CAMEL BRUCELLOSIS IN THREE NORTHERN STATES OF NIGERIA

BY

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DECLARATION

I declare that this thesis is a product of my handwork. The work has neither in part nor whole been submitted anywhere else for the award of a similar or any other degree, certificate and diploma. All quotations are specifically acknowledged by means of references.

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The thesis entitled *Camel Brucellosis in three Northern States of Nigeria* by Dr. N. B. Adamu meets the regulations governing the Award of the Degree of Master of Science in Veterinary Public Health and Preventive Medicine of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to

my dear Parents,

Mallam Bala Adamu & Mallama Rabi Bala Adamu

and to

members of my beloved family

Mallama Asmau, Hamidat, Muhammad Ashafa

and those yet unborn.

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ABSTRACT

Serological and bacteriological investigation for Brucellosis in slaughtered camels was carried out to determine the prevalence of the disease, isolate and biotype the *Brucella* species responsible for the disease in one-humped camels (Dromedary camels) in three Northern States (Kaduna. Kano and Borno) of Nigeria. Two serological tests viz; Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) were used as screening and standard tests respectively to test the sera obtained from the blood of camels. Milk samples were tested by Milk Ring Test (MRT) while heperinized whole blood, milk and foetal Stomach contents were cultured using specific *Brucella* medium. Direct Ziehl-Neelsen smear Stain examination of urine and faecal samples were made.

Of the overall total of 1047 sera samples tested. 291(27.79%) were RBPT positive while 200(19.10%) were SAT positive. Out of 215. 400 and 432 serum samples tested in Kaduna. Kano and Borno States respectively. 60(27.91%). 97(24.25%) and 134(31.02%) were positive by RBPT while 40(18.60%). 50(12.50%) and 110(25.46%) were positive by SAT respectively. Milk Ring Test (MRT) revealed overall prevalence rate of 12(17.65%) out of the 68 milk samples tested, The highest number and percentage by MRT reactors 6(21.43%) was from Borno State, followed by Kaduna State 2(18.18%) and then Kano State 4(13.79%).

In all the states sampled, female camels were more at risk of contacting Brucellosis compared to male camels, female camels in Kano, Kaduna and Borno States have relative risk values of 1.31. 1.26 and 1.02 compared to their male counter-parts with relative risk of 0.76. 0.70 and 0.98 respectively. There is however, no significant difference between the rates of infection in male and female camels in Kaduna. Kano and Borno States when data was subjected to X^2 analysis at P = 0.05.

None *of* the blood, milk and foetal stomach contents samples cultured yielded *Bruce/la* organisms. However, bacteria such as *Staphylococcus* sp. *Streptococcus* sp. *Bacillus* sp. *Pasteurella* sp. *Corynebacicrium* sp. *Escherichia coli, Klebsiella* sp and *Proteus* sp were however isolated from these samples.

Based on these findings, it is recommended that further studies should be earned out to isolate the *Brucella* species that might be responsible for brucellosis in other camel raising areas in Nigeria. Such isolates when obtained should have their strains determined and biotyped. Further studies on camel brucellosis especially in range camels in addition to slaughtered camels should be encouraged. Brucellosis control programmes for Nigeria should embrace the camel.

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LIST OF ABBREVIATION

B	-	Blood
FSC	-	Foetal Stomach Contents
ml	-	Millilitre
RR	-	Relative Risk
mcl	-	Microlitre
X^2	-	Chi-square
w/v	-	Weight per volume
+VE	-	Positive
-VE	-	Negative
%	-	Percentage
+	-	Isolate
-	-	No Isolate
^oC	-	Degree Celsius

Chapter one

1.1 INTRODUCTION

The Arabian one-humped Camel (*Camelus dromedarius*) belongs to Family: Camelidae, Order: Artiodactyla (even-toed ungulates), Suborder: Tylopoda (pad-footed), Genus: Camelus (True or old world camels), Specie: *Camelus dromedarius* (Dromedary camel or one-humped or Arabian camel). It is an important livestock specie that uniquely adapts to desert and semi-desert areas. It is a primary inhabitant of the northern half of Africa, the Middle East, Pakistan, India and some parts of West Africa, including Nigeria. In Nigeria the one- humped camel is found in the Northern States namely: Adamawa, Bauchi, Borno, Yobe, Kano, Kaduna, Katsina, Kebbi, Gombe, Plateau, Sokoto and Zamfara. Bourn *et al* (1994) estimated the population of these animals in Nigeria at about 90,000 herds.

Despite the advent of modern transportation system and farm machineries, the camel is important as a traditional means of transportation and for agricultural purposes in Northern parts of Nigeria. The camel plays a vital role in the agro-industries economy in carting farm produce, threshing, traction and transportation. It provides highly nutritious milk, meat and by-products such as wool, hair and hides. Camel milk is very rich in vitamin C and is considered to have therapeutic properties (Burgemeister, 1978). The meat from young camel in good condition has been described as equal to the best of beef (Mc Knight, 1969). The resentment of people towards camel meat is dwindling; as much of the

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meat in various parts of Nigeria is actually derived from camels among other livestock species (Anon. 1984). Camel has an added advantage of feeding in areas where other species do not thrive and perhaps do not survive. Sustenance can be obtained by the camel from almost anything that grows (Mc Knight. 1969). It can survive on poor quality native pasture and because it has long legs and neck, is capable of browsing on trees and shrubs which are beyond the reach of other animals.

Diseases are major factors limiting camels production in most countries where they are found. A number of disease conditions have been described in one-humped camel in Nigeria (Garba and Maigandi. 1995). Some of the disease conditions described are zoonotic and can cause great economic losses through abortion, infertility, reduced weight gain and death. One of such diseases is brucellosis, a zoonosis usually encountered through ingestion or contact with contaminated animal or animal by-products such as meat, milk, hide or wool. Brucellosis has also been documented in other livestock species in Nigeria and also in other African countries (Eze, 1977; Chukwu, 1985; Ocholi *et al*, 1993). There is however a dearth of information on camel brucellosis in Nigeria.

This present study is therefore designed to fill this vacuum and to investigate the role of camels in the epidemiology of brucellosis in Nigeria.

1.2 Objectives of the Study

The objectives of the study are:

i) To determine the prevalence, by serology, of camel brucellosis in camel rearing states of Kano, Kaduna and Borno states of Nigeria.

ii) To determine the prevalence by cultural isolation of brucella, in camels in these chosen States.

1.3 Significance of the Study

The present study would help in elucidating the presence and epidemiology of Brucellosis in camels in the selected Northern states of Nigeria so that necessary control measures and interventions could be instituted.

Chapter two

LITERATURE REVIEW

2.1 Historical Perspective on the Genus Brucella

Brucellosis, also known as Malta, Mediterranean or Undulant fever in man and Bang's disease in animals, is zoonotic and a disease of warm blooded animals caused by bacterium of Genus *Brucella*. The Genus has six well documented species namely: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae*. The species are further divided into biotypes (Alton, *et al*, 1975).

Brucellosis was first recognized on the Mediterranean Island of Malta. Sir David Bruce (1887) was the first to isolate the causative organism then known as *Micrococcus melitensis* from the spleens of dying military and naval officers on Her Majesty's services. Later on, in Denmark, Bang and Stribolt (1897) discovered a causative agent of abortion from aborted bovine foetuses and foetal membrane and named it "Bacillus of abortion". The same organism was subsequently recognized and named *B. abortus*. A similar organism was also reported by Horrocks in 1905 from the milk and urine of goats and was named *Brucella melitensis*.

The relationship of the disease in man and animals was not well understood until when the disease was of great concern to the British Government which then set up the Mediterranean Fever Commission mandated to look into effective control measures. By 1905, Zammit, a Maltese Bacteriologist, demonstrated *Brucella* agglutinins in the sera of goats and also isolated the organism from blood and spleens of slaughtered goats at an abattoir.

In the same year, Horrocks isolated a similar organism from the milk and urine of apparently healthy goats. After series of investigations, the Commission came up with the conclusion that the sources of human infection was the maltese goats excreting *Brucella* organisms into their milk, this was substantiated by the decline in infection rate sequel to a prohibition of the consumption of raw goats milk.

Other species of *Brucella* of importance have since been isolated. For instance, *Brucella ovis*, a cause of epididymitis and abortion in sheep was isolated by Buddle and Boyes (1953) in New Zealand and Australia. *Brucella neotome* was isolated by Stoener and Lackman (1957) from the desert wood rat (*Neotoma lepida*) in Arizona U.S.A. and *B. canis* the cause of canine epizootic abortion and epididymitis, was isolated from Beagle dogs in the same U.S.A. by Carmichael (1967). Other isolates were *B. suis* isolated by Trauma (1914) from aborted porcine foetuses in a premature delivery. The same organism was recognised by Keefer (1924) as the cause of Undulant fever in man in the United States.

For many years there was confusion regarding the nomenclature of these organisms for not only are they morphologically identical but they differ only in small degree in their cultural requirements and serological reactions. Furthermore, different strains show varying biochemical reactions. Evans (1918) was however able to point out the similarity of *Micrococcus melitensis* and Bang's bacillus but stated that they could be separated serologically. Meyer and Shaw (1920) suggested that the genus be named *Brucella* in honour of Bruce, the first discoverer of the disease in man.

2.2 Epidemiology of Brucellosis

The disease is widespread in nature and is of major economic and public health importance in most countries where it occurs (Eze, 1978; Alton, 1981). From the point of view of human health, the disease is important because the organism causes the characteristic intermittent waves or undulating fever in man. Incidence of brucellosis in animals varies considerably between herds, between areas and between countries (Meyer, 1964; Nuru, 1974). The epidemiology of a disease such as brucellosis is not static, as changes in agent, host or environment affects the complex interrelationship that exist, thereby influencing the epidemiology of the disease.

2.2.1 Aetiology

The species of Brucella known to infect camel are *B. abortus* (Thorpe *et al.*, 1965) and *B. melitensis* (Bilal *et al.*, 1991; Gameel *et al.*, 1993).

Brucella is a generic name for a group of small non-motile, non-spore forming, pleomorphic coccobacilli or short gram-negative rods with straight or slightly convex sides and rounded ends measuring 0.5 - 0.7 wide by 0.6 - 1.5 milli-micron long. The organism occur singly or more rarely in groups of short chains or cluster.

2.2.2 Resistance of Brucella to Environmental Factors

Brucella organisms are susceptible to sunlight and common disinfectants. The organisms thrive in a wide range of hydrogen ion concentration (pH). The maximum

multiplication was observed between pH 6.6 and 7.4 with growth as from between pH 6.1 - 8.4 (Marr and Wilson, 1951). They have been found to survive drying in dust or soil for up to 10 weeks and in water for 35 days when not exposed to sunlight (Merchant and Packer, 1975). According to FAO/WHO (1986), the organisms can survive for 120 days in bovine faeces, 913 days in liquid manure, 200 days in uterine exudate and at least 75 days in aborted foetuses provided the temperature is kept near 0°C though Plommet (1973) estimated the survival of *B abortus* for up to 8 months.

Survival of the organisms is prolonged at low temperature and they can remain viable for many years in frozen tissues. Kronenwett *et al* (1954) studied the thermal death point of the organisms and found that pasteurization at 160°F for about 4 seconds would readily kill any *Brucella* present in a raw milk. *B. abortus* dies out fairly rapidly in raw milk kept under room temperature. Similarly, it was documented that brucellae in milk do not survive the long distance from the field to the laboratory even when transported in 1% boric acid solution (Eze,1978). Attributable factors could be that the organisms are moderately sensitive to acid so that they tend to die out in sour milk and in cheese that have undergone lactic acid fermentation for sometime. They are likely to be destroyed by the acid secretions of the stomach (Cruickshank *et al*., 1973). The organisms have been isolated from butter, cheese and ice-cream prepared from infected milk. The organism survives in carcass meat, pork and ham and may remain viable after refrigeration for several weeks and after pickling. They are however killed in 10 minutes at a temperature of 60°C (Cruickshank *et al*., 1973).

2.2.3 Relationship between Brucellae and other organisms

The exposure of the host to some organisms can provoke diagnostically significant titres in antibodies cross-reacting with Brucella antigens used in diagnostic tests such as the agglutination, complement fixation, precipitation and antiglobulin test (FAO/WHO, 1986). For instance, *Francisella tularensis* cross-react with *B abortus* as shown by immunodiffusion technique by Ohara *et al*, (1974). *Yersinia enterocolitica* serotype IX has been reported to cross-react extensively with various *Brucella* species (Marx *et al*., 1975). This cross-reaction was demonstrated by means of agglutination tests, complement fixation tests and immunodiffusion techniques using hyperimmune sera. *Brucella spp* have also been demonstrated to cross-agglutinate with *Pasteurella* sp (Mallman, 1930) and *Vibrio* (*Campylobacter*) sp (Morse *et al*, 1953). Furthermore, Eisele *et al* (1946) reported increases in *Brucella* titres in human patients vaccinated against typhoid and cholera, and Corbel (1975) showed that natural infection of cattle with *Salmonella urbana* coincided with low grade increase in titres to *B. abortus*. *Escherichia coli* has been shown to cause false serological reaction to *B. abortus* by unknown mechanism (Nielsen *et al*., 1980).

The methods developed to assist in differentiation of these infections have tended to rely on the detection of antibodies to two antigens characteristic of *Y. enterocolitica* IX. These are flagella (H) and entero- bacterial common antigen (ECA). In addition, Mitral and Tizard (1979) have reported that in general, homologues titres tend to exceed heterologues titres. Thus, in *Brucella* infected animals *Brucella* O agglutinin titres tend to exceed *Yersinia* O agglutinin titres while the reverse is the case in Yersiniosis. The latter

phenomenon they believe is rather more consistent than the presence of antibodies to *Yersinia* specific antigens since *Yersinia* flagellation is not a stable characteristic.

According to FAO/WHO (1986) immunoelectrophoresis studies of the antigenic relationship between *Brucella* protein antigen and proteins from other organisms have indicated that there are no common protein antigens, so that it can be accepted that antibodies against *Brucella* protein antigens are *Brucella*-specific. Therefore, according to them, immuno-precipitation test can be useful in the differential serological diagnosis of brucellosis and other infections producing antibodies cross-reacting with *Brucella*.

2.2.4 Regional Distribution of Brucellosis

Brucellosis is distributed world wide. Several developed countries have however succeeded in eradicating the disease and others are well on the way to doing so (Alton, 1981). At the same time, some less developed countries are reporting an increased incidence of the disease (Chukwu, 1985; FAO/WHO, 1986; Ocholi *et al.*, 1993). In a review of the control of *B. melitensis* infection in sheep and goats, Alton (1987) reported that the disease is absent from Canada and the USA, most of Northern Europe and South East Asia, Israel, Australia and New Zealand. In camels, the incidence and prevalence of clinical brucellosis appear to be low, but serological reports indicate that it is widespread from Asia to African Countries (Abu-Damir *et al.*, 1984; Ajmal *et al.*, 1989; Yagoub *et al.*, 1990; Faraj *et al.*, 1991; Gameel *et al.*, 1993; Afzal and Sakkir, 1994;). In Nigeria there is

limited serological evidence of the prevalence of the disease in camels, the overall incidence is also still unknown (Okoh, 1979).

2.2.5 Host Range and Susceptibility

All domesticated animals are susceptible to *Brucella* species; cattle, sheep, goats, horses, camels, pigs and dogs have been incriminated as being natural host of brucellae (FAO/WHO, 1986). The secondary host usually is not important in the maintenance of the disease, the primary host is the reservoir of infection for each particular specie.

The various species of *Brucella* differ in the frequency in which they infect particular host animals. *Brucella abortus* is principally associated with cattle. It is also found in camel, deer, elk, reindeer, water buffalo, rabbit and mice (Thorpe *et al*., 1965). Fay (1972) reported that antibodies to *Brucella abortus* have been detected in sera from Eland, Herte beast, Wildbeast, Giraffe, Baboons and Buffaloes. Meyer (1977) has found foxes to be infected with *B. abortus*, while Condy and Vickers (1969) isolated *Brucella* from water buck and also from Eland in Rhodesia (now Zimbabwe). It has also been isolated from hygromas of cattle (Eze, 1978; Bale and Kumi-Diaka, 1981) and sero-prevalence evidence has been reported in horses (Bale and Kwanashe, 1984). Farm dogs are not generally considered to be a major reservoir of *B. abortus*, however, the organism has been isolated from dogs on farm where several cattle were serologically positive for brucellosis (Prior, 1976).

Dogs are primary hosts for *B. canis* but accidental infection has been documented in man (Cruickshank *et al* 1973).

Brucella melitensis principally affects sheep and goats, it transmits readily to other animals in contact with these species (Alton, 1987: Abdulkadir, 1989). Nevertheless, it has not been definitely established whether the infection can maintain itself in other animal species in the absence of infected small ruminants. Alton (1981) is of the opinion that it seems unlikely. Gameel *et al* (1993) isolated *B. melitensis* biovar I from camels in Libya.

Brucella neotomae, a rodent strain, has not been reported to infect other species of animals.

Brucella ovis displays high degree of host specificity as its primary host is the sheep, no natural infection has been reported in man and other animals.

Brucella suis infects pigs primarily but may be transmitted to man, cattle, horses, guinea pigs, mice, rabbits, dogs and fowl. Susceptibility of turkey, pigeon, pheasant, duck, goose and chickens (local and exotic) to *Brucella* disease has been reported (Emmel, 1930; Bale and Nuru 1982; Adesiyun and Abdu 1984).

2.2.6 Age, Breed and Sex Susceptibility

Brucellosis occurs in animals of all ages but the infection persist most commonly in sexually matured animals. The degree of susceptibility increases from calfhood to sexual maturity in cattle (Morgan, 1977). Calves born of reactor dams are usually seropositive for 4-6 months due to colostral antibodies and later usually become seronegative even though a

latent infection may exist in a small proportion of these calves (Blood and Radostits, 1989). These may remain unnoticed and potentially serve as a source of infection later.

Breeds of cattle are not associated with susceptibility of brucellosis infection: this was the conclusion drawn by Cedro *et al.* (1967) from their studies. Esuruoso (1974) however found N'Dama breeds of cattle which show varying degrees of resistance to trypanosomosis and streptothricosis to be equally susceptible to brucellosis. Agunloye *et al* (1988) indicated a relatively low infection rate in surveillance of 2 N'Dama breeding herd in Nigeria. Ajogi (1984) reported that Bunaji breed of cattle are more susceptible to *B. abortus* infection compared with Rahaji and Gudali than red Bororo, Adamawa Gudali and Sokoto Gudali.

Spink (1956) reported that brucellosis is practically a disease of males than females but concluded that there is no definitive proof that females are more resistance to the disease than males. Ajogi (1984) reported, on slaughtered cattle in Northern Nigeria, that females are more susceptible than males. Similarly, examination of 110 human serum samples (44 males, 66 females) for brucellosis Faraj *et al.* (1991) showed 29.1% of positive cases. The prevalence in males was higher (38.6%) than in females (22.7%). Likewise, Adekolu-John (1989) reported 22.3% of 394 humans sero-positive for brucellosis with higher prevalence among the adolescent and young adult males than females. Yagoub *et al* (1990) reported a contrasting report in camels with prevalence of 13.76% of 270 adult females compared to 4.94% of 1153 adult males in four areas of Eastern Sudan. Similar finding in caprine and

ovine species have shown that sero-prevalence is relatively higher in females than in males (Brisibe *et al.*, 1993; 1996).

2.2.7 Transmission

Generally, transmission of brucellosis is either through direct or indirect contact with brucellae organism. The FAO/WHO (1986) Expert Committee on Brucellosis reported a high rate of brucellosis infection in camels kept in close contact with other ruminants in Mongolia. Angus *et al* (1971) reported natural transmission of *B. abortus* from cattle to chicken in India while Gameel *et al* (1993) isolated *B. melitensis* biotype I from milk, aborted foetus and vaginal swab of camel in Libya. These demonstrated that camels can acquire infection not only from infected camels but also from contact with other domestic animals and vice versa.

Bilal *et al* (1991) reported close contact with camels, other ruminants and consumption of raw milk as the main risk factors identified in contracting brucellosis in human. Similarly, Cooper (1992) in a study to determine risk factors in transmission of brucellosis from animals to humans reported the greatest risk to be associated with indirect contact with animals (the consumption of unpasteurized milk products), as opposed to direct contact to animals. When specific animal products were considered of greatest risk was associated with the consumption of milk and butter milk "Laban" as opposed to cheese or uncooked liver. When specific animal species were considered, greatest risk was associated with products derived from sheep and goats as opposed to camels and cattle.

When direct contact with animal was considered, the study found a very high risk associated with assisting in animal parturition, but no significant risk associated with other direct unspecified) animal contact.

Brucella excretion by infected animals could be through colostrum or milk, genital discharge, urine, faeces, placentas, membranes and fluids, these and other major sources such as the aborted foetuses, the foetal membrane and uterine fluid are sources of infection to the remainder of the herd. The organism is found in greatest concentration in contents of the gravid uterus, foetus and placenta; therefore the primary hazard within a herd is the aborting animals in the first days of abortion, while at the time of abortion, the foetus and its membranes contain about 10^{14} and the fluids about 10^{12} bacteria (Alton, 1981). It is this material that provides the contamination of the environment and allows transmission to other animals. Infection can also be passed from infected dams to calves through *in utero* (Lapraik, 1975). Other sources of infection are; via the conjunctiva, the skin (broken or unbroken) and contamination of udder during milking (Blood and Radostits, 1989); or through the genital system from contaminated semen (Radwan *et al.*, 1984). Nuru (1974) and Alton (1981) reported that venereal transmission by natural service is rare. Salman and Meyer (1984) added that the chance of spread from the infected bull is very great if the semen is used for careless artificial insemination practices, than by being exposed by natural insemination.

Bruce (1962) indicated that there is possibility of mechanical transmission within a short distance by dogs or other scavengers carrying infected foetus or its placenta from one

herd to another or by personnel from a neighbouring herd. Meyer (1977) felt there is a possibility that flies congregating on infected materials such as foetus could mechanically transmit the organisms to nearby animals in the herd. *Stomoxys calcitrans*, *Tabanus spp* and *Musca domestica* have been shown to transmit the disease experimentally to cows, sow and goats (Wellman, 1952). *Brucella abortus* was cultured in large numbers from face flies (*Musca autumnalis*) for 12 hours after feeding on *Brucella* infected materials but not after 72 hours (Cheville *et al*., 1989). Similarly, a small percentage of ticks collected yielded the organism (Tovar, 1947; Thorpe *et al*., 1965). Heavy contamination of up-stream watering places could spread the infection (Bruce, 1962)

2.2.8 Management Practice

The system of husbandry as well as environmental condition greatly influence the spread of infection. Banerjee and Bhatty (1970) reported that the relationship between brucellosis and husbandry methods accounted for the differences in the prevalence rate of infection in institutional and local herds. In institutional flock, animal that aborts due to brucellosis tends to have high chance of infecting other animals within the flock (Nuru, 1975; Bale, 1980; Bale *et al*., 1982) than free range and nomadic type of husbandry animals which have less chance of remaining or returning to the contaminated environment for sometime. A cursory observation on the nomadic and semi-nomadic cattle herds may suggest this but a closer examination by Banerjee and Bhatty (1970) and Nuru (1974) revealed contrary findings. The former reported incidence of bovine brucellosis in private

fulani cattle herds to be higher (8.06%) than in government herds (3.74%). The latter rported 7.1% in fulani herds and 3.6% in government herds.

The husbandry methods and management practice in extensive husbandry conditions are primitive. Since proper sanitary measures are not practiced, in the event of an abortion occurring in a herd due to bruceilosis the aborted foetus, placental membranes and persistent vaginal discharges which are extremely rich in virulent *Brucella*, play an important role in a dissemination of the infection through contamination of grazing pastures and common water reservoirs (Manthei, 1968). The animals which have aborted or infected animals calving to term are never isolated, and are thus left to disseminate infection throughout the herd. This is why according to Salman and Meyer (1984) use of maternity pens, type of housing, source of replacement animals, livestock intermix, and use of artificial insemination are important management factors in the epidemiology of brucellosis. The incidence of brucellosis in the rainy season was found to be significantly correlated with abortion while in the dry season it was related to close contact and crowding of animals around grazing areas, watering points, vaccination and treatment centres. Banerjee and Bhatty (1970) suggested that the prevalence of the disease may have been lowered in government ranches due to prophylactic vaccination in the past.

2.3 Pathogenesis

Brucellae are obligate parasites of animals and humans and characteristically located intracellularly (Jawetz *et al*., 1989). The organism invades body tissue through the mucus

membrane of the oropharynx, upper respiratory tract, and the conjuctiva. It subsequent spread via lymphatic channels and regional lymph nodes to the thoracic duct and the blood stream, which distributes them to other tissues including bones and joints. Following invasion and bacteraemic spread, the main predilection sites are the pregnant uterus, udder, testicle and accessory male sex glands, lymph nodes, joint capsules and bursae (Blood and Radostits, 1989). According to Lambert *et al* (1960), localization in the supramammary lymph nodes and udder occur in 93% of infected animals. Manthei (1968) reported the order of frequency of *B. abortus* localising in the lymphoid tissue as supramammary lymph node, udder, retropharyngeal and mandibular, internal and external iliac lymphnode, other regional lymph nodes and lastly, the uterus. But experiment with dromedary camels in Sudan by Abu-Damir *et al*. (1989) reveals that the lymph nodes from which *Brucella* organism were mainly recovered were the lymph nodes of the head and genital tract.

The main site for localization of the organism in the male are the testes, epididymis and the seminal vesicles. Others include the carpal and other bursae where hygromas containing large amount of brucellae may be present (FAO/WHO, 1986). Congenital infection can occur in newborn calves as a result of *in utero* infection and the infection may persist in a small proportion of calves which may also be serologically negative until after their first parturition or abortion (Blood and Radostits, 1989). In adult, non-pregnant animal, the localization occur in the udder and the uterus.

Abortion is due to enormous multiplication of *Brucella* in the uterus caused by Erythritol$^{(R)}$, a polyhydric acid substance produced by the foetus. The substance occurs

naturally in greatest concentration in the placenta and foetal fluid of animals and probably is responsible for the localization of the infection in these tissue (Keppie *et al* 1965; Blood and Radostits, 1989). There is no erythritol[(R)] in human placental and abortion is not part of Brucella infection of human (Jawetz *et al*., 1989). In the pregnant animal uterus, the organism invades the epithelium of the embryonic chorionic villi and causes its detachment from the maternal placenta as a result of pathological changes ranging from degeneration and necrosis of the villi with formation of fibrino-purulent exudate. The organism invades the chorion, allantois and umbilical cord of the foetus, gaining entry to the latter via the blood stream and by the taken in of the amniotic fluid into the stomach. Separation of the foetal cotyledon from the maternal caruncles causes death of the foetus and usually foetal expulsion results at this stage. The expulsion of the foetus occurs principally in the last trimester (Roberts, 1971). Brunner and Gillespie (1973) believes that the abortion is a sequel of inflammation of the chorionic villi which interferes with circulation of the foetus. Morgan (1970) reported that occurrence of abortion lies on the degree of infection, the virulence of the organism, the stage of pregnancy when infection occurred and or the resistance of the animal.

2.4 Pathology

The establishment of *Brucella* infection in a sexually mature animals is characterised by acute lymphadenitis and subsequent enlargement of the glands which frequently bear small or large medullary haemorrhages. This correspond to the findings of

Abu- Damir *et al* (1989) where they reported generalised lymphadenitis and focal granulomata in the liver of experimentally infected dromedaries. They observed no gross lesion on post-mortem examination.

Brucellosis is essentially a necrotic placentitis and ulcerative endometritis (Morgan, 1970). In studies of the gross and or histopathological picture of the genital organs in female camels those with ovario-bursal adhesion and hydrobursitis had higher incidences of brucellosis (87.5% and 70%) than those with ovarian degeneration and endometritis (33.3% and 26.3%) respectively (Nada and Ahmed, 1993).

Camels seropositive for brucellosis had distinctly hard texture and thick walls of the uterotubal junctions when compared with brucella-negative camels. Granulomas were also found in *Brucella* - positive camels, even in non-pregnant females. In male, the lesions are confined to the genital organs. These may include orchitis, epididymitis or seminal vesiculitis (Roberts, 1971). Localization does occasionally occur in synovial structure to produce purulent tendovaginitis, arthritis, or bursitis.

Histopathologically, there is marked leukocytic infiltration especially with lymphocytes and plasma cells, congestion and cellular infiltration leading to necrosis. In the uterus, there is an interstitial inflammation leading to a severe ulcerative endometritis.

2.4.1 Clinical Findings

Temperature fluctuation, anorexia and depression are signs characteristic of the septicemic stage of brucellosis in animals (FAO/WHO, 1986). However, fluctuation in

body temperature is commonly observed in camels with the range of normal values from 35.5 - 41.0°C (Dioli *et al.*, 1992). Higgins and Kock (1984) indicated that a healthy camel will have a lower temperature in the early morning (average 36 to 36.5°C) rising steadily throughout the day to about 39°C in the evening and then dissipated during the cooler nights, though, he pointed out that normal values are not absolute. The temperature may vary from day to day.

High temperature above 41.01°C during the morning are indicative of fever. Accompanying symptoms of fever are hyperlacrimation, congested conjunctivas, increased respiratory and pulse rates of 5 - 12 and 32 - 50 per minute respectively, loss of appetite, dull and depressed appearance, decrease urine production and rapid weight loss.

There may be no clinical symptoms in brucellosis in camels (FAO/WHO 1986), but abortion are observed sporadically. This observation of abortion is similar to observation made in other animal species. Roberts (1971) and Blood and Radostits (1989) indicated that the Clinical signs of the disease in female animal are abortion most commonly in the last trimester of pregnancy, birth of weak or dead full-term foetus, retained foetal membranes, muco-purulent vaginal discharge usually dirty grey to brownish-red in colour, metritis and temporary or permanent infertility are a common sequela to abortion. In certain herds abortion is uncommon but retained placenta constitutes the only evidence of the disease possibly with decreased milk production (Carpenter and Hubbert, 1963).

Bruce (1962) was of the opinion that the retained placenta in the female is due primarily to inflammatory swelling of the chorionic villi in crypts of the caruncles.

Wernery and Wernery (1990) reported that there was no evidence of ill health in female dromedary they found positive for brucellosis in United Arab Emirates. For male animals, Ahmad and Nada (1993) conducted a serological survey of affected male camels sera with predominantly lesions of epididymitis. Orchitis and testicular degeneration in Cairo abattoir and reported 11.54% of 360 camels positive for brucellosis. In 1989, Abu-Damir *et al* experimentally infected six dromedary camels, 4 with S.19 and 2 with a field bovine strain. The result showed that no clinical signs were observed in the 4 camels inoculated with S.19, slight, non-specific symptoms were observed in those infected with field bovine strain. Both the field strains and the S.19 were shown to cause abortion in cows and orchitis in bulls with moderately to severely reduced fertility. Blood and Radostits (1989) reported that other than the occasional orchitis, epididymitis and possibly seminal vesiculitis, clinical signs of brucellosis manifest in bulls are usually not seen in camel.

The symptoms in human include fever (with or without sweating), weakness, generalized body pain, headache, backache, joint pain, abdominal pain, coughs, insomnia and nervous disturbance. According to Alausa (1984), *Brucella* infection should be taken into consideration in every cases of pyrexia of unknown origin. The incubation period, which is often prolonged, is reported by Cruickshank *et al*. (1973) to be about 10 - 30 days, but sometimes symptoms are delayed for several months. Abu-Damir *et al* (1989) reported detection of antibody titres in experimentally infected camels within 6 to 11 days.

2.5 Laboratory Diagnosis of Brucellosis

A wide variety of methods have been used for the diagnosis of brucellosis in different animal species in various countries of the world. The methods vary depending on the type of sample of specimen, the urgency of the result, vaccination status, purposes for screening or surveillance and the condition of the specimen (fresh or putrefying). In general, the following specimens and methods are acceptable for brucellosis diagnosis.

2.5.1 Useful Specimens for Diagnosis of Brucellosis

The most useful specimens for the laboratory diagnosis of brucellosis have been found to include;

(a) Aborted foetus
   - i) The stomach contents
   - ii) Foetal Lungs
   - iii) Foetal spleen
   - iv) Foetal membranes and cotyledons

(b) Aborting animal (Dam)
   - i) Uterine discharge
   - ii) Serum samples (taken 2 weeks apart)
   - iii) Whole blood
   - iv) Milk/colostrum
   - v) Hygroma fluid

vi) Vaginal swabs and placenta (especially from aborted animal 2 - 4 weeks post abortion.

(c) Bulls

i) Semen or seminal plasma.

ii) Preputial sheath washing

iii) Blood or serum

(d) Carcasses of animals

i) Lymphnode (supramammary, internal iliac, submaxillary and retropharyngeal).

ii) Spleen

iii) Uterus

iv) Testicular and seminal vesicles

v) Bone marrow

vi) Lesion of bones and joints.

2.5.2 Direct Smear

Smear made directly from fresh samples and stained by *Brucella* differential staining techniques. Stamp *et al* (1950) advocated Gram's, modified Koster or modified Ziehl-Neelsen staining while Cottral (1978) reported that positive diagnosis may be made by direct examination of tinctorial or flourescent antibody staining of tissue impression smears. None of these techniques is specific for brucellosis but can be used as screening

tests. The organisms appear under the microscope in clumps intracellularly as well as extracellularly, as long rods or coccobacilli.

2.5.3 Laboratory Animal Inoculation

Guinea pigs are believed to be the best laboratory animals for use in diagnosing brucellosis. It is more satisfactory than culture techniques for isolation of brucella organism (Alton and Jones, 1967). However, mice are susceptible and may be substituted for guinea pigs (Hull 1963). Routes of administration such as subcutaneous, intramuscular and conjunctival can be used. In positive cases, the organisms are recovered from the spleen of the animal killed 3 - 6 weeks post inoculation.

2.5.4 Serology

A variety of serological tests are widely used for the diagnosis of brucellosis. The tests have been based on the demonstration of antigen-antibody reactions, presence of IgG and IgM (Cottral, 1978). The commonly used serological tests for sera are the Rose Bengal plate test (RBPT), Serum agglutination test (SAT), complement fixation test (CFT) and Rivanol test (RT) (Eze, 1977; Falade, 1983). Other useful serological tests are Coomb's (Antiglobilin) test, Heat inactivation test, 2-mercapto-ethanol agglutination test (ME), precipitation (gel diffusion) test, Flocculation test, Fluorescent antibody test, immunoassay and Radio immunoassay, Fluoroimmunoassay and Enzyme-linked Immunosorbent Assay. Whole milk plate agglutination test, whey tube or plate agglutination test in addition to milk

ring test. Coombs and ELISA have been used for milk samples. Tube or plate agglutination tests are also useful tests for Vaginal discharge and seminal plasma.

Serological tests are influenced by factors such as long and variable incubation periods during which sero-test results are negative, vaccination status of the animal involved, exposure, variable responses of vaccinated and infected animal at the time of exposure to infection (FAO/WHO, 1971).

(a) Serum Agglutination Test (SAT)

The serum agglutination test (SAT) using standardized antigen has been used extensively for the diagnosis of brucellosis in man and animals and is particularly valuable for use in the unvaccinated animals or on farm where calfhood vaccination is practiced. It detects both IgM and IgG and therefore cannot differentiate reactors due to infection and those due to recent vaccination with S.19. In a proportion of chronically infected animals, the test may be inconclusive or negative (Morgan *et al.*, 1978). The test is also less effective in detecting early infections. The reaction of the test is mediated mainly through IgG_1, IgM and IgA immunoglobulin fractions (Nielsen *et al.*, 1988).

The serum agglutination test performed by the tube method is the most widely used procedure for the measurement of *Brucella* antibodies for purposes of international trade and other certification. The titre is the highest dilution of the serum that causes a given degree or agglutination; for any serum, the titre depends on the sensitivity of the antigen (Morgan, 1967). The sensitivity of the test is a function of the concentration of the cells in

the antigen. A titre of at least 50% agglutination at 1:40 serial dilution or more is indicative of infection.

In view of the occasional occurrence of a highly positive serum showing negative reactions (Agglutination) at lowest dilutions (titres) and becoming positive at higher dilutions otherwise known as Prozone phenomenon, at least four tubes are normally used for each serum under test.

The plate agglutination test is a modification of the tube test adapted for detection of rapid agglutination on a glass plate with colour background. The techniques and interpretations have been described by Alton *et al* (1975).

(b) Rose Bengal Plate Test (RBPT)

The test (RBPT) also known as card test or Buffered *Brucella* antigen test is a modification of plate agglutination test. It is a simple rapid test using an acidified Rose Bengal stained antigen (pH 3.65) to prevent agglutination of non-specific antibodies (IgM) that may be present in the serum. It is a convenient test used for routine or large screening job where incidence of brucellosis is low and percentage of animals to be screened is high.

Nicoletti (1967) demonstrated a close correlation between the results of the RBPT and the isolation of the *Brucella* from naturally positive animals. But Buxton and Fraser (1977) found where vaccination with *B. abortus* S.19 was widely used, a number of false positive reactions to be due to residual antibody activity from the vaccination.

Morgan *et al* (1978) observed that the test (RBPT) seems to identify infected animals at an earlier stage than the SAT and is often positive when SAT is negative or inconclusive. However, false-negative reactions are observed during early incubation of the disease and immediately after abortion. Corbel (1972) observed that the sensitivity of the test (RBPT) was associated with fractions containing immunoglobulin lgG especially the lgG_1.

(c) Complement Fixation Test (CFT)

Complement fixation test (CFT) is a very sensitive and specific test. The test is useful for routine tests on vaccinated animals. It is particularly useful for the detection of chronically infected animals in which complement fixing antibodies fade less rapidly than agglutinins. It is fast, accurate and definitive test for brucellosis but has the disadvantages of being cumbersome and failure to detect lgG_2, a relevant antibody isotype, which might result in "false negative" reactions (Nielsen *et al*., 1988).

(d) Milk Ring Test (MRT)

The milk ring test has been used considerably, particularly in survey and eradication schemes as a bulk milk test to locate infected herds. The test depends on the ability of the fat globules in milk to collect agglutinated *Brucella* organisms and carry them up to the cream ring. The antigen can be detected visually by staining with haematoxylin. A positive reaction being indicated by white milk and a deeply coloured cream layer. A negative

reaction is shown where the milk is coloured and the cream layer remain white. Roekpe *et al* (1949) adapted the test to cream samples and in this way increased its range of usefulness for field examination.

The test like the previous tests, has limitation for use. It is affected by fat globules and the time of sampling (Morgan, 1967). In addition, the presence of mastitis can cause reactions to be produced to the Milk Ring Test due to leakage of serum protein into the milk. False positive reactions due to vaccination of animal with *B. abortus* strain-19 is not uncommon.

(e) The Antiglobulin (Coombs Test)

The method detects the presence of lgG and lgA antibodies and has been applied to the diagnosis of chronic infection when antibodies associated with lgM immunoglobulin have disappeared (Kerr et al., 1967). It is useful in epidemiological survey of brucellosis with the advantage of detecting both incomplete and complete antibodies. It is very useful where prozone phenomenon is suspected.

(f) 2-Mercaptoethanol Test (2MET)

The procedure for serial dilution, incubation and addition of antigen are carried out as for SAT only that here the lgM is destroyed by 2-mercaptoethanol at the disulphide bridge bond leaving only lgG to be detected. The test is used in vaccinated herd to eliminate the effect of vaccination where lgM persist longer. It is used as a presumptive

evidence for the present of IgG by comparing the titres before and after treatment (Morgan *et al*., 1978).

(g) Rivanol Test (RT)

This test (RT) like 2 MET, precipitate other antibody except IgG. It is performed in tube or plate form. It is capable of distinguishing between vaccinated and infected animal and to chronic carriers (Abdulkadir 1989). The test is however difficult to interprete.

(h) Indirect Immunoflourescence Test (IFAT)

It is a specific, rapid and sensitive test for detecting antibodies on sera of human. The test and ELISA are used as confirmatory test.

(i) Heat Inactivation Test (H.I.T)

The test is based on the observation that two types of *Brucella* agglutins IgM and IgG were found in sera and could be differentiated on the basis of stability at 65°C for 15 min., cooled to 18°C and centrifuged. The deposited cells are resuspended in saline and examined for action. The test is carried out just like in the tube agglutination test. The only difference being the heating. It is very sensitive at early stages of infection in case of experiment; the only disadvantage being the necessity to centrifuge and resuspend the deposited cells in saline.

(j) Immuno-Assays

The need for development of a simple, universal antibody test system, capable of rapidly and accurately detecting humoral antibodies to brucellosis to overcome the limitations of the conventional methods led to the development of primary binding assays techniques such as Fluoro Immuno Assay (FIA), Radio-Immuno-Assay (RIA) and recently, Enzyme-Immuno-Assays (EIA) techniques.

(k) Radio-immuno-Assay

The test is highly sensitive and permits precise quantification although the conjugate have short shelf life. It is well suited to large screening tests. It requires complex and sophisticated equipments; highly trained personnel added with medical hazards involved in their use (voller *et al.*, 1976). These and other factors might have limited their use to centralised and specialised laboratories.

(l) Fluoroimmuno Assay

The techniques have been applied to brucellosis tests (Raybould and Chantler, 1979). It is a simple test that involve a flourescent dye conjugating to antibody. It is a tedious technique that is difficult to quantify for antibody assays. It depends on a degree of subjective visual assessments of flouresecence and the results are expressed as the serial dilution of serum that gives the least flourescence (Voller *et al.*, 1976). The technique is not suitable for use in large screening programme.

(m) Enzyme-Linked Immunosorbent Assay (ELISA)

The test has been developed and standardized as an efficient serological test for diagnosis of a number of bacterial and viral infections (Carlson and Lindburg, 1978). It has been used for detecting antibodies to *B. abortus* in serum and milk and found to be rapid, sensitive, specific and reproducible (Ocholi 1990; Majeed and Akhtar, 1993). The method allows a solid-phase antigen to react with serum specific antibody present in the serum to form an immune complex with the antigen. It is useful for mass screening of serum samples of livestock and human being (Irshad *et al*., 1990) and detects more infected herds than the ring test. It however has the disadvantages of not being able to distinguish antibodies arising from vaccination with attenuated *B. abortus* strain-19 from those resulting from field strain infection (FAO/WHO, 1971).

2.5.5 Cultural and Biochemical Characterization of Brucella

A confirmatory diagnosis of brucellosis is by isolation of the causative organism from a suitable specimen. The procedures for isolation and typing of brucellae have been described by many workers (Alton *et al*., 1975; Brodie and Sinton, 1975; Corbel *et al*., 1978). Various culture media primarily made up of basal medium, serum and antibiotics have been used in isolating *Brucella* organisms (Kuzdas and Morse, 1953). Media containing extracts of the bovine uterine wall, foetal membranes and foetuses, and in addition amniotic fluid agar, blood clot agar and glycerine agar have been found useful in isolating *Brucella* (Robertson *et al*., 1977). Liver agar plates are prepared containing

Thionin, basic fuchsin, and methyl violet. *Brucella melitensis* grows in the presence of all dyes. *Brucella abortus* is inhibited by thionin but grows in the presence of the other dyes. The organisms are catalase and oxidase positive, they produce hyrogen sulphide and reduce nitrates to nitrites.

Alton and Jones (1967) described the media, in use for the isolation of brucellae, to include serum-dextrose agar, Tween-dextrose agar, glycerol-dextrose agar, potato infusion agar, and the dehydrated media trypticase soy agar, tryptose agar and albimi agar. Kuzdas and Morse (1953) advocated addition of antibodies bacitracin (25 units/ml), polymyxin D (6 units/ml), circulin (15 unit/ml), cycloheximide (100 ug/ml) and bacteriostatic dye crystal violet. The addition of the antibiotics reduces the amount of contamination by both yeast and fungi as well as limiting bacterial overgrown. Morgan (1960) concluded that serum-dextrose agar containing the antibiotics polymyxin, bacitracin, and cycloheximide, was the only selective medium which could support the growth of all the strains of *B. abortus*, *B. melitensis* and *B. suis* which were examined. Farrel (1974) described the development of a selective medium incorporating bacitracin, polymyxin, nalidixic acid, vancomycin, cycloheximide and nystatin.

In Nigeria, most of the earlier media used in the culture of *Brucella* were one described by Alton and Jones, 1964; Alton *et al* 1975; Brodie and Sinton, 1975; Corbel, Gill and Thomas, 1978. These media have been successful in isolating *B. abortus* and *B. melitensis* from the Nigerian livestock (Eze, 1978; Bale, 1980; Bale and Kumi-Diaka, 1981; Falade, 1981).

The variability of the growth promoting properties of many of the media described may have been due to the method of incubation employed. The organism are aerobic and no growth occurs under strictly anaerobic condition. They grow best when incubated in 10% CO_2 at 37°C. Due to the fastidious nature of the organism, incubation may take as long as 5 - 10 days. And due to the long incubation period, this method is not very often used in disease survey.

2.6 Immune Response, Vaccine and Vaccination

Brucellae infection usually results in the induction of both humoral and cell-mediated immune responses. The magnitude and duration of these responses can be affected by many factors that include virulence of the infecting strain, size of innoculum, sex, pregnancy, species, immune status of the host and the age (FAO/WHO, 1986). Age resistance to the disease can be demonstrated, calves are highly immune. According to Bruner and Gillespie (1973), calves infected *in utero* or from their surrounding after birth, remain infected ordinarily for only a short time unless they are raised on infected milk or kept in the presence of the infection. Merchant and Packer (1975) stated that the duration of the disease and the amount of immunity acquired depends on the character of the particular strain with which the animal is infected.

Immunity usually depends entirely on phagocytosis and processing of *Brucella* organism by the macrophages and cell-mediated immune response by T-lymphocytes (Holland and Pickett, 1958). The pattern of humoral immune response is the same in

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vaccinated and naturally infected animals and man. The pattern of immunoglobulin appearance show that in some cases, in the very early antibody response, IgM may be the main antibody class found in the serum while in others it is the IgG isotypes that dominate. The presence of IgG in the absence of IgM is taken as evidence of active infection (Morgan, 1967). Immunoglobulin produced in the response to *B. abortus* infection include IgM, IgG_1, IgG_2 and IgA (Beh, 1974). The IgG_1 is usually present in serum in greater amount than IgM and IgG_2 and these antibody isotypes are present in higher proportion in naturally infected than in vaccinated animal.

2.6.1 Vaccine

Two major vaccines used to control *Brucella* are the live attenuated *Brucella* strain-19 (S19) and the modified killed vaccine K/45/20A (Duphavac). *Brucella abortus* Strain-19 vaccine is a viable culture of S.19 that is agglutinogenic, low virulence and comes in a lyophilised form. While the vaccine is said to be the best available for an immunizing agent when comparing the compatibility of *Brucella* to the livestock management practice, there has been reported isolation of S.19 - isolate and also abortion from vaccinated animals (Beckett and Mac Dairmid, 1985). These and other factors such as post-vaccination reaction that include local granuloma, systemic reaction, allergic reaction and death are the main disadvantages of the use of this vaccine.

The vaccine however, has the advantage of protecting uninfected animals living in a contaminated environment, enabling infected animals to be disposed off gradually (Blood

and Radostits, 1989). Studies by Beckett and MacDiarmid (1985) concluded that vaccination of adult animal with reduced dose S.19 should not be used in uninfected herds because of the resultant interference with serological and milk ring agglutination tests. Similarly Meyer and Nelson (1969) reported shedding of *B. abortus* S.19 in milk of calves nursed to dams vaccinated with S.19.

The killed Adjuvant vaccine K/45/20A, which is a modification of smooth strain 45/20 has been found to be more protective in cattle than did S.19 (Cunningham, 1977). The major advantage of the vaccine, however lies in its lack of influence on SAT result thus proving to be a basic diagnostic tool. It causes anamnestic response in infected or prevaccinated animals. The main disadvantage is its ability to reverse to smooth form, thus causing infection.

2.6.2 Vaccination

Vaccination of adult animal with the currently recommended dose of S.19 (4 to 12 x 10^{10} organism/dose) will provide a degree of protection against infection and will prevent abortion which according to Beckett and Mac Diarmid (1985) is usually caused by erythritol tolerant variants of S.19. It interfere with serological tests for such a long time as to render its use unacceptable in an eradication programme. Recent studies have indicated that the vaccination of adult cattle with a reduced dose of S.19 (2 x 10^8 to 3 x 10^9 organisms/dose) provides worthwhile immunity yet does not interfere unduly with subsequent serological tests (Alton and Corner, 1981). Vaccination of bulls is not recommended because of

possible persistent agglutinins, excretion of brucellae via genital tract, orchitis and lowered fertility.

According to Esuruoso (1974) and Ajogi (1984) vaccination of animals with S.19 in Nigeria is irregular and in some cases unjustified. Both authors suggested that the *Brucella* serotype causing the disease in an area be isolated, identified completely and propagated for further study and vaccine production.

The optimal age for vaccination in cattle is between 4 and 8 months and there is no significant difference between the immunity conferred at 4 and 8 months of age.

2.7 Treatment

Abdulkadir (1989) reported that treatment of brucellosis especially in cattle is of no value. Nicoletti *et al* (1989) found in treatment of naturally infected cattle, the most effective regimen consisted of two intramammary infusion of streptomycin liposomes and two doses of oxytetracycline administered intramascularly. Radwan *et al* (1989) showed that long term treatment with a high dose of tetracycline alone had succeeded in eliminating *B. melitensis* from a group of sixteen sheep. Marin *et al* (1989) found similar result especially long acting oxytetracyline combine with dihydrostreptomycin sulfate, but the treatment failed to cure epididymitis in rams.

FAO/WHO (1986) recommended tetracycline 500 mg orally, six hourly in combination with intramuscular injection of streptomycin 1mg daily for three weeks to be effective in treatment of acute brucellosis. Similarly, Co-trimoxazole (trimethoprim, 160

mg and sulfamethazole 800 mg) is also effective, given three times daily for two weeks with subsequent increment to twice daily. Though success has been reported, they were complicated by relapses.

The drug of choice in treatment of acute brucellosis is Rifampicin 600-900 mg daily alone or in combination with Doxycline at 200 mg daily (FAO/WHO, 1986). Both drugs are given in the morning as a single dose and relapse is unusual after a course of treatment continued for at least six weeks.

2.8 Control and Prevention

Control of brucellosis should aim at raising the standard of environmental sanitation in order to lower the intensity of exposure of animals to infection. Attention should be paid to housing conditions, isolation of aborting animals, disposal of aborted materials, disinfection of floors of infected areas including contaminated materials, segregation and quarantine of infected animals. Other useful control measures include restriction of animal movement both within and from the adjacent border countries and abroad unless adequately screened for brucellosis. Promotion of Public awareness through the mass media and where mass media facilities are not available according to Falade (1981) such information can be relayed especially to rural areas through schools, churches, mosques and community leaders. McDiarmid (1957) recommended the adoption of a compulsory calfhood vaccination scheme as a preliminary step to reduce the incidence of *Brucella* infection.

Human brucellosis is primarily an occupational disease, it is important therefore to educate people at risk. Animal attendants should wear protective clothing and take care in personal hygiene. Animal products likely to contain brucellae should be rendered safe by heat treatment or not consumed at all. More hygienic measures of preparing milk and milk products should be encouraged. Therefore fresh milk is made safe by pasteurization or boiling (Hendricks and Meyer, 1975). The present standard of pasteurization by holding methods; namely 145°F for 30 minutes or the short time high temperature, procedure; namely 161°F for 15 minutes, are effective in destroying *Brucella* and rendering milk and dairy products prepared from pasteurized milk safe for human consumption (Hull, 1963). Cheese are dangerous especially fresh cheese, and in areas where sheep and goat brucellosis occur, they should be avoided. Meat can be made safe by ordinary cooking process. Vaccination as a supplement to sanitary and hygienic measures may be considered for protecting humans where there is a greater danger of occupational infection for abattoir workers, Veterinarians, Laboratory workers, herdsmen and Shepherds (Cruickshank *et al.*, 1973).

2.9 Situation of Camel Brucellosis in Nigeria

There is paucity of information on brucellosis in camel in Nigeria. The only published information was one from abattoir in Kano (Okoh, 1979) were overall incidence of 2 (1%) of 232 camels was reported.

2.10 Public Health Significance of Brucellosis

Brucellosis is a disease of Public health significance that include infection to man and animals. the consequent illness. physical incapacity and loss of man-hour and manpower. It also includes the serious delimination of much needed foodstuffs especially animal proteins. which are essential to human health and well-being.

The disease in humans produces an undulating and debilitating fever which in the absence of expensive antibiotic treatment may persist for months and sometimes even years; chronic lesions in bones and joints may follow (Alton, 1973).

Vaccination has considerably reduced the economic loss to farmers in form of abortions but it has not eliminated the dangers of human infection due to animals shedding *Brucella* in their milk. Brucellosis can be disseminated in milk from animals which have no clinical symptoms (Bruce, 1962). Davies (1957), a medical practitioner in Wales, who himself contracted brucellosis, reported on 11 chronic brucellosis cases in one year from a rural area and implicated raw milk as the sources of infection. Cooper (1992) reported that the greatest risk was associated with the consumption of unpasteurized milk products.

Humans acquire the infection by direct or indirect contact with infected animals, their tissues or by consuming improperly cooked animal products and unpasteurized milk and milk products contaminated with *Brucella* organisms. It is an occupational disease of those who raise and care for livestock, or are employed in the slaughter of meat animals and processing of the products. Bilal *et al* (1991) reported that close contact with goats, sheep, cows and camels, and raw milk consumption were the main risk factors identified in

contacting the disease. Schwabe (1969) reported that the infection in man is rarely acquired by eating infected meat as cooking destroys the organism. However, partially cooked meat has been incriminated as a source of human infection.

In Nigeria, the commonest source of public exposure to infection is most likely from consumption of raw milk and milk products from infected animals in both rural and urban areas (Nuru and Schnurrenberger, 1975).

2.11 Economic Importance of Brucellosis

Brucellosis is widespread and is of major economic importance in most countries of the world. Like other abortion-causing diseases of animals, it causes economic losses in animal industry through calf losses from abortion, stillbirth, early death, death from acute metritis, infertility in both cases, and increased cost of animal replacements as well as lowered sale value of infected animals (Bothwell, 1962). Following abortion, there is generally much reduced milk production which is especially detrimental to the owner whose livelihood depends on sale of the milk. Other losses included prolonged inter calving period, heavy culling of valuable cows following permanent infertility. The average delay in conception following an infected calving has been estimated at 63 days (Hugh-Jones *et al*., 1975) or may remain barren for 3 years (Esuruoso, 1982). The disease in man has some social implications such as pain and suffering, cost of medication and treatment, wrong diagnosis at early stage, as well as man-hour losses from frequent absence from work and hospital visits in terms of Gross National Product (GNP) are important economic considerations of the disease (Ellis *et al*., 1979).

The economic loss resulting from *Brucella* infection among a nation's livestock populations can be truly staggering. For instance, the loss has been estimated at 350 million dollars annually in America (Anon, 1972) and 16 million pounds in Great Britain (Bothwell, 1962). In Sweden, the economic loss due to bovine brucellosis was more than 8 million dollars annually before the successful eradication programme (Schwabe, 1969).

In Nigeria, although accurate figure on economic losses due to brucellosis are not available, it has been estimated that abortion due to disease in cattle may cause losses of up to 3.5 million Naira per annum (Nuru, 1974); in addition to financial losses from infertility and lower milk yield. Esuruoso (1982) has estimated the gross cost of the control and eradication project feasibility and labour inclusive at 15 million Naira for the first period subject to the variations that may occur due to revaluation or devaluation. Rikin (1988) estimated that bovine brucellosis control in Nigeria over a span of 15 years to cost 29.394 million Naira, with a total benefit of 65.5 million Naira, a benefit cost ratio of 3.2 (using a 10% discount rate) and an interval rate of return of 32%.

Chapter three

MATERIALS AND METHODS

3.1 The Study Areas

The three Northern States of Nigeria from where samples (Blood, Serum, Milk, Stomach Contents of wasted Camel foetuses faeces, urine) were collected for the study comprise Kano (8°28'E, 11°57'N), Kaduna (7°28'E, 10°31'N) and Borno (11°13.5'E) states. The vegetation of the study areas are predominantly Guinea, Sudan and Sahel Savannah with relatively short rainy and a longer dry season. There are temperature variations which are much wider and hotter due to a high intensity of radiation combined with a longer day (Williamson and Payne, 1978) and this is suitable for adaptation of one-humped camel. The occupation of the people is mainly farming, trading and animal rearing.

3.2 Inspection of Camels at the Abattoirs

There are sections designated as camel slaughter slabs within the abattoirs visited. Ante-mortem inspection of the camels, which is an indispensable part of meat inspection is not being routinely practiced. The camels were usually brought into the abattoirs as early as 5.30 a.m local time on the day they were to be slaughtered. Flaying and evisceration of the slaughtered camels were carried out on the floor of the abattoirs. The hygienic conditions of the abattoirs were deplorable.

3.3 The Animals, Sources and Vaccination Status

The samples (Blood, serum, stomach contents of wasted camel foetuses, faeces, urine) were obtained from one-humped camels (*Camelus dromedarius*) of unknown age and health status which were daily purchased by butchers from camel markets, within a radius of 8 kilometers away from the abattoirs. Reports indicated that the camels were not previously vaccinated against Brucellosis. The milk samples were obtained from the abattoirs, camel markets and within herds. The origin of the animals in addition to those from Nigeria, was reported to be Chad, Cameroon, Niger and Libya.

3.4 Sex Determination of the Animals

The sexes of the camels were determined as they were brought into the abattoirs by quickly observing the perineal and genital organs. The exact ages could not be determined due to lack of routine ante-mortem inspection prior to slaughter of camels, coupled with the impatience of the butcher hurrying up to meet the early morning market.

3.5 Sampling Procedure: Sample Collection and Analysis

3.5.1 Blood

From each slaughtered camel, approximately 10mls of venous blood was collected into a sterile heparinized vacutainer tubes for culturing and isolation of brucella organisms along with another non-heparinized 10mls for serology. Similarly, about 10 mls of whole blood in Heparin vacutainer tubes were collected to determine the haematological parameters. As soon as the samples were collected, labelled with identification number, the

sex of the camels and date of collection were marked on them. The blood samples for cultural isolation of brucellae organisms and determination of haematological parameters were kept on ice in thermos flask and those for serology were allowed to clot at ambient temperature and serum decanted from the clotted blood. The samples were then brought back to the laboratory as soon as possible for analysis. In the laboratory, all blood samples were cultured and the sera were further centrifuged at 3,000G for 15 minutes, thereafter clarified sera were decanted into clean labelled universal bottles and stored at -20°C until they were tested. The blood samples for determination of haematological parameters were all analysed on the very day of collection.

3.5.2 Milk

Ten millitres of milk samples were obtained from the quarters of individual lactating female camel found within abattoirs and camel markets in Kano, Kaduna and Borno States of Nigeria. The milk samples were taken into a labelled sterilised vacutainer tubes. Before taking a milk sample from an individual camel, the hands of the milker and the teats of the lactating animal (Camel) were disinfected with 70% ethyl alcohol. The first few jets of milk from the animals were also discarded. A drop of 1% w/v boric acid as a preservative solution was added to each milk sample and the samples packed inside an ice-packed box and transported immediately to laboratory for analysis.

3.5.3 Stomach Contents of Wasted Camel Foetuses

Wasted camel foetuses were collected and brought back to the laboratory in an ice packed box where they were carefully opened and the surface of the abomasun seared with hot sterile platinum spatula. About 1-2mls of abomasal contents were removed with a sterile Pasteur pipette and put in sterile container containing Brodie and Sinton's liquid medium (BSM). The samples were then immediately analysed. In situation were analysis could not be conducted on the same day, the samples were stored at -20°C to following day.

3.5.4 Urine

Approximately 15 mls of urine samples were collected into sterilised universal bottles from slaughtered camels of both sexes in Borno State. The samples were labelled and packed inside an ice-packed box and transported immediately to the laboratory for analysis.

3.5.5 Faeces

About 5 gms of feacal samples were collected into a sterilised plastic bag from slaugthered camels of both sexes. The samples were labelled and brought back to the laboratory in an ice-packed box as soon as possible for analysis.

3.6 Serology

All the sera were tested for presence of antibodies by two serological methods; Rose Bengal Plate test (RBPT) as a screening test and Serum Agglutination Test (SAT), as a standard test.

The Rose Bengal Plate test essentially consists of adding equal volumes (0.03 mls) of the antigen to the test serum on a clear white ceramic tile, mixed thoroughly with a sterile tooth pick and the box handrocked for 4 minutes (Alton *et al*., 1975) and observed for distinct pink agglutination. Evidence of agglutination is indicative of the presence of the infection in the animal species.

Equally, all sera tested by RBPT were subjected to SAT in order to determine the sensitivity and specificity of each test on camel brucellosis. The recommendation of WHO as described by Morgan *et al*. (1978) was strictly followed. The end point of a titration was taken as the highest dilution giving a reading of 50 I.U. The serial dilution of the test sera was carried out in 4 labelled agglutination tubes and an automatic pipette. Exactly 0.8mls of phenol saline was placed in the first tube and 0.5mls in each succeeding tube. Similarly, 0.2mls of the serum under test was transferred to the first tube and thoroughly mixed with the phenol saline. Half a mililitre of the mixture transferred over to the second tube using all glass tuberculin syringe to which narrow tube was attached. There after, 0.5mls the mixture was transferred to the third tube after mixing. The process was continued until the last tube from which after mixing 0.5mls of the serum dilution is discarded. Then to each tube was added 0.5mls of antigen (diluted 1:10) thus giving final dilutions of 1:10; 1:20; 1:40 and 1:80. The tubes were hand-rocked briefly to

ensure proper mixing of antigen and the serum and incubated along with a positive and negative control at a temperature of 37°C in an incubator for 20 ± 2 hours. The results were read against black background with a source of light coming above and behind the tubes.

A positive reaction was one in which the serum-antigen mixture was clear with precipitate at the bottom, not disrupted by gentle agitation. An incomplete reaction was one in which the serum-antigen mixture was partially clear and gentle shaking did not disrupt the precipitate at the bottom. A negative reaction was one in which the serum-antigen mixture was turbid and gentle shaking revealed no precipitate at the bottom (Anon, 1981).

It is a quantitative test to determine the titres of all the reactors. Any sera with serum agglutination at a dilution of 1:40 (50 International Units) (Alton *et al.*, 1975) and above was considered as positive sample. The camels were not known to have been previously vaccinated.

3.7 Milk Ring Test (MRT)

The milk samples and MRT antigen were placed at room temperature for one hour to attain the room temperature and on each, the MRT was carried out as follows: One millilitre of a gently but thoroughly mixed milk sample was placed in a narrow Kahn test tube and 0.03ml or a drop of a haematoxylin-stained brucella antigen was added by a micropipette. The contents were gently mixed within few seconds of addition of the antigen.

The mixture was incubated at 37°C for one hour and read thereafter. Positive reaction was indicated by formation of a blue-coloured cream ring at the top of the tube (Morgan *et al*., 1978).

3.8 Culture and Isolation

All the samples were analysed according to the procedures for isolation of brucellae as previously described (Farrel, 1974; Alton *et al*., 1975; Brodie and Sinton, 1975; Corbel *et al*., 1978; Bale, 1980; Bale and Kumi-Diaka, 1981).

3.8.1 Blood

The heparinised blood was mixed and 0.2ml inoculated into Brodie and Sinton's liquid medium (BSM). This was incubated at 37°C in candle jars and air respectively for five days. After this, 0.2ml of BSM culture were each plated onto serum dextrose Agar (SDA) with antibiotics and incubated in duplicate plates at 37°C in air and in candle jars for another five days. Plates were then examined for growth and biochemical tests conducted.

3.8.2 Milk

Five millilitres of individual milk sample were centrifuged at 3000G for 15 minutes. The milk under the cream layer were poured off using a sterile applicator stick. The cream was thoroughly mixed with the sediments and a loopfull of the cream sediment mixtures was inoculated into Brodie and Sinton's liquid medium (BSM). The samples were labelled and incubated at 37°C in candle jars and air for 5 days. A loopful from the broth was then

plated onto serum dextrose agar (SDA) with antibiotics and incubated under the same conditions as above. Growth were observed for typical colonies of *Brucella*.

3.8.3 Abomasal Contents of Wasted Camel Foetuses

One millilitre of aspirated abomasal stomach contents was removed with sterile Pasteur pipette and 0.2ml of which was inoculated into Brodie and Sinton's liquid medium (BSM). This was incubated at 37°C in candle jars and air for five days. After this, a loopfull of BSM was plated onto serum dextrose Agar (SDA) with antibiotics and incubated at the same conditions for 5 days.

3.9 Identification and Biochemical Tests

The growth on culture media (SDA) were observed for colonies morphology and the morphology of individual organism by Gram-stain and modified Ziehl Neelsen stain.

The biochemical identification was carried out according to techniques of Corbel *et al*. (1978) and FAO/WHO (1986). The tests carried out here were catalase, oxidase, urease, nitrate, citrate, Voges - Proskauer, H_2S production, Dye sensitivity and CO_2 requirements.

3.10 Smear Examination

Smears were made directly from urine and faecal samples onto clean slides, air dried and heat-fixed over a bursen burner flame. The smear were stained by the modified Ziehl

Neelsen staining technique as described by Morgan *et al.* (1978) for presumptive evidence of Brucella organism.

3.11 Determination of Heamatological Parameters

The total red blood cell (RBC), total white blood cell (WBC), packed cell volume (PCV) and haemoglobin (Hb) concentration were determined from the whole blood at one time in Borno State by the standard methods (Schalm *et al.*, 1975)

3.12 Statistical Analysis

Prevalence rates of brucellosis in male and female camels were subjected to Chi-square (X^2) test to determine the significance of the differences between the males and females. The relationship and association between sex and prevalence rates of brucellosis were also determined by calculating relative risk.

Chapter four

RESULTS

4.1 Serology

A total of one thousand and forty-seven (1047) camel sera samples comprising 400, 215 and 432 from Kano, Kaduna and Borno State abattoirs respectively were tested by Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT). Two hundred and ninety-one (27.79%) and 200 (19.10%) of the 1047 samples were positive by RBPT and SAT respectively (Table 4.1).

Prevalence rate is highest in Borno (25.46%) followed by Kaduna (18.60%) and then Kano with 12.50% by SAT.

Table 4.1 **Prevalence of Camel brucellosis in Kano, Kaduna and Borno States, Nigeria.**

State	Sample Number	No. +ve	%+ve	No. -ve	% -ve
		Rose Bengal Plate Test (RBPT)			
Kano	400	97	24.25	303	75.75
Kaduna	215	60	27.91	155	72.09
Borno	432	134	31.02	298	68.98
Total	1047	291	27.79	756	72.21
		Serum Agglutination Test (SAT)			
Kano	400	50	12.50	350	87.50
Kaduna	215	40	18.60	175	81.40
Borno	432	110	25.46	322	74.54
Total	1047	200	19.10	847	80.90

+ve = Positive
-ve = Negative
No. = Number
% = Percentage

4.2 Prevalence of Brucellosis by Sex

Table 4.2 shows variation in prevalence of camel brucellosis by sex. Higher prevalence of infection was observed in females than males in all the states sampled. The differences were not statistically significant (Table 4.2).

The SAT results revealed that out of the 197 sera of female camels examined in Kano, 28(14.21%) were positive compared to 22(10.84%) of 203 males. In Kaduna State the prevalence were 20(21.05%) of 95 females compared to 20(16.67%) of 120 males. Similarly, the sero-positive results in Borno State were 51(25.76%) of 198 females to 59 (25.21%) of 234 males.

- Of the 490 females and 557 males examined, 99(20.20%) females and 101(18.13%) males were positive by the SAT. Similar trend was observed by the RBPT.

Female camels are more at risk than males judging from the samples examined (Table 4.2). However the differences between the prevalence rates in females and males were not statistically significant ($0.5 > P > 0.1$) in any of the three States studied.

Table 4.2 **Prevalence of Camel brucellosis by sex using RBPT and SAT.**

State	Sex	Samples tested No. %	RBPT +ve No. %	SAT +ve No. %	Relative Risk (RR)	X^2
KANO	Male	203 50.75	41 20.20	22 10.84	0.76	1.06
	Female	197 49.25	56 28.43	28 14.21	1.32	
	Sub total	400 100.00	97 48.63	50 25.05		
KADUNA	Male	120 55.81	30 25.00	20 16.67	0.79	0.66
	Female	95 44.19	30 31.58	20 21.05	1.26	
	Sub total	215 100.00	60 56.58	40 37.72		
Borno	Male	234 54.17	70 29.91	59 25.21	0.98	0.02
	Female	198 45.83	64 32.32	51 25.76	1.02	
	Sub total	432 100.00	134 62.23	110 50.97		
Total	Male	557 53.20	141 25.31	101 18.13	0.90	0.72
	Female	490 46.80	150 30.61	99 20.20	1.10	
		1047 100.00	291 55.92	200 38.33		

RBPT = Rose Bengal Plate Test
SAT = Serum Agglutination Test
RR = Relative Risk
X^2 = Chi-square value at degree of freedom of 1.
+ve = Positive
-ve = Negative

4.3 Milk Ring Test (MRT)

Table 4.3 shows the results of the MRT test. Twelve (17.64%) of the 68 milk samples were positive to brucellae by MRT. The highest MRT positive reactor of 6 (21.43%) of 28 milk samples tested was from Borno State. Kaduna State followed by 2(18.18%) out of 11 milk samples. The least was Kano State which recorded 4 (13.79%) MRT positive out of 29 samples. Overall prevalence rate was 12(17.65%) out of 68 milk samples tested in the three sampled States.

Table 4.3 **Investigation of Camel Milk for evidence of brucellosis using milk ring test (MRT).**

Source of Milk	Total No. Examined	No. +ve	% +ve	No. -ve	% -ve
Kano	29	4	13.79	25	86.21
Kaduna	11	2	18.18	9	81.82
Borno	28	6	21.43	22	78.57
Total	68	12	17.65	56	82.35

+ve = Positive
-ve = Negative
No. = Number
% = Percentage

4.4 Culture and Isolation

Out of a total of 518 samples comprising blood (350), milk (68) and stomach contents of wasted abattoir foetuses (70) cultured for evidence of brucellae, none yielded *Brucella* sp. During the investigation for brucellae, other bacteria namely; *Staphylococcus, Streptococcus, Bacillus, Pasteurella, Klebsiella, Clostridium, Corynebacterium, Escherichia coli and Proteus* species were isolated and identified based on their colonial appearance, morphology, biochemical tests and staining reaction of bacteria (Table 4.4). *Staphylococcus, Streptococcus, Bacillus* and *Klebsiella* were isolated from all the three samples. *Pasteurella* and *Proteus* were isolated from milk and blood only. *Escherichia* was isolated from foetal stomach contents and milk. *Staphylococcus* has the highest prevalence, 86 (16.6%) followed by *Streptococcus*, 49 (9.5%) while the least was *Proteus* 2 (0.4%) (Table 4.4).

Table 4.4 **Distribution of bacteria isolates from 380 Blood, 68 Milk and 70 Foetal Stomach contents of camel samples from Kano, Kaduna and Borno states.**

Bacteria isolated	Sample			Total No Positive	Location		
	B	M	FSC		Kano	Kaduna	Borno
Staphylococccus sp	+	+	+	86(16.6%)	48(9.3%)	5(1.0%)	33(6.4%)
Streptococcus sp	+	+	+	49(9.5%)	32(6.2%)	3(0.6%)	14(2.7%)
Bacillus sp	+	+	+	33(6.4%)	18(3.5%)	2(0.4%)	13(2.5%)
Pasteurella sp	+	+	-	29(5.6%)	14(2.7%)	1(0.2%)	14(2.7%)
Escherichia coli	-	+	+	14(2.7%)	12(2.3%)	1(0.2%)	1(0.2%)
Klebsiella sp	+	+	+	12(2.3%)	4(0.8%)	2(0.4)	6(1.2%)
Corynebacterium sp	+	-	-	5(1.0%)	3(0.6%)	1(0.2%)	1(0.2%)
Clostridium sp	+	-	-	4(0.8%)	3(0.6%)	0	1(0.2%)
Proteus sp	+	+	-	2(0.4%)	2(0.4%)	0	0

B = Blood
M = Milk
FSC = Foetal Stomach Content
+ = Isolate
- = No Isolate

4.5 Smear Examination

All the 62 faecal and 22 urine samples examined using modified Zielh Neelsen staining in Borno State were negative for Brucella organisms.

4.6 Haematological Parameters

The result of fifteen whole blood each from male and female camels is shown in Table 4.5 RBC, PCV, Hb, basophils and lymphocytes range and mean values are higher in males than females as observed in this study. The females have higher WBC value compared to males in neutrophiles and eosinophils. With the exception of high number of monocyte and lymphocyte these parameters were within the normal values for the dromedary.

Table 4.5 **Summary of haematological parameters of male and female camel blood from Borno State.**

Sex	No.of Camels	RBC (10^6/mm^3)	WBC (10^3/mm^3)	PCV (%)	Hb (g/dl)	Neutrophil (%)	Basophil (%)	Eosinophil (%)	Lymphocytes (%)	Monocyte (%)
Male	15	6.4-11 (8.3)	10-37 (15.6)	21-39 (29.6)	10-16 (13.3)	40-63 (52.1)	2-17 (7.9)	0-5 (1.2)	17-49 (28.6)	3-19 (9.5)
Female	15	4.2-7.9 (6.6)	10-59 (16.4)	19-31 (26.9)	9-15 (12.4)	42-79 (52.2)	1-15 (7.9)	0-6 (1.7)	15-45 (27.7)	2-20 (10.4)
Total	30	10.62-18.9 (14.9)	20-96 (32.0)	40-70 (56.5)	19-31 (25.7)	82-142 (104.3)	3-32 (15.8)	0-11 (2.9)	32-94 (56.3)	5-39 (29.9)

Averages of the 15 camels in each sex group are indicated in parenthesis

Chapter five

DISCUSSION AND CONCLUSION

5.1 Sample Collection in Slaughtered Camels

Collection of samples from slaughtered camels is easier, cheaper and less laborious than from live ones. Camels are usually slaughtered in a sternal recumbency to facilitate butchering. This method of slaughtering made blood samples collection very easy in the camels. Sometimes the butchers and the camel owners resisted sample collection.

The unco-operative attitude of the butchers and the camel owners during sample collection can be minimised by proper education.

5.2 Slaughter Record and Sex Distribution of Camel Brucellosis

Records in this study show that more male than female camels are being slaughtered in the abattoirs. The serological tests on the other hand indicated that more females than males were at higher risk of *Brucella* infection. This findings of higher prevalence in females than males apppear to agree with those of AbuDamir *et. al.* (1984) and Yagaoub *et. al.* (1990) in Sudan. The two investigators respectively reported 5.6% of 287 and 13.76% of 270 females being more infected than 4.5% of 447 and 4.94% of 1153 male camels. Similar findings of higher sero-prevalence in females than males have been reported in caprine and ovine species (Ogundipe *et al.*, 1993, Brisibe *et al.*, 1993; 1996) and in bovine Ajogi (1984). Generally, more males where infection is usually chronic may be

culled for slaughtered compared to females. In the males the clinical disease presents with observable signs of epididymitis, orchitis and testicular degeneration to the farmers and the clinicians leading to the culling of more infected male animals than the females in which the only noticeable sign of the disease may be abortion. Abortion is due to enormous multiplication of *Brucella* in the uterus caused by erythritol, a polyhydric acid substance produced by the foetus. The substance occurs naturally in higher concentration in the placenta and foetal fluid of pregnant animals than in seminal vesicles and testis of the males and is probably responsible for the localization of the infection in the female than male animals (Keppie *et al*., 1965) The epidemiological and zoonotic implication resulting from abortion are enormous. Brucellosis if present will spread at a faster rate to susceptible animals and humans through aborted foetuses, uterine discharges and milk especially under the prevailing range management system of camels.

Another reason for the discrepancies in the number of male and female slaughtered might have to do with selection. In most cases more male camels are sold. Ajogi (1984) reported that the nomadic fulani must have been aware of the unproductive and economically disadvantageous practice of keeping the male cattle within their herd and so the tendency to sell out more of the males than the females. The same reasons may apply for the camels.

5.3 The Serological Tests

The prevalence of 19.10% out of 1047 camels demonstrate that brucellosis in camels in Nigeria is on the increase when compared with findings of 2(1%) out of 232 camels examined in Kano (Okoh, 1979). The results are however comparable and are in agreement with those obtained elsewhere in other African countries such as 20% in Egypt (Zaki, 1948) and 14% in Kenya (Waghela *et. al.*, 1978). The increase is probably as a result of differences in sample size, time of collection and as reported by Chukwu (1985) increase in indiscriminate influx of animals into Nigeria from where control measures are inadequate or absent. In addition, in most cases, control and prevention against diseases such as brucellosis are not strictly adhered to.

Camel owners migrate out of Nigeria during the peak of rainy season and come back during the onset of dry season. It is possible during the movement, the animals come back with diseases, brucellosis inclusive. There is no herd health programme targetted at camels and vaccination status of these camels were unknown but camels in Nigeria are not usually vaccinated against brucellosis. The co-existence of camels with other animals could expose them to *Brucella* infection (Bilal *et al.*, 1991).

Brucella infection was more common in camels sampled in Borno State than in Kano and Kaduna States. Maiduguri, capital of Borno State, draws animals from neighbouring border countries. The high density of the susceptible animals, movement of the stock for grazing, congregration of the animals at closer proximity around water points in Borno State may explain the difference in higher infection rates in Borno State than Kano

and Kaduna States which are in the hinterland. Borno State, an arid zone, is noted for its weather extreme. The State is characterised with predominantly dry climate and inadequate water supply. Therefore, it is not uncommon to find animals of all species congregrating together at water point. This practice predisposes the animals including the camels to high infection rate.

Kaduna State unlike Kano and Borno States lies mainly in the Guinea Savannah and has comparatively denser vegetation, more rainfall and cooler weather all of which favour the survival of *Brucella* organism in the environment long enough to serve as a source of infection to other herd of camels. This may explain why higher prevealence rate were observed in Kaduna compared to Kano State.

The detection of higher antibody titres by RBPT (27.29%) than the SAT (19.10%) is in agreement with previous studies (Nicoletti, 1967; Morgan *et al*., 1969) and show that RBPT to be more sensitive than the SAT. 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 SAT is valid in this study with camels.

5.4 Milk Ring Test (MRT)

The presence of *Brucella* organism in raw milk depends on the level of infection in the dairy herd.

Though the 68 samples analysed in this study are few, the incidence of brucellosis in camels was demonstrated using Milk Ring Test. Camel milk is an important component of

the diet of nomads and it is consumed by the owners and herders in Nigeria. Camel milk is also consumed in many parts of the world (Knoes, 1977). In Nigeria, Nuru (1974) reported that over 95% of raw cow milk in the Northern States is untreated and the sale of milk in the market is an everyday affair; camel milk is among these. The demonstration of antibodies against brucellosis in camel's milk in this study is of public health importance. The presence of brucellosis in animals is an indication of its presence in man especially in those taking raw untreated milk and milk products. It is also dangerous to milk handlers and those who handle such infected animals. The overall prevalence rate of brucellosis by MRT of 17.64% is very close to that of 19.10% by SAT. This appear to further suggest the presence of brucellosis in these herds.

5.5 Culture and Isolation

None of the cultured samples of blood, milk and foetal stomach contents of abattoir wasted foetuses and the smear examination of urine and faeces yielded brucellae. Blood is a more suitable specimen for easy isolation of *B. Melitensis* and *B. suis* which principally infect goats and pigs respectively than *B. abortus* (Cruickshank, 1973, FAO/WHO, 1986). The bacteraemic stage of brucellosis in camels is unlikely to last long because of the high body temperature. Dioli *et. al.*, (1992) reported that in camels, unlike other large animals, the body temperature fluctuates widely with a normal values varying between 35.5 - 41.0°C.

Eze (1978) and Falade (1981) reported that brucellae were more readily isolated from milk samples as found in cattle, sheep and goats than from any other sources. But in camels, even with these samples, Alkhalaf and El-Khaladi (1989) who successfully isolated

B. abortus from foetuses of aborted camels could not isolate the organism from the culture of 209 milk samples in Kuwait. In this study, no brucellae were isolated from the milk samples. Isolation of brucellae are more probable in strongly MRT-positive samples (Bale, 1980) and is only useful when the organism is being shed in the milk at the time of sampling. It is possible at the time of taking these samples that the organisms were not being shed in the milk. Brucellae in the milk do not survive the long distance travel from the field to the laboratory even when transported in 1% boric acid solution (Eze, 1978). One of the best material for isolation of Brucellae include abomasal contents of aborted foetuses (FAO/WHO, 1986). In this study abomasal contents of abattoir wasted foetuses were used. This may further explain the lack of isolation reported in this study. *Escherichia coli, Bacillus sp, Klebsiella sp and Streptococus sp* were among the bacteria isolated during this study. Similar findings in goats in Nigeria have been reported by Falade (1981). Hashim *et al.* (1987) similarly, reported isolation of *E. coli* in pure form from two aborted cattle foetuses during a study in cattle, sheep, goats and camels in Al-Hassan, Saudi Arabia.

The feeding habit of the camel and some of the plants it grazes on may have medicinal effect and inhibit or kill some microorganisms, *Brucellae* inclusive. These might further strengthen the difficulty experienced in isolating Brucella from the camels samples investigated.

Some of the bacteria such as *E. coli* and *Staphylococcus* isolated in this study are of zoonotic importance. It will appear that antibacterial ingredient in brucella media may not

be absolutely effective against some of these bacteria which normally grow faster than brucellae.

5.6 Haematological Parameters

The haematological parameters of RBC, WBC Neutrophils, eosinophils in this study were within the normal ranges reported for one-humped camels (Allen *et al*., 1992); though the monocyte and lymphocytes values were higher than the reported normal values. Increase in lymphocytes and monocytes have been associated with chronic infection such as brucellosis (Saror, 1989).

5.7 Conclusions And Recommendations

Serological investigation of slaughtered camels have shown from this study that the prevalence of brucellosis in camels is on the increase.

Brucellae were however not isolated from any of the samples cultured. The findings suggest the need to control the disease in Nigeria.

Recommendation is made for further studies to isolate the organism, determine the strain and biotype(s) from camels. Effort should be made at investigating the disease in range camels to further elucidate the epidemiology of the disease in this specie of animals. Camel owners and those involved in slaughtering and processing of camels at the abattoirs may be harbouring the organism suggesting the need for a study of the disease in these occupational hazard group.

REFERENCES

Abdulkadir, I. A. (1989). *Infectious Diseases of Livestock in Nigeria*: An outline. Ahmadu Bello University Press Limited. 54 - 66.

Abu-Damir, H., Kenyon, S. J., Anbae Khalafalla and Idris,O. F. (1984). *Brucella* antibodies in Sudanese Camels. *Tropical Animal Health and Production* 16: 209 - 212.

Abu-Damir, H., Tageldin, M. H., Kenyon, S.J., Idris, O.F. and Damir, H. A. (1989). Isolation of *Brucella abortus* from experimentally infected dromedary c amels in Sudan; A Preliminary Report. *Veterinary Research Communications* 13: 403 - 406.

Adekolu-John, F. O. (1989). A serological survey of human brucellosis in Kainji Lake population of Nigeria - *Tropical Veterinarian* 7: 141 - 148.

Adesiyuw, A. A. and Abdu, P. A. (1984). *Brucella abortus* agglutinins in chickens in Nigeria. Bulletin Animal Health and Production in African. 32: 311 - 312.

Afzal, M. and Sakkir, M. (1994). Survey of antibodies against various infectious disease agents in racing camels in Abu Dhabi, United Arab Emirates. *Revue-Scientifique-et-Technique-office-Internattional- des-Epizooties* 13: 782 - 792. (English summary)

Agunloye, C. A., Esuruoso, G. O., Ajogi, I., Ogundipe, G.A.T., Agbede, S. A., Oyekole, O. D. and Ezegwu, R. U. (1988). Bovine Brucellosis surveillance in 2 N'Dama breeding herds in Nigeria. *Bulletin of Animal Health and Production in Africa*. 36: 63 - 68.

Ahmed, W.M. and Nada, A. R. (1993). Some pathological affections of testes and epididymis of slaughtered camels (*Camelus dromedarius*). *International Journal of Animal Science* 8: 33 - 35.

Ajmal, M., Ahmad, M. D. and Arshad, M. (1989). Sero-Surveillance of brucellosis. *Pakistan Veterinary Journal*, 9: 115 - 117.

Ajogi, I. (1984). Investigation of cattle slaughtered in various parts of Northern Nigeria for evidence of brucellosis. (Short title: Current Status of Bovine Brucellosis in Northern Nigeria). M.P.V.M. Thesis University of Ibadan, Nigeria. 1 - 122.

Alausa, K. O. (1984). Human Brucellosis in Nigeria. *International Journal of Clinical Microbiology*. 1: 5 - 11.

Al-Khalaf, S. and El-Khaladi, A. (1989). Brucellosis of Camels in Kuwait. *Comparative Immunology and Microbiology and Infectious Disease* 12: 1-4.

Allen, W. R., Higgins, A. J., Mayhem, I. G., Snow, D. H. and Wade, J. F. (1992). Proceeding of the first International Conference. 55 - 60.

Alton, G. G. (1973). Brucellosis in Goats and Sheep. *World Animal Review* 5: 16 - 20.

Alton, G. G. (1981). The control of bovine brucellosis. Recent developments. World Animal Review; FAO 39: 17 - 24.

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

Alton, G. G. and Corner, L. A. (1981). Vaccination of Heifers with a reduced dose of *Brucella abortus* strain 19 vaccine before first meeting. Australian Veterinary Journal. 57: 548 - 550.

Alton, G. G. and Jones, L. M. (1964). Typing of Brucellae. Laboratory Technique in Brucellosis (Animal Health Monograph No 7) F.A.O. Rome.

Alton, G. G. and Jones, L. M. (1967). "Laboratory Techniques in brucellosis". World Health Organization, Monograph Series WHO Geneva Switzerland 55: 27 - 92.

Alton, G. G., Jones, L. M. and Pietz, D. E. (1975) Laboratory Techniques in Brucellosis, 2nd edn. WHO Monograph Series No. 55, WHO Geneva 11 - 59.

Anon (1972) WHO programme Review Veterinary Public Health. EB51/9:5.

Anon (1981): Procedure for incubating, observing and reading the tube agglutination test. NADL Diagnostic Reagents Manual No. 650 (United States Department of Agriculture, Ames, IOWA).

Anon (1984). Consumption. Federal Livestock Department (FLD). Annual Report. 53 - 66.

Angus, R. D., Brown, G. M. and Gue, C. S. (1971). Avian brucellosis: A Case Report Natural Transmissn from cattle. *America Journal of Veterinary Research* 32: 1609 - 1612.

Bale, O. O. J. (1980). Cultural and Serological Study of Ovine and caprine brucellosis in Northern Nigeria. M.Sc. Thesis. Ahmadu Bello University, Zaria. 1 - 120.

Bale, O. O. J. and Kumi-Diaka, J. (1981). Serological and bacteriological study of bovine brucellae from Livestock Investigation and Breeding Centres in Nigeria. *British Veterinary Journal*. 137: 256 - 261.

Bale, J. O. O. and Kwanashie, G. G. (1984). Sero-prevalence of brucellosis among horses in Northern Nigeria. *Journal of Animal Production and Research*. 4: 161 - 164.

Bale, J.O. and Nuru, S. (1982). Serological Study of brucellosis in local fowls in Northern Nigeria. *Journal of Animal Production and Research*. 1: 53.

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

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 - 338.

Bang and Stribolt (1897). The aetiology of epizootic abortion. *Journal of Comparative Pathology and Therapeutics*. 10:125 - 149.

Beckett, F. W. and Macdiarmid, S. C. (1985). The effect of reduced dose of *Brucella abortus* strain 19 vaccination in accredited dairy herds. *British Veterinary Journal*. 141: 507 - 514.

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

Bilal, N. E., Jamjoom, G. A., Bobo, R. A., Aly, 0. F. M.and El-Nashar, N. M. (1991). Brucellosis in the Asir region of Saudi Arabia. *Saudi Medical Journal* 12: 37 - 41.

Blood, D. C. and Radostits, O. M. (1989). Disease caused by *Brucella* species in "Veterinary Medicine" 7th edn. Bailliere Tindall Ltd London. 677 - 690.

Bothwell, P. W., Macdiarmid Barthram, H. G.,Mackenzie, H.A. and Williamson, A. R. H. (1962). Brucellosis control and eradication: Notes and proposals of the Oxford working group. *Veterinary Record*. 74: 1091 - 1101.

Bourn, D; Wint, W; Blench, R. and Woolley, E. (1994). Nigeria livestock resource survey. *World Animal Review*. 78: 49 - 58.

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

Brisibe, F., Nawathe, D. R. and Bot, C. J. (1996). Sheep and goats brucellosis in Borno and Yobe states of arid North Eastern Nigeria. *Small Ruminant Research* 20: 83 - 88.

Brodie, J. and Sinton G. P. (1975). Fluid and solid media for isolation of *Brucella abortus*. Journal of Hygiene. 74: 359 - 367.

Bruce, D. (1887). Note on the discovery of a Micro-organism in Malta fever. *Practitioner*. 39: 161 - 170.

Bruce, W. (1962). Brucellosis. *Bulletin of Epizootic Disease of Africa*. 10: 187 - 201.

Bruner, D. W. and Gillespie, J. H. (1973). The Brucellaceae. In: Hagan's infectious diseases of domestic animals; with special references to etiology, diagnosis and biologic therapy. 6th edition. Comstock publishing associates, Cornell University Press, Ithaca and London. 173 - 232.

Buddle, M. B. and Boyes, B. W. (1953). A *Brucella mutans* causing genital infection in sheep in New Zealand. *Australian Veterinary Journal*. 29: 145 - 153.

Burgemeister, R. (1978). Distribution and use of dromedaries. *Animal Research and Development*. 7: 39 - 48.

Buxton, A. and Fraser, G. (1977). *Brucella*. In: Animal Microbiology Blackwell scientific publications Oxford London Edinburgh Melbourne 1: 133 - 140.

Carlson, H. E. and Lindburg, A. A. (1978). Application of Enzyme-linked Immunosorbent Assay for the diagnosis of bacteria and mycotic infections. *Scandavanian Journal of Immunology*. 8: 97 - 110.

Carmichael, L. E. (1967). Canine brucellosis: Isolation diagnosis, transmission. Proceeding of U.S. Livestock Sanit. Association. 71: 517 - 527.

Carpenter, C. M. and Hubbert, W. T. (1963). Brucellosis in: Hull T. G.(Ed.) Disease Transmitted from Animals to Man. Charles C. Thomas, Springfield, Illinois, U.S.A. 126 - 167.

Cedro, V. C. F., Cisale, H. O. and Fart, D. E., Meraldo, L. R. (1967). Brucellosis in Bulls. Agglutination titres and bacteriological diagnosis. *World Health Organisation*. WHO/Bruc/67: 302.

Cheville, N. F., Rogers, D. G., Deyoe, W. L., Krafsur, E. S. and Cheville, J. C. (1989). Uptake and excretion of *Brucella abortus* in tissues of the Facefly (*Musca autumnalis*). *America Journal of Veterinary Research*. 50: 1302 - 1306.

Chukwu, C. C. (1985). Brucellosis in Africa Part I: The prevalence. *Bulletin Animal Health and Production in Africa*. 33: 193 - 198.

Condy, J. B. and Vickers, D. B. (1969). The isolation of *Brucella abortus* from a waterbuck (*Kobus ellipsipsiprymnus*). *Veterinary Record*. 85: 200.

Cooper, C. W. (1992). Risk factors in transmission of brucellosis from animals to humans in Saudi Arabia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 86: 206 - 209.

Corbel, M. J. (1972). Characterisation of antibodies active in the Rose Bengal Plate Test. *Veterinary Record*. 90: 484 - 485.

Corbel, M. J. (1975). Stimulation of protective immunity to Salmonella kauffman - white group N'serotype by *Brucella abortus* and *Yersinia enterocolitica* antigens. *British Veterinary Journal*. 131: 625 - 626.

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

Cottral, L. G. (1978). *Manual of standardized methods for Veterinary microbiology*. Comostock, Ithadh, London. 395 - 403.

Cruickshank, R., Duguid, J. P., Marmion, B. P. and Swain, R. H. A. (1973). *Medical microbiology*. A guide to the laboratory diagnosis and control of infection. 12th edn. Vol. 1 ELBS and Churchill Livingstone Edinburgh London and New York. 350 - 354.

Cunningham. B. (1977). Experiences with strain 45/20 vaccines in Ireland. In: CRAWFORD, R. P. and HIDALGO, R. J. (Ed) Bovine Brucellosis. An international symposium London: Texas A and M University Press. 189 - 200.

Davies. J. E. (1957). Chronic Brucellosis in General Practice. British Medical Journal 11: 1082 -1087

Dioli. M.. Schwartz. H. J. and Stimmelmayr (1992). Management and handling of the camel. In: Schwartz. H. J. and Dioli. M. (Ed). The one humped camel in Eastern Africa: A pictorial guide to diseases, health and management. Verlag Josef Margraf Scient. books, Schonwald, drunk. Berlin Germany. 62 - 152.

Eisele. C. W.. McCullough. N. B., Beale, G. A. and Burrows, W. (1946). Development of *Brucella* agglutinins in humans following vaccination for cholera. *Proceedings of Society for Experimental Biology and Medicine*. 61:89 - 91.

Ellis. P. R. and James. A. D. (1979). Economics of animal health (1). Major disease control programmes; Economics in farm practice. *Veterinary Record*. 105: 504 - 506; and 523 - 526.

Emmel. M. W. (1930). The susceptibility of the turkey, pigeon, pheasant, duck and goose to *Brucella* disease. *Journal of American Veterinary Medical Association*. 30: 185 - 197.

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

Esuruoso. G. O. (1982). Current status of brucellosis in Nigeria and a preliminary evaluation of the probable costs and benefits of a proposed brucellosis control programme for the country. A Text of paper presented at the second international symposium of Veterinary epidemiology and economics. Canberra. Australia. 644 - 649.

Evans. A. C. (1918). Further studies on *Bacterium abortus* and related bacteria II. A comparison of *Bacterium abartus* with *Bacterium bronchosepticus* and with organism which causes Malta fever. *Journal of Infection Diseases*. 22: 580 - 593.

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

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. (1981). Caprine abortion with special reference to *Brucella* infection in Nigeria. Ph. D. Thesis. University of Ibadan. 1 - 194.

Falade, S. (1983). Some observations on the use of the Rose Bengal Plate, Tube agglutination heat, inactivation and Rinoval tests in caprine brucellosis. *Tropical Veterinarian*. 1: 49 - 53.

Faraj, B. S. M., Azwai, S. M., Gameel, S. E., Sharehs, A.M., Benhaj, K. M., Rayes, H. M., Nayil, A. A., Wardeh, M. F., Wilson, R. T., Zaied, A. A. (1991). Camel and human brucellosis in Libya.(English summary) *Proceedings of the International Conference on Camel Production and Improvement*, Tobruk, Libya. 224 - 227.

Farrell, I. D. (1974). The development of a new selective medium for the isolation of *Brucella abortus* from contaminated sources. *Research in Veterinary Science*. 16: 280 - 286.

FAO/WHO (1971) Joint expert Committee on brucellosis fifth report WHO Technical Report series No. 464, Geneva. 1 - 92.

FAO/WHO (1986). Joint expert Committee on brucellosis sixth report WHO Technical Report series No. 740, Geneva. 1 - 131.

Fay, L. D. (1972). Report to the government of Kenya on wildlife diseases Research UNDP FAO programme Report No TA 3049. 1 - 30.

Gameel, S. E. A. M., Mohammed, S. O., Mustafa, A. A. and Azwai, S. M. (1993). Prevalence of camel brucellosis in Libya. *Tropical Animal Health and Production*. 25: 91 - 93.

Garba, H. S. and Maigandi, S. (1995). Diseases of camels (*Camelus dromedarius*) encountered at slaughter at the abattoir in Sokoto, Nigeria. *Tropical Veterinarian*. 13: 95 - 102.

Hashim, N. H., Galil, G. A., Hulaibi, M. A. and Saleem, E. M. (1987). The incidence of brucellosis and species of *Brucella* organisms isolated from Animals in Al-Hassan, Saudi Arabia *World Animal Review*. 61: 32 - 35.

Hendricks, S. L. and Meyer, M. E. (1975). Brucellosis. In: Hubbert, W. T., McCulloch, W. F. and Schnurrenberger, P. R. (Ed). Diseases transmitted from Animals to man. 6th edn. Charles C. Thomas Pub. Springfield, Illinois, U. S. A. 10 - 32.

Holland, J. J., Pickett, M. J. (1958). A cellular brucella infection. *Journal of experimental Medicine*. 108: 343 - 359.

Horrocks, W. H. (1905). A preliminary note on goats as a means of propagation of mediterranean fever. Report of the commission on mediterranean fever. Part III, Harrison and Sons, London. 84.

Hugh, Jones, M. E., Ellis, P. R., Felton, M. R. (1975). An assessment of the eradication of bovine brucellosis in England and Wales. *University of Reading Study* No. 19.

Hull, T. G. (1963). Disease Transmitted from Animals to Man 5th edn. Charles C. Thomas Pub. U.S.A. 126 - 169.

Irshad, H. M., Ajmal and Akarm, A. (1990). Enzyme-linked immunosorbent assay for the diagnosis for brucellosis in animals and human beings. 3rd *International Congress Veterinary Medical Association*. (Islambad) 28 - 29.

Jawetz, E., Melnick, J. L., Adelberg, E. A., Brooks, G.,Butel, J. S. and Ornston, L. N. (1989). Medical Microbiology. A LANGE medical book 18th edn. International edn. Prentice-Hall international Inc. 230 - 232.

Keefer, C. S. (1924). Report of a case of Malta fever originating in Baltimore, Maryland. Bulletin of the Johns Hopkins Hospital. Baltimore. 35: 6.

Keppie, J., Williamson, A. E. and Smith, H. (1965). The Role of Erythritol in the tissue localization of the *Brucella*. *British Journal of Experimental Pathology*. 46: 104 - 108.

Kerr, W. R., Payne, D. G. H., Roberson, L. and Coombs,R. R.A. (1967). Immunoglobulin class of *Brucella* antibodies in human sera. *Immunology*. 13: 223 - 225.

Knoess, K. H. (1977). The camel as a meat and milk animal. *World Animal Review*. 22: 39 - 44.

Kronenwett, F. R., Lear, S. A. and Metlger, H. J. (1954). Thermal death time studies of *Brucella abortus* in milk. *Journal of Dairy Science*. 37: 636 - 640.

Kuzdas, C. D. and Morse, E. V. (1953). A selective medium for the isolation of brucellae from contaminated materials. *Journal of Bacteriology*. 66: 502 - 504.

Lambert, G., Amerault, T.E., Manthei, C.A. and Goode, E. R. (1960). Further studies on the persistence of *Brucella abortus* infection in cattle. *Proc. U.S. livestock San. Ass.* 109 - 117.

Lapraik, R. D. (1975). Isolation of *Brucella abortus* from a month old heifer reared in Brucella free environment from 6 weeks of age. *Veterinary Record.* 97: 52 - 54.

Majeed, I. U., and Akhatrr, S. (1993). Relative diagnostic parameters of Enzyme-linked Immunosorbent Assay for bovine brucellosis. *Pakistan Veterinary Journal.* 13: 114 - 115.

Mallmann, W. L. (1930). The interagglutinability of members of the *Pasteurella* and *Brucella* genera. *Journal of American Veterinary Medical Association.* 77: 636 - 640.

Manthei, C. A. (1968). Brucellosis as a cause of abortion today in: "Abortion diseases of livestock" Ed. by L. C. Faulkner; Charles C. Thomas Co. springfield, Illinois.

Marin, C. M., Jimenez De Bagues, M. P., Barberan, M. and Blasco, J. M. (1989). Efficacy of long - acting oxytertracyline alone or in combination with streptomycin for treatment of *Brucella ovis* infection of rams. *American Journal of Veterinary Research.* 50: 560 - 563.

Marr, A. G. and Wilson, J. H. (1951). Fixation of carbon^{14} dioxide in amino acids by *Brucella abortus*. Arch. Biochemistry and Biophysics. 34: 442 - 448.

Marx, A., Sandulache, R. Pop, A. and Cerbu, A. (1975). Biochemical basis of the serological cross-reactions between *Brucella* and *Yersinia enterocolitica* serotype 09 Ann. *Microbiology*. 126B: 435 - 445.

McDiarmid, A. (1957). The degree and duration of immunity in cattle resulting from vaccination with S 19 *Br abortus* vaccine and its implication in the future control and eventual eradication of brucellosis. *Veterinary Record.* 69: 877 - 879.

McKnight, T. L. (1969). The camel in Australia. Melbourne University Press, Australia. 1 - 154.

Merchant, I. A. and Packer, R. A. (1975). *Veterinary Bacteriology and Virology* 7th edn. IOWA state University Press, U. S.A. 320.

Meyer, M. E. (1964). The epizootiology of brucellosis and its relationship to the identification of *Brucella* organisms. *American Journal of Veterinary Research*. 105: 553 - 557.

Meyer, M. E. (1977). Non-bovine reservoirs of *Brucella abortus In*: *Brucellosis*. An International Symposium. edited by CROWFORD and HIDALGO.

Meyer, M. E. and Nelson, C. J. (1969). Persistence of *Brucella abortus* strain in immunized cattle. U.S. livestock sanitary Ass. 159 - 164.

Meyer, K. F. and Shaw, E. R. (1920). A comparison of the morphologic, cultural and biochemical characteristics of *Brucella abortus* and *Brucella melitensis*. Studies on the genus *Brucella*. *Journal of Infectious Diseases*. 27: 173 - 184.

Mitral, K. R. and Tizard, I. R. (1979). A simple technique to differentiate between animals infected with *Yersinia enterocolitica* IX and those infected with *Brucella abortus*. *Research in Veterinary Science*. 26: 248 - 250.

Morgan, W. J. B. (1960). Comparison of various media for the growth and isolation of *Brucella*. *Research in Veterinary Science*. 1: 47 - 56.

Morgan, W. J. B. (1967). The Serological diagnosis of bovine brucellosis. *Veterinary Record*. 80: 612 - 621.

Morgan, W. J. B., Mackinnon, D. J., Lawson, J. I. And Cullen, C. A. (1969). The Rose Bengal Plte Test in the diagnosis of brucellosis. *Veterinary Research*. 85: 636 - 641.

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

Morgan, W. J. B. (1977). In: Crawford R. P. and Hidalgo, R. J.(ed). Bovine Brucellosis. An International symposium. Texas, A and M University Press.

Morgan, W. J. B., Mackinnon, D. J., Gill, K. P. W., Gower, S. G. M. and Morris, P. I. W. (1978). Brucellosis diagnosis: Standard Laboratory Techniques 2nd Edn. Central Veterinary Laboratory Weybridge, England. 1 - 53

Morse, E., Ristic, M., Robertsiad, G. and Schreider, D. (1953). Cross-agglutination reactions among *Brucella, vibrio* and other micro organisms. *American Journal of Veterinary Research*. 14:324 - 330.

Nada, R. A. and Hamad, W. M. (1993). Investigation of brucellosis in some genital abnormalities of She-camels (*Camelus dromedarius*). *International Journal of Animal Science*. 8: 37 - 40.

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

Nicoletti, P., Lenk, R. P., Popescu, M. C., Swenson, C. (1989). Efficacy of various treatment regimens, using Liposomal streptomycin in cows with brucellosis. *American Journal Veterinary Research*. 50: 1004 - 1007.

Nielsen, K. Samagh, B. S., Specbmann, G. and Stemshorn, B. (1980). The bovine immune response to *Brucella abortus* II. Elimination of some sporadic serological reactions by chelation of divalent cations. *Canadian Journal of Comparative Medicine*. 43: 420 - 425.

Nielsen, K., Wright, P. F., Kelly Cherwonogrodzky, J. H. (1988). A review of Enzyme immunoassay for detection of antibody to *Brucella abortus* in cattle. *Veterinary Immunology and immunopathology*. 18: 331 - 347.

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

Nuru, S. (1975). Serological survey of brucellosis in slaughtered cattle in North Central State of Nigeria. *Journal of Nigerian Veterinary Medical Association*. 4: 9 - 13.

Nuru, S. and Schnurrenberger, P. (1975). The present status of bovine brucellosis in Nigeria: it prevalence, significance and areas of research need. *Bulletin Office International Des epizootics*. 83: 1113 - 1123.

Ocholi, R. A. (1990). Prevalence of brucella Antibodies in fulani cattle herds in Kaduna State. M.Sc. Thesis Ahmadu Bello University, Zaria. 1 - 81.

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

Ogundipe, G. A. T., Nwaichi, H. N. and Ayanwale, F. O. (1993). serological survey for the prevalence of *Brucella* antibodies in slaugheted goats in Ibadan, Nigria. Tropical Veterinarian 11: 143 - 149.

Ohara, S., Sato, T. and Homma, M. (1974). Serological studies on *Francisella tularensis, Francisella novicida, Yersinia philomaragia* and *Brucella abortus*. *International Journal of System. Bacteriology*. 24: 191 - 196.

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 Nortehrn Nigeria. *Bulletin of animal Health and Production in Africa*. 36: 251 - 254.

Okoh, A. E. J. (1979). A survey of brucellosis in camels in Kano Nigeria. *Tropical Animal Health and Production*. 11: 213 - 214.

Plommet, M. (1973). Survival of *Brucella abortus* in liquid manure from cattle. Disinfection by Xylene. *Veterinary Bulletin*. Abstract. 43: 407.

Prior, M. G. (1976). Isolotion of *Brucella abortus* from two dogs in contact with bovine brucellosis. *Canadian Journal of comparatine Medicine*. 40:117 - 118.

Radwan, A. I., Bekairi, S. I., Al-Mukayel, A. A. and Abuagla, O. S. (1984). Control of Ovine brucellosis in Naida sheep in Saudi Arabia. *Tropical Animal Health and Production*. 16: 213 - 218.

Radwan, I., Hafez, M., Karim Al-Aska, A., Al-Yamani, M. J., Bekairi, S. I., Al-Jalaifi, M., Al-Mukayel, A. A. (1989). Experimental treatment of *Brucella melitensis* infection in sheep with oxytetracycline alone or combined with streptomycin. *Tropical Animal Health and Production*. 21: 211 - 216.

Raybould, T. J. C. and Chantler, S. (1979). serological differential between infected and vaccinated cattle by visual quantitative immunofluorescence using *Brucella abortus* antigen coupled sepharose beads. *Journal of Immunology Methods*. 30: 37 - 46.

Rikin, U. M. (1988). Brucellosis of cattle in Nigeria: Proposals for a control programme under intensive and extensive husbandry system. *Acta Veterinary Scadinvica*. (Suppl.) 84: 94 - 97.

Robert, S. J. (1971). Veterinary Obstetrics and Genital Diseases. 2nd Edn. Ithaca, New York. 320 - 321.

Robertson, L., Farrell, I. D. and Hinchliffe, P. M. (1977). The isolation of brucellae from contaminated sources. A review. *British Veterinary Journal*. 133: 193 - 200.

Roepke, M. H., Clausen, L. B. and Walsh, A. L. (1949). The milk and cream ring test for brucellosis. Proc. 52nd Ann. Meet., U.S. Livestock Sanit Ass. 1:147 - 159.

Salman, M. D. and Meyer, M. E. (1984). Epidemiology of bovine brucellosis in the Mexicali Valley, Mexico: Literature review of disease-associated factors. *American Journal of Veterinary Research*. 45: 1557 - 1560.

Saror, D. I. (1989). *Principles of general veterinary Pathology*. Ahmadu Bello University Press. 40 - 44.

Schalm, O. W., Jain, N. C. and Carrol, E.J. (1975). *Veterinary Haematoloty*, 3rd Ed. Lea and Febiger, Philadephia.

Schwabe, C. W. (1969). *Veterinary Medicine and Human Health*. 2nd Publ. Williams and WILKINS comp. 428E Preston str. Baltimore, M. D., U.S.A. 1 - 713.

Spink, W. W. (1956). The Nature of brucellosis. University of Minnesota Press, Minneapolis. 464.

Stamp, J. T., Mcewen, A. D., Watt, J. A. A., and Nisbet, D. (1950). Enzootic abortion in Ewes transmission of the disease. (Possible rickettsial infection). *Veterinary Record*. 62:251 - 254.

Stoenner, H. H. and Lackman, D. R. (1957). A new species of Brucella Isolated from the desert wood rat: Neotoma lepida. *American Journal of Veterinary Research*. 18:947.

Thorpe, B. D., Ssdwell, R. W., Bushman, J. B., Smart, K. L. and Moyes, R. (1965). Brucellosis in Wildlife and livestock of West Central Utah. *Journal of American Veterinary Medical Association*. 146: 225 - 232.

Tovar, R. M. (1947). Infection and transmission of Brucella by ectoparasites. *American Journal of Veterinary Research*. 8: 138 - 140.

Trauma, J. (1914). Infectious abortion investigations in pigs. Annual Report of the Chief, Bureau of animal industries, U.S. Department of Agriculture, Washington, D.C. 30.

Voller, A., Bidwell, D. E. and Bartlett, A. (1976). Enzyme immunoassays in diagnostic medicine: Theory and practice. *Bulletin of WHO*. 53: 55 - 65.

Waghela, S., Fazil, M. A., Gathuma, J. M. and Kagunya, D. K. (1978). A serological surve of brucellosis in camels in North-Eastern Province of KENYA. *Tropical Animal Health and Production*. 10: 28 - 29.

Wellman, G. (1952). Experimental transmission of brucellosis by insects. Zentralblath Fur Bakterizlogie, parasitenlunde infektionskraudheiten and Hygiene I (Originate) 159: 71 - 86. (English summary). Abstract - 1953. *Veterinary Bulletin*. 23: 308, No. 2212.

Wernery, U. and Wernery, R.(1990). Seroepidemiological investigations in female camels (*Camelus dromedarius*) for antibodies against *Brucella*, Chlamydia, leptospira, BVD/MD, IBR/IPV and enzootic bovine leukosis viruses. Deutsche - Tierarztliche - woch-enschrift. 97(3): 134 - 135. (English summary). Abstract, 1992. *Veterinary Bulletin*. 062 - 04268.

Williamson, G. and Payne, W. J. A. (1978). *An Introduction to Animal Husbandry in the Tropics* 3rd edn. Publ. Longman, London and New York. 755.

Yaqoub, I. A., Mohammed, A. A., Salim, M. O. (1990). Serological survey of *Brucella abortus* antibody prevalence in one-humped camel (*Camelus dromedarius*) from Sudan. revue-d' elevage et-de-medecine-veterinaire-des-pay' Tropicank. 43: 2,167, 169 - 171. (English summary). Abstract 1991, *Veterinary Bulletin*. 061 - 05329.

Zaki, R. (1948). Brucellosis infection among Ewes, camels and pigs in Egypt. *Journal of Comparative Pathology*. 58: 145 - 159.

Zammit, T. (1905). A preliminary note on the examination of blood of goats suffering from mediterranean fever, report of commission investigation of mediterranean Fever, Part III. Hamson and Sons, London 85.

APPENDIX

Preparation of Culture Media and Reagents.

i) **Antigen:** MRT, RBPT and SAT were obtained from Central Veterinary Laboratory (CVL), Weybride, Surrey, England.

The Serum Tube Agglutination Test Stock antigen was further diluted 1ml of the antigen into 9mls of phenol saline (i.e. 1:10 dilution).

ii) **Culture Media:**

(a) Brodie and Sinton's Liquid medium (BSM)

Oxoid tryptone soya broth	30g
Distilled water	1litre
Equine Serum, inactivated at 56°C for 30 min	50ml
Bacitracin (20,000 units)	12.5ml
Polymyxin B Sulphate (5,000 units)	1.2ml
Nalidix acid	5mg
Vancomycin hydrochloride	20mg
Nystatin	25mg
Cycloheximide	100mg
Amphotericin B (Fungizone)	4mg
D-cycloserine	312.3mg

The medium is prepared by dissolving the tryptone soya broth powder in the distilled water and autoclaved at 121°C for 15 minutes. After cooling the broth to 50°C the sterile horse serum is added aseptically, followed by the antibiotics. The medium is dispensed into bijou bottles and stored at 4°C until use.

(b) Brucella medium Base.

This dehydrated medium contains:

Peptone	10g
'Lab-Lemco' Powder	5g
Dextrose	10g
Sodium chloride	5g
Aga No.3	15g.

(c) Serum dextrose agar (SDA)

Brucella medium base (Oxoid)	45g
Distilled water	1000ml
Horse serum, inactivated at 56°C for 30 minutes	50ml
25 percent w/v Dextrose antoclaved at 109°C/10 minutes	40ml

Brucella medium base was added to 1 litre of distilled water and soaked for 15 minutes. It brought boiling to dissolve completely and then sterilised by

autoclaving at 121°C for 15 minutes. P^H 7.5 (approximately). After cooling the medium to 50°C the sterile horse serum was added aseptically. Mixed well and plates are then poured.

(d) Dye Sensitivity test media

These are prepared by the addition of the relevant dye to a serum dextrose agar basal medium. About 0.1g dye was dissolved in 100ml distilled water to produce stock solutions. These were sterilised by steaming for one hour. The volume of dye solution to produce the required final concentration is then added to the SDA basal medium. Plates are poured from this immediately.

(iii) **Antibiotics**

The various antibiotics used are prepared according to manufacturers recommendations as follows:

(a) Bacitracin: The 500000 units are dissolved in 25 ml of sterile distilled water: this was further diluted 1/10 to give a concentration of 2000 units/ml. It was stored at 4°C when not needed or incorporated in the basal medium at a concentration of 25000 units per litre (25 units/ml).

(b) Polymyxin B Sulphate: 500000 units was diluted 1/100 by dissolving in 10 ml sterile distilled water, and further diluting in 1/10

to give a concentration of 5000 units/ml. It was dispensed in 2ml in plastic specimen Vials and stored in the ice chamber of the refrigerator, and used in a concentration of 6,000 units per litre (6 units/ml).

(c) Cycloheximide: One gm was dissolved in 20 ml acetone and diluted 1/20 with sterile distilled water to give a concentration of 10000 microgram per ml. It was stored at 4°C and used in a concentration of 100 mg per litre (100 microgram/ml).

(d) Vancomycin hydrochloride: 0.5g was dissolved in 10 ml of sterile distilled water and stored at-20°C and used in a concentration of 20 microgram per ml.

(e) Nalidixic acid: 5 percent w/v was dissolved in equal parts N/1 sodium hydroxide and sterile distilled water and stored at 4°C. Immediately before use a 1/10 dilution was made in distilled water and added to the medium at 5 microgram per ml.

(f) Nystatin: 500,000 units was dissolved in 10 ml distilled water for each batch of medium and incorporated at 100 units per ml.

(g) D-Cycloserine: A stock solution containing 50 mg/ml was made by dissolving 0.5g in 10 ml of sterile distilled water, seitz filtered and 6.25 ml added to each litre Volume of the medium.

(h) Amphotericin B (Fungizone): A stock solution containing 10 mg/ml was made by dissolving the sterile powder in sterile distilled water and this was used immediately.

(iv) **Chemicals**

(a) Phenol saline (8.5g $Nacl_2$ plus 5g phenol in 1 litre of distilled water)

(b) 70% Methyl alcohol

(c) Carbol fuchsin (Basic fuchsin 10g plus 100ml of 95%. Ethanol). The dye was dissolved in the ethanol and stored at 37^{oc} overnight.

(d) Methylene blue 1% w/v in distilled water.

(e) Crystal violet (2g crystal violet in 20ml of 95% v/v ethanol plus 0.8g ammonium oxalate in 80ml of distilled water). The two solutions are mixed to form a stock solution which was then diluted 1/40 in distilled water for use.

(f) Gram's iodine (iodine 1g plus potassium iodide 2g). The two were grinded and dissolved slowly in 300ml distilled water.

(g) Safranin O (2.5g/100ml alcohol as a stock solution). The working solution is consituted by adding 10ml stock solution to 90ml distilled water (1:10 dilution).

(v) **Reagents**

(a) Christenson's Urea Agar

Pepton	1.0g
Nac1	5.0g
KH_2PO_4	2.0g
Agar	20.0g
Distilled water	1000 ml

The ingredients is dissolved by heating, adjust to P^H 6.8, filtered and sterilized at 115°C for 20 min.

Glucose	1.0g

Phenol red, 0.2 percent aqueous solution 6ml was added to the melton base steam for 1 hr and cool for 50 - 55°C. Then 100ml of urea 20 percent aqueous solution added. Steriled by filtration and added aseptically to the base.

(b) Carbohydrate Fermentation

Base Medium: Andrate peptone water (oxoid. cm 61)

Peptone	10.0g
Nacl	5.0g
Andrade's indicator	0.1g
Distilled Water	1000 ml
P^H.	7.4

The constituents were suspended in 1000ml of distilled water, throughly mixed and 10ml of 1 percent (W/V) of the carbohydrate substrate added. The mixture was distilled in 4ml volume into bottles containing Durham's tubes. The bottles and their contents were sterilized by heating at 80°C for 30 minutes for 3 consecutive days. The carbohydrate stock solutions were prepared and sterilization by Seitz filtration and included the following: Glucose 50 percent (W/V), Galactose 25% (W/V), Xylose 50% (W/V), Mannitol 25 percent (w/v) and Sobitol 50 percent (w/v).

(c) Koser's Citrate Medium (Oxoid CM 65)

Sordium ammonium phosphate	1.5g
Potassium dihydrogen phosphate	1.0g
Magnesium sulphate	0.2g
Bromo-thymol Blue	0.016g
Distilled water	1000 ml
P^H.	6.8

The solids were dissolved in 1000 ml of distilled water, mixed and dispensed in 3ml volumes into Bijou bottles. The bottles and their contents were autoclaved for 15 minutes at 121°C.

(d) Hydrogen Peroxide

3 percent aqueous solution (10 volume) was prepared in sterile distilled water. The solution was stored at 4°C in a glass stoppered bottle.

(e) Lead Acetate Paper

Filter paper was cut into 10mm x 50mm strips. The papers were impregnated with hot saturated lead acetate solution and dried overnight at 37°C.

(f) Oxidase Reagent:

N N N 'N' - tetramethyl-p- phenylene-diamine dihydrochloride (BDH). A spatula tip of the reagent was dissolved in 3ml distilled water in a dark bottle just before use. The solution was colourless.

(vi) **Modified Ziehl-Neelsen's Staining technique.**

Procedure:

(a) Air-dried, heat-fixed smears were prepared. The slides were flooded with diluted carbon fuchsin and stained for 10 minutes.

(b) Each slide was washed thoroughly in water and the smear decolourized with 0.3 percent v/v acetic acid for up to 30 seconds taking care not to over differentiate. The slide was again thoroughly washed with water.

(c) The counter stain (methlene blue) was then applied for 30 seconds and the slide blotted dry.

(d) The slides were then examined under the microscope for the presence of micro organism.