CULTURAL AND SEROLOGICAL STUD OF OVINE AND CAPRINE BRUCELLOSIS IN NORTHERN NIGERIA

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THESIS APPROVAL

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	i
INTRODUCTION	1
CHAPTER I	3
GENERAL LITERATURE REVIEW ON THE GENUS *BRUCELLA*	
Historical Background to the diseases caused by genus Brucella	4
GENERAL CHARACTERISTICS OF THE GENUS *BRUCELLA*	5
Growth requirements and characteristics	5
Morphology and Staining reaction	9
Gaseous requirement	9
pH requirement	11
Temperature requirement	11
Dissociation property	11
Dye sensitivity	12
Biochemical reactions	12
Resistance to environmental factors	13
Antigenic structures and phage lysis	13
HOST SUSCEPTIBILITY	14
Mode of transmission	15

PATHOGENESIS OF BRUCELLOSIS 16

CLINICAL FINDINGS 17

PATHOLOGY OF BRUCELLOSIS 19

LABORATORY DIAGNOSIS OF BRUCELLOSIS 21

Serological diagnosis 22

Cultural diagnosis 26

IMMUNITY 28

Vaccines 28

PUBLIC HEALTH SIGNIFICANCE OF BRUCELLOSIS 29

ECONOMIC IMPORTANCE OF BRUCELLOSIS 33

CHAPTER II

SEROLOGICAL STUDY OF OVINE AND CAPRINE BRUCELLOSIS IN NORTHERN NIGERIA 36

Introduction 37

Materials and Methods 38

Results 40

Tables 42

Discussion 48

Summary 52

CHAPTER III

BACTERIOLOGICAL INVESTIGATION OF OVINE AND CAPRINE BRUCELLOSIS IN GOVERNMENT FARMS IN NORTHERN NIGERIA 53

Introduction 54

Background to the Livestock Improvement and Breeding Centre (L.I.B.C.). 56

Materials and Methods 58

Results 61

Tables 63

Discussion 68

Summary 68

GENERAL DISCUSSION 69

APPENDIX 73

REFERENCES 90

ABSTRACT .. ..

INTRODUCTION

The socio-economic significance of small ruminants, sheep and goats in Nigeria needs no emphasis. It is estimated that there are over 10 million of these animals in the humid zones of West African of which Nigeria has the largest number of each species. Within the Savannah and Sahel zone, there are an estimated 27-30 million heads of goats and 8 million sheep in Nigeria. (Agric. Dev. Nig. 1973-85). The short legged dwarf breeds of sheep and goats are predominant in the humid zone of Nigeria while the long legged types are common in the Northern States.

In all parts of Nigeria, these species of animals have been virtually neglected by research scientists, veterinarians and livestock enterpreneurs, yet the two species are very much a part of the rural and urban community. Socially, they play an important role in the life of the community being either loaned or exchanged among relatives and friends, or slaughtered for social, religious and festive occasions. Economically, they are readily available for sale by the farmers to boost family income or meet financial emergencies. Their meat is widely accepted by all and thus free from religious or tribal taboos except in few areas where goat meat is not consumed regularly.

A major obstacle to improved production of these highly prolific species of livestock however is disease. Sheep and goats suffer from helminth parasites as well as a number of bacterial and viral diseases which limit their production. Of great importance both from economic

and human health point of view is brucellosis which is common to these species the world over.

Understanding of the role and significance of brucellosis is therefore the primary objective of this investigation, not only because it limits production though abortion, and infertility but also because it is of public health significance.

No published information is available on species and biotypes of *Brucella* in sheep and goats in Nigeria. The overall incidence of the disease in these animals in Nigeria livestock is still unknown. In many parts of the world the disease is considered among the major maladies of enormous economic importance.

A limited survey of ruminants in the past showed the presence of the disease in Nigeria. The reports were based on serological findings from milk ring test, Rose Bengal plate test, or card test, serum agglutination test and on a few occasions, complement fixation test.

The present study was designed therefore:

1. To determine the prevalence by serological survey of brucellosis in sheep and goats of Northern Nigeria where these animals occur in largest numbers.
2. To determine the species and biotypes of *Brucella* involved in sheep and goats infection by cultural, biochemical and antigenic studies.

