a proposed brucellosis control programme for the country. *Veterinary Epidemiology and Economics*. 644-649.

Esuruoso G.O. (1979b). Control of Bovine brucellosis in Nigeria. *Paper presented at the 2nd International Symposium on Veterinary Epidemiology and Economics.* Camberra; Australia.

Eze E.N. (1977a). Brucellosis in Nigeria. A review. *Bulletin of Animal Health and Production in Africa.* 25: 371-379.

Eze E.N. (1977b). The fate of *Brucella abortus* and associated antibodies in traditionally soured milk. *Bulletin of Animal Health and Production in Africa*. 23(1): 5-8.

Eze E.N. (1978). Isolation of *Brucellae* from the Nigerian livestock and typing of such isolates. *Bulletin of Animal Health and Production in Africa*. 26:29-36.

Falade S. (1974). *Brucella* agglutination antibodies in the sera of persons dwelling in Ibadan and surrounding districts. *Journal of Nigerian Veterinary Medical Association*. 3: 21-23.

Falade S. (1980). Caprine brucellosis. Serological studies and objectives for control in Nigeria. *Bulletin of International Epizootics*. 92: 111-127.

Falade S. (1981). Studies of *Brucella melitensis* Rev. 1 vaccine in goats. *Veterinary Medicine Bulletin.* 28: 749-758.

Farouk U.M. (2006). Sero-prevalence of bovine brucellosis in cattle herds in Jigawa State, Nigeria. *MSc Thesis*. Ahmadu Bello University, Zaria, Nigeria. 12-28.

Fensterbank R. (1978). Congenital brucellosis in cattle associated with localization in a hygroma. *Veterinary Record*. 103: 283-284.

Forbes L.B. (1990). *Brucella abortus* infection in 14 farm dogs. *Journal of the American Veterinary Medical Association.* 196(6): 911-916.

Franz D.R., Jahrling P.B. and Friedlander A.M. (1997). Clinical recognition and management of patients exposed to biological warfare agents *Journal of the American Medical Association.* 278(5): 399-411.

Halle P.D. and Ajogi I. (1997). Brucellosis in Nigeria: A review of recent developments. *Israel Journal of Veterinary Medicine.* 52(4): 125-131.

Hellmann E., Staak C. and Baumann M. (1984). Bovine brucellosis among two different cattle populations in Bahr el Ghazal province of southern Sudan. *Tropenmedizien und Parasitologie.* 35: 123-12.

Henry N.K., Wilson W.R. and Henley J.O. (1998). Infections caused by Brucella, Francisella, Tularensis, Pasteurella, Yersinia species and *Bordetella pertussis*. In; *Stein's Internal Medicine*, 5th edition. 1604-1607.

Herr S., Ribeiro L.M.M. and Chapparro F. (1990). Persistent infection with *Brucella abortus* biotype 1 in a cow. *Journal of the South African Veterinary Association.* 61(2): 77.

Hill W.K.W. (1963a). Die gleichzeitige Anwendung mehrere serologische Untersuchungsverfahren dei der Diagnose der Brucellose de Rinder unter besondere BerAcksichtigung der Differentzierung Zwischen Impf-und Infektionsreakionen. *Zentralblat fAr Veterin rmedizin.* 10: 127-172.

Hill W.K.W. (1963b). Standardization of the Complement Fixation Test for Brucellosis. *Bulletin de l' Office International des Epizooties*. 60: 401-417.

Hill W.K.W. (1966). Een onderzoek over de waarde van de Abortus Bang-Ringtest bij melkmonsters uit melktanks. *Tijdschrift voor Diergeneeskdunde.* 14: 885-895.

Hook E.W. (1990). Salmonella species (including typhoid fever). In: Mandell GL, Douglas RG and Benietti JE. *Principles and practice of infectious diseases*, 3rd Edition. New York, Churchill Livingstone. 1700-1716.

Hoover D.L. and Friedlander A.M. (1997). Medical Aspects of Chemical and Biological warfare. In : *Textbook of Military Medicine*. 513-521.

Hope R.A. (1998). Oxford Handbook of Clinical Medicine. 4th edition. 13-18. Oxford University Press. 13-18.

Joni Scheftel (2003). Brucellosis. *Compendium*. 25(11): 846-852.

Jubb K.U.F. and Kennedy P.C. (1963). Brucellosis. In : *Pathology of Domestic animals.* New York, USA. Academic Press. 124-127.

Keer W.R., Pearson J.K.L. and Rankin J.E.F. (1959). The Bovine udder and its agglutinins. British Veterinary Journal. 115: 105-109.

Kerimov C.H. (1983). Survival of *Escherichia coli* and *Brucella abortus* in cattle manure. Veterinariya. Moscow, USSR. 12: 23.

Kerkhofs P., Bottom Y., Thiange P., Dekeser P. and Limet J.N. (1990). Diagnosis of bovine brucellosis by enzyme immunoassay of milk. *Veterinary Microbiology.* 24(1): 73-80.

King N.B. (1957). The survival of *Brucella abortus* (USDA strain 2308) in manure. *Journal of the American Veterinary Medical Association.* 131: 349-352.

Kiss Z. (1971). Value of antiglobulin test in brucellosis eradication in a one-year field
study. *Veterinary Bulletin.* 5609.

Kocak I., Dundar M., Culhaci N. and Unsal A. (2004). Relapse of brucellosis simulating testis tumor. *International Journal of Urology*. 11(8): 683-5.

Levieux D. (1974). Bovine immunoglobulins and brucellosis. Activity of serum IgG_1, IgG_2 and IgM in agglutination, Coombs, Complement Fixation and Rose Bengal Tests. *Annales de Recherches Veterinaires* 5: 343-353.

Levine M.M., Grados O., Gilman R.H., Woodward W.E., Soliplaza R. and Waldman W. (1978). Diagnostic value of the widal test in areas endemic for typhoid fever. *American Journal for Tropical Medicine and Hygiene*. 27: 795-800.

Luchsinger D.W. and Anderson R.A. (1967). Epizootiology of Brucellosis in a flock of sheep. *Journal of the American Veterinary Medical Association.* 150: 1017-1021.

MacMillan A. (1990). Conventional serological tests. *Animal brucellosis.* 153-197.

Manthei C.A. and Carter R.W. (1950). Persistence of *Brucella abortus* infection in cattle. *American Journal for Veterinary Research.* 11: 173-180.

Manthei C.A. and Deyoe B.L. (1970). Brucellosis. In: Gibbons W.J., Catcott E.J. and Smithcors J.F. (editors), Bovine Medicine and Surgery and Herd Health Management. *American Veterinary Publications*. 104-121.

Mantur B.G., Akki A.S. and Mangalgi S.S. (2004). Childhood brucellosis – a microbiological, epidemiological and clinical study. *Journal of Tropical Pediatrics*. 50(3): 153-157.

Mathur T.N. (1967). Isolation of *Brucella abortus* from goats and sheep in the Punjab. *Indian Journal of Veterinary Science and Animal Husbandry.* 37: 277-286.

McCaughey W.J. and Purcell D.A. (1973). Brucellosis in bulls. *Veterinary Record.* 93: 336-337.

Ministry of Agriculture, Kaduna State, (2008). Population of cattle in Kaduna State (Personnal information).

Montaraz J.A. and Winter A.J. (1986). Comparison of living and non-living vaccines for *Brucella abortus* in BALB/c mice. *Infection and Immunity.* 53: 245-251.

Moore C.G. and Schnurrenberger P.R. (1981). A review of naturally occurring *Brucella abortus* infections in wild mammals. *Journal of the American Veterinary Medical Association.* 179: 1105-1112.

Morgan W.J.B. (1967). The Serological diagnosis of bovine brucellosis. *Veterinary Record.* 80: 612.

Morgan W.J.B. (1970). Brucellosis. In : Fertility and infertility in Domestic Animals. JA LAING (Editor). 2nd edition. Bailliere Tindall and Cassel, London. 241-268.

Morgan W.J.B. and Corbel M.J. (1990). Brucella infections in man and animal. In: Smith G.R. and Easman C.S.F. (editors), *Topley and Wilson's principles of bacteriology, virology and immunity.* 8th edition. US. Edward Arnold. 3: 548-570.

Nayamanda A.N. (1994). A serological investigation of bovine brucellosis in cattle slaughtered at Bodija abattoir, Ibadan. *A dissertation for the degree of Master in Preventive Veterinary Medicine.* University of Ibadan. 28-40.

Nicoletti P. (1967). Utilization of the card test in brucellosis eradication. *Journal of American Veterinary Medical Association*. 151: 1778-1783.

Nicoletti P. (1980). The Epidemiology of Bovine Brucellosis. Advance in Veterinary Science *and Comparative Medicine.* 24: 69-98.

Nuru S. (1974). Infectious bovine abortion in Northern Nigeria, *PhD Thesis.* Ahmadu Bello University, Zaria. 91-103.

Nuru S. (1975). Serological survey of bovine brucellosis in slaughtered cattle in North Central State of Nigeria. *Journal of Nigerian Veterinary Medical Association.*
4: 1-13.

Nuru S. and Dennis S.M. (1975). Serological survey of brucellosis in slaughtered Cattle in North Central State of Nigeria. *Journal of Nigerian Veterinary Medical Association*. 4: 3-8.

Ocholi R.A. (1990). Prevalence of *Brucella* antibodies in Fulani cattle herds in Kaduna State. *M.Sc. Thesis*, Ahmadu Bello University, Zaria. 24-26.

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

Ocholi R.A., Kwaga J.K.P., Ajogi I. and Bale J.O.O. (2004). Phenotypic characterization of *Brucella* strains isolated from livestock in Nigeria. *Veterinary Microbiology*.
103: 47-53.

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

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

Oladosu L.A., Falade S. and Akpokodje U. (1984). Equine brucellosis in Nigeria. *Zaria Veterinarian*. 1: 129-133.

Olascoaga R.C.(1976). Serologic diagnosis of brucellosis. *Boletin de centro Panamericano de zoonosis Ramos mejia*. Buenos Aires, Argentina. 28: 165-192.

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

Oyedipe E.O., Buvanendran V. and Eduvie L.O. (1981). Factors affecting the reproductive performance of Fulani Cattle. *NAPRI Seminar.*

Peelman J. and Dekeyser P. (1987). De verspreiding van de *Brucella* infectie bij viaamse weeknemers professioneel in contact met runderen. *Vlaams Diergeneeskundug Tijschrift.* 56: 314-23.

Philippon A., Renoux G. and Plommet M. (1970). Experimental Bovine brucellosis. Vaginal excretion of *Brucella abortus* before and after calving. *Annales de Recherches Veterinaries.* 1: 215-224.

Pietz D.E. (1970). Brucellosis Epidemiology course for Veterinarians. *United States Department of Agriculture.* Iowa. 37-41.

Plommet M. (1972)). Survie de *Brucella abortus* dans le lisier de bovins. Desinfection par le xylene. *Annales de Recherches Veterinaire.* 3: 621-632.

Plommet M. (1991). New animal vaccines. In: Emel TAmbay, SAleyha Hilmi, czdem Ang, (editors) *Brucella* and brucellosis in man and animals. *Proceedings of a symposium held in Izmir, Turkey.* 77-85.

Prior M.G. (1976). Isolation of *Brucella abortus* from two dogs in contact with bovine brucellosis. *Canadian Journal of Comparative Medicine*. 40: 177-118.

Rankin J.D. and Taylor R.J. (1969). A study of some diseases hazards which could be associated with the system of applying cattle slurry to pasture. *Veterinary Record.* 85: 578-581.

Ray W.C. (1979). Brucellosis (due to *Brucella abortus* and *Brucella suis*). In: Steele J., (edition) Handbook Series in Zoonoses. Section A: Bacterial, Rickettial and Mycotic Diseases. Roca Raton. Florida, USA: CRC Press. 99-127.

Roberts S.J. (1971). *Veterinary obstetrics and genital disease*. 2nd edition. Ithaca, New York. 154-156.

Robertson F.J., Milne J., Silver C.L. and Clark H. (1973). Abortion associated with *Brucella abortus* (Biotype 1) in the TB Mare. *Veterinary Record.* 92: 480-481.

Rodostits O.M. and Blood C. (2000). Veterinary Medicine. *A textbook of the diseases of cattle, sheep, pigs, goats and horses*. W.B. Saunders Co. Ltd. 867-882.

Rose J.E. and Roepke M.H. (1957). An acidified antigen for detection of nonspecific reactions in the plate agglutination test for bovine brucellosis. *American Journal for Veterinary Research.* 18: 550-55.

Rossi C. and Cantini G. (1969). Mercaptoethanol test in the diagnosis of bovine brucellosis. *Veterinary Bulletin.* 39: 4874.

Ruppanner R., Meyer M.E., Willeberg P. and Behymer D.E. (1980). Comparison of the enzyme-linked immunosorbent assay with other tests for brucellosis, using sera from experimentally infected heifers. *American Journal for Veterinary Research.* 41: 1329-1332.

Schnurrenberger P.R., Walker J.F. and Martin R.L. (1975). *Brucella* infections in Illinois Veterinarians. *Journal of the American Veterinary Medical Association.* 167: 1084-1089.

Schnurrenberger P.R., Brown R.R., Hill F.P., Scanlan C.M., Altiere J.A. and Wykoff J.T. (1985). *Brucella abortus* in wildlife on selected cattle farms in Alabama, *Journal of Wildlife Disease.* 21(2): 132-136.

Snedecor G.W. and Cochran W.G. (1980). *Statistics Methods*. 7th Edition. Iowa State University Press.

Stemshorn B.W. and Nielsen K.H. (1980). Evaluation of an enzyme labeled antiglobulin test for anti-*Brucella* immunoglobulin G among three cattle populations. *American Journal for Veterinary Research*. 41: 779-784.

Stevens M.G. (1995). Comparative analysis of immune responses in cattle vaccinated with *Brucella abortus* Strain 19 or Strain RB51. *Veterinary Immunology and Immunopathology.* 44: 223-235.

Stevens M.G. and Olsen S.C. (1996). Antibody responses to *Brucella abortus* 2308 in cattle vaccinated with *Brucella abortus* RB51. *Infection and Immunity.* 64(3): 1030-1034.

Stevens M.G., Olsen S.C., Pugh G.W.(Jr) and Palmer M.V. (1994). Immune and pathologic responses in mice infected with *Brucella abortus* 19, RB51, or 2308. *Infection and Immunity.* 62: 3206-3212.

Sutherland S.S. (1984). Evaluation of the enzyme-linked immunosorbent assay in the detection of cattle infected with *Brucella abortus*. *Veterinary Microbiology.* 10(1): 23-32.

Sutherland S.S., Evans R.J. and Bathgate J. (1986). Application of an enzyme-linked immunosorbent assay in the final stages of a bovine brucellosis eradication program. *Australian Veterinary Journal.* 63(12): 412-415.

Taliani G., Bartoloni A. and Tozzi A. (2004). Lumbar pain in a married couple who likes cheese: *Brucella* strikes again!. *Clinical Experimental Rheumatology*. 22(4): 477-80.

Tanwani S.K. and Pathak P.N. (1971). Studies on abortus bang ring test: Factors affecting the nature of reaction in different milk samples. *Indian Journal for Animal Science.* 41: 1037-1040.

Thomsen A. (1950). Experimental studies on the incubation period of infectious abortion in cattle. *British Veterinary Journal.* 106: 41-45.

Tobias L., Schurig G.G. and Cordes D.O. (1992). Comparative behavior of *Brucella abortus* Strain 19 and RB-51 in the pregnant mouse. *Research in Veterinary Science.* 53: 179-183.

Topley C. and Wilson M. (1998). Brucellosis. *Principles of bacteriology, virology and immunity.* 8th edition. United States Edward Anold. (3): 341-353.

Verger J.M. and Grayon M. (1985). Present classification of the genus *Brucella melitensis* (Verger & Plommet). *Dordrecht: Martinus Nijhoff*. 41-47.

White S.R. and Eitzen E.M. (2000). Hazardous materials exposure In: Emergency medicine: *A comprehensive study guide.* 5th edition. 1201-1214.

WHO (1986a). Joint FAO/WHO Expert Committee on Brucellosis (5th Report). *World Health Organisation Technical Report* Series. Geneva. 646: 86-88.

WHO (1986b). Joint FAO/WHO Expert Committee on Brucellosis (6th Report). *World Health Organisation Technical Report* Series. Geneva. 740: 71-74.

WHO Weekly Epidemiological Record, (1995). Brucellosis associated with unpasteurized milk products abroad. United kingdom. 70(43): 13-15.

Wray C. (1975). Survival and spread of pathogenic bacteria of veterinary importance within the environment. *Veterinary Bulletin.* 45: 546.

Yinnon A.M. and Morali G.A. (1993). Effect of age and duration of disease on clinical manifestation of brucellosis – a study of 73 consecutive patients In Israel. *Israel Journal of Medical Science.* 29: 11-16.

Young E.J. (1995). *Brucella* species In: *Principles and Practices of Infectious Diseases.* 3rd edition. 2053-2057.

APPENDIX 1

QUESTIONNAIRE SAMPLE

Please tick [ ] or write in the box or on line as appropriate

BOVINE BRUCELLOSIS SCREENING AMONG MIGRATORY CATTLE HERDS IN KADUNA STATE

Name of owner----------

Location of herd----------

Is it a migratory herd? Yes [ ] No [ ]

If yes, from where?----------

Where next? ----------

Number of animals in the herd----------

Number of females (>3yrs) ------- (2-3yrs)--------(1-2yrs)---------(<1yr)----------

Number of males (>3yrs) ---------- (2-3yrs)---------(1-2yrs)----------(<1yr)----------

Have you vaccinated your herd against brucellosis before? Yes [ ] No [ ]

Have you noticed any of the following signs in your herd before?

Signs	Yes	No	No. of animals
1. Abortion	[ ]	[ ]	[ ]
2. Still-birth	[ ]	[ ]	[ ]
3. Weak calves	[ ]	[ ]	[ ]
4. Infertility	[ ]	[ ]	[ ]
5. Hygroma/Swollen knees	[ ]	[ ]	[ ]
6. Orchitis	[ ]	[ ]	[ ]

If Yes to any signs, what treatment did you give?----------

Do you seek veterinary assistance when your animals are sick? Yes[ ] No[ ]

Do you cull sick animals? Yes[ ] No[ ]

How did you dispose the dead carcass? _____

Have you had any replacement animal? Yes [ ] No [ ]

Do you quarantine newly purchased animals before addition to herd? Yes[ ] No[ ]

Is there contact between your herd and another herd? Yes [ ] No [ ]

Do you boil your milk before you drink? Yes[ ] No[ ]

Do you know that brucellosis can be transmitted from animal to man? Yes[ ] No[ ]

THANK YOU.