CHAPTER I

GENERAL LITERATURE REVIEW ON THE GENUS *BRUCELLA*

Historical Background to the Diseases Caused by Genus Brucella

Brucellosis is a disease of all warm blooded animals caused by the genus *Brucella*. Members of this genus are mammalian facultative intracellular parasites with a wide range of hosts including man. The six well known species of this genus are *B . abortus, B . melitensis, B . suis, B . ovis, B . canis* and *B . neotomae.*

Historically, Bruce discovered the disease in man which he called "Malta fever" and which was known to be associated with milk and milk products of infected goats (Bruce 1930, Zammit 1905). This is one of the epidemiologically important diseases whose clinical courses in man were very well known long before the causative organisms were identified. Bruce later identified the causative agent and named it *Micrococcus melitensis*. Later, Bang (1897) discovered a causative agent of abortion in cattle and he named it "Bacillus of abortion". The organism has since been recognised as *B.. abortus*. Similarly, Dubois (1911) reported abortion, lameness, mastitis and orchitis in goats and this was confirmed by Taylor and Hazeman in 1932 and by Holth and Reynolds in 1935. Traum (1914) isolated *Br. suis* from cases of abortion in swine.

With these discoveries in various species of animals, Meyer (1920) suggested that the genus be named *Brucella* in honour of Bruce, the first discoverer of the disease in man. The disease in sheep has been reported by various authors: in France by Dubois (1910),Southern Europe by Allessandrini (1939), North Africa by Renoux (1958), South Africa by Van Drimmelin (1953), in the Middle East by Unel, William and Stableforth

(1969) and Entassar (1967), in Britain by Allsup (1969) and in United States by Stoenner (1951). The middle and later part of the twentieth century witnessed more discoveries of species of the genus *Brucella* as a cause of disease in various animals. Thus Buddle (1956) isolated and described *B . ovis* in sheep, while Carmichael (1967) isolated and identified *B . canis* as an aetiological agent of contagious abortion in dogs.

GENERAL CHARACTERISTICS OF THE GENUS BURCELLA

Growth requirements and characteristics

Brucella colonies generally become visible after the cultures have been incubated for 3 days, but the plates are examined on the fourth or fifth day because there may be a delay of growth on selective media. Smooth colonies of *Brucella* species are convex, about 2mm in diameter with smooth edge and a blue, glistening and homogeneous appearance when viewed from indirect sunlight. Rough colonies appear as yellow, opaque, granular and break up when touched with a needle. Intermediate colonies have appearance intermediate between smooth and rough, that is slightly more opaque and granular than the smooth colonies. Mucoid colonies are glistening, greyish in colour and have a slimy consistency when touched with a wire. Only *B. canis* form a mucoid sediment in liquid media. (*Corbel et al.*, 1978).

Sir David Bruce in 1887 isolated causative agent in human brucellosis on peptone-beef extract. Bang (1897) and other workers after Bruce's work

observed that freshly isolated *Brucella abortus* requires an added amount of carbondioxide for growth. Detailed study by Zobell and Meyer 1932 threw more light on the growth requirements of brucellae in defined media.

Koser and Rettger (1919) were among the first of several workers who unsuccessfully attempted to grow brucellae in a synthetic medium. The comprehensive investigation of Zobell and Meyer (1932) and Zobell and Zobell (1932), resulted in successful cultivation of brucellae in chemically defined media. However, relatively large inocula were required, the growth period was prolonged and the actual cell yields were low, while serial transfer was not possible. The immediate cause of this was not known. McNutt and Purwin (1931), Olitzki and Bromberg (1931), and Huddleson (1932) also reported partial success with synthetic media.

Probably the first fully defined and nutritionally adequate medium was published by Koser, Breslove and Dorfman in 1941, at which time the specific preformed vitamin requirements of the brucellae were discovered.

Different undefined media, usually meat infusion comparable to that originally employed by Bruce, have been principally used in the cultivation of Brucellae. The beef liver infusion medium of Stafseth (1920) is typical and earlier found considerable use. Potato infusion agar was recommended by the Bureau of Animal Industry, U.S. Department of Agriculture, and is still in use for the preparation of standardized antigen and of vaccine for cattle.

Modifications of these complex media have appeared from time to time in the literature and in the manufacturers' formulae. Yeast and liver extracts and hemin have been reported to enhance growth in some cases, and

the growth-stimulating effect of thiamin when this vitamin is added to some undefined media suggests that it may sometimes be a limiting factor (McCullough, Mills Herbst and Roessler, 1947). The addition of iron and higher concentrations of glucose increased the growth of *B. suis* in one medium (McCullough, 1947). When the supply of oxygen is greatly increased, the usual concentrations of peptone and glucose may become limiting (Sanders, 1950). Yeast extract, thiamin, sodium bisulfite, and phosphate have been included in various commercial preparations along with the usual peptones, salt and glucose.

The growth of brucellae on undefined media may be inhibited by certain fatty acids. Boyd and Casman (1951) found that such components probably account for the toxicity of a commercial peptone. These toxic materials may be extracted by fat solvent or neutralized by starch.

McCullough *et al.*, (1947) and Sanders, Highuchi and Brewer (1953) designed media that will specifically support growth of strains of *B. suis* and *B. melitensis* respectively. In contrast, the medium of Rode, Glenda and Schuhardt (1950) was designed to promote growth from small inocula of large number of strains of the three species of Brucella. Of the 61 strains tested, including 47 that required additional CO_2, all grew in this medium, although wide differences were observed in the nutritional requirements of the various strains.

In retrospect, the development and application of synthetic media have tended towards either of two types; media that contain multiple sources of nitrogen and give maximum growth or media that contain a single source of nitrogen and have maximum simplicity compatible with

moderate growth. The choice of a medium, then depends on the purpose of the study in which it is to be used.

The basic inorganic materials needed in the media are met in various ways: Sodium ions usually have been added at the same concentration as in the traditional "physiological saline"(0.85%). The requirement for sulphur may be met by any of a number of inorganic or organic sources; thiosulphate and cystine have been used most commonly. Calcium is added in the form of calcium pantothenate. Nitrogen source is from mixtures of amino acids. Glucose traditionally is included in most bacteriological media as a source of carbon and energy. The concentration generally employed for brucella has been 0.1 to 0.4 percent. Other source of carbon and energy includes erythritol, L-glutamate and asparagine.

Accessory growth factors required by brucellae include: thiamin, nicotinamide, biotin, Vitamins B_{12} and Vitamin A (McCullough and Dick, 1942a, b). Several other supplements have been reported to accelerate the initiation and rate of multiplication of brucellae. These supplements include ribose nucleic acid, a mixture of its constituents, or adenine, guanine or cytosine (McCullough and Dick, 1942a, b).

Living animals, cell suspensions and body fluids constitute exceedingly important "media" for growth of brucellae. Embryonated chickens' eggs, leucocytes and tissue cultures prove especially useful *in vivo* systems (Gay and Damon 1955).

Morphology and Staining Reaction

The microorganisms are coccobacilli or short gram-negative rods measuring 0.5 - 0.7 by 0.6 - 1.5 milli-micron, occuring singly or more rarely in short chains.

Modified acid-fast (Stamp *et al.*, 1950) staining technique is used for staining Brucella organisms. With this method, Brucella organisms appear as red cocci or coccobacilli against a blue background, while other organisms are stained blue.

Gaseous requirement

The brucellae are aerobic and do not grow under anaerobic conditions. As with most aerobes, growth initiation of brucellae is favoured by a reduced air supply but considerable increases of both the rate and and extent of growth result when cultures are adequately aerated by either shaking or sparging (McCullough *et al.*, 1947). Agitation alone is ineffectual but turbulence is necessary for a high rate of oxygen transfer (Sanders, 1950). The type of sparger and the antifoam agent used are important factors in sparger aeration systems for mass production of brucellae. It is important to recognize that high aeration rates from shaking or sparging of cultures often result in a considerable and variable amount of evaporation during the usual incubation period unless the atmosphere is humidified.

The CO_2 requirement of *B . abortus* apparently was first studied by Bang in 1897; observing a sub-surface layer of growth in semisolid medium incubated in air and using different atmospheres of O_2 and CO_2, he thought that growth of the organism was influenced by lowered pO_2.

This idea of a reduced oxygen tension also was used to explain why primary isolates grow well in closed containers with cultures of *Bacillus subtilis* (Nowak, 1908) and in sealed tubes (Nowak, 1908). Huddleson (1921), clearly showed that the increased pCO_2 rather than reduced pO_2 was responsible for stimulating growth of *B . abortus*, but he concluded that this CO_2 in part was necessary to produce the pH change in the atmosphere. Wilson (1931) demonstrated unequivocably that CO_2 was required per se.

Many primary and freshly isolated strains of *B . abortus* differ from others of the group in requiring a quantity of CO_2 in excess of that needed by most heterotrophs. Five percent CO_2 in an air atmosphere seems adequate for such dependent strains (Huddleson, 1954); Ten percent frequently is used but may result in unfavourable pH changes (Huddleson, 1954), although either these increased levels or complete exclusion inhibits the growth of dependent strains (McAlpine, and Slanetz, 1928).

On continued subculture, isolates of *B . abortus* frequently lose the requirement for added CO_2 and may be grown in air alone. Marr and Wilson (1950) found that this acclimmatization consists in the selective establishment of spontaneously occuring mutants rather than the gradual adaptation of the culture as a whole.

The biochemical basis of the CO_2 requirement is unknown. Unlike comparable requirements in some other bacteria, the added CO_2 needed by *B . abortus* apparently cannot be replaced by cell extracts, complex nutrients, kreb cycle intermediates, or purines or pyrimidiness (Marr and Wilson, 1951).

pH requirement

The brucellae thrive in a rather wide range of hydrogen ion concentration. The maximum multiplication was observed between pH 6.6 and 7.4 with some growth as from pH 6.1 to 8.4 (Marr *et. al.*, 1951). Hydrogen ion concentration has a more specific effect on the viability of the organisms suspended in salt solutions.

Temperature requirement

Tradition and convenience often have dictated the use of an incubation temperature of 37^{oc} for brucellae. A scant literature (Copeland, 1955) indicates that both the rate and extent of growth are greater at 34^{oc}.

Dissociation property

Mutation in brucellae is readily observed as smooth-rough with virulence. There is ample evidence that such changes represent spontaneous mutations and that the emergence of a particular type in a population results from environmental selection (Braun, 1946). Population changes in originally smooth brucella cultures were found to be suppressed by normal serum and globulin fractions of susceptible animals (Braun, 1949), pyrophosphate (Cole and Braun, 1950), or chelating agents (Cole, 1952), and to be enchanced by homologous antiserum (Braun, 1946, 1947), pH, Eh and temperature (Braun, 1946), penicillin (Braun, 1946; Kraft *et al.*, 1952), metallic ions (Braun, 1946), sugars and breakdown products of deoxyribonucleic acid (Braun, *et al.*, 1954) uniquely favor change from rough to smooth colonial types.

Dye sensitivity

Brucella abortus usually grows in the presence of basic fuchsin, methyl violet, pyronin and safranin 0 but not thionin, at standard concentrations. *B . melitensis* grow in the presence of basic fuchsin, thionin, methyl violet, pyronin and thionin blue at the standard concentrations. *B . ovis* grow in the presence of basic fuchsin and thionin but are inhibited by methyl violet at standard concentrations. *B . suis* grow in the presence of thionin but most strains are inhibited by basic fuchsin, methyl violet, pyronin, safranin 0 and malachite green at standard concentrations. *B . canis* growth is inhibited by basic fuchsin but not by thionin at the standard concentrations. *B . neotomae* will grow in the presence of thionin only (Alton, 1975).

Biochemical reactions

Brucella organisms exhibit oxidative metabolism and usually show little fermentative action on carbohydrates in conventional media. Multiple amino acids, thiamin, biotin and nicotinamide are required. Haemin and nicotinamide adenine dinucleotide are not required. They are catalase positive; oxidase positive with the exception of *B . ovis* and *B . neotomae*; they produce hydrogen sulphide and urea is hydrolysed to a variable extent; nitrates are reduce to nitrites except for *B . ovis*; citrate is not utilized for growth; indole is not produced; methyl red and Voges-Proskauer tests are negative. They do not liquefy gelatin or lyse blood.

Resistance to environmental factors

Brucella organisms are susceptible to sunlight. Carpenter and Boak (1928) found that *B. abortus* was destroyed in 30 to 60 seconds at 160°F during pasteurisation. Kronenwett, Lear and Metzger (1954) studied the thermal death of brucellae and have shown that pasteurization at 160°F for about 4 seconds would destroy any brucellae present in the raw milk. They are readily killed by the common disinfectants. *B. abortus* dies out fairly rapidly in raw milk held at room temperature (Smith, 1934; Pullinger, 1935). *Brucella* organisms could survive in cream, butter and cheese at 4°C for 4-6 weeks (Guerra, 1957) and are able to survive in soil for 70 days if not exposed to sunlight; and in water for 35 days if not exposed to sunlight (Merchant and Packer, 1967).

Antigenic structures and phage lysis

The *smooth strains of B. abortus* may have A, M or A and M surface monospecific antigen, depending upon biotype. Cultures in the smooth or smooth-intermediate phase are lysed by brucella-phages of the Tbilisi (Tb), Weybridge (Wb), Firenze (Fz), M51-S708, Berkeley (Bk), MC/75 and D groups at routine test dilution (RTD) and 10^4 RTD. Non-smooth cultures are lysed by brucella-phage R at RTD.

Smooth strains of *B. melitensis* are lysed by BK phage at RTD and 10^4 RTD, but are not lysed by the other phages. Non-smooth cultures are resistant to lysis by all of phages. Smooth cultures may have the A, M or both A and M surface antigens reactive in tests with monospecific sera (Alton, 1975).

Brucella ovis does not have a true smooth phase, cultures are always in the rough phase on primary isolation. Rough (R)-specific surface antigens cross-reacting with other non-smooth brucellae are predominant. The cultures are lysed only by phage R at RTD and 10^4 RTD.

Brucella canis does not have a smooth phase at all, cultures are always in the rough or mucoid phase on primary isolation. It forms a mucoid sediment in liquid media. Rough-(R)-specific surface antigens cross-reacting with other brucella are predominant. It *is* not lysed by any of the phages.

Smooth cultures of *B . suis* have A surface antigen predominantly except for those of biotype 4 which react with A and M of monospecific antisera. Smooth or smooth-intermediate cultures are lysed by phages Wb, M51-S708, BK, MC/75 and D groups at RTD and 10^4 RTD. Non-smooth cultures are not lysed by any of the phages.

Brucella neotomae in smooth cultures have the A surface antigen predominant. Smooth or smooth-intermediate cultures are lysed by brucella-phages of the Wb, Fz, BK, M51-S75 and D groups at RTD. Rough or mucoid cultures are not lysed by any of the phages.

HOST SUSCEPTIBILITY

The various species differ in the frequency with which they infect particular host animals. *B . abortus* primarily infects cattle but sometimes buffaloes, camels, deer, dogs, horses, sheep, yaks and man may be infected (Boyd, 1950). Primary host for *B. canis* is the dog but accidental infection occurs in man. *B . melitensis* primarily infects

sheep and goats but may be transmitted to cattle and man. *B. neotomae* infect desert wood rat (Neotoma Lepida Thomas) only under natural conditions and no cases of natural infection occurs in other species. *B. ovis* displays a high degree of host specificity as its primary host is the sheep and natural infections have not been reported in any other species, including man. *B. suis* affects pig primarily but may be transmitted to dogs, hares, rodents and man. The secondary host usually is not very important in the maintenance or spread of the disease, the primary host is the reservoir of infection for each particular species.

Mode of transmission

Brucellosis is a zoonotic disease. All breeds and sexes of animals and man are susceptible to brucellosis and animal from endemic area appear to possess more resistance to the disease than those from brucellosis free areas.

Brucella excretion by infected animals could be through milk or vaginal discharge for three weeks after kidding or abortion (Taylor, Lisbonne, Vidal and Hazemann, 1938; Polding, 1943). However, Meyer and Eddie (1935) found at least one case where vaginal excretion lasted for 41 days.

The portal of infection include: the alimentary tract in contaminated feed or water; the genital system in contaminated semen; the unbroken skin, conjuctival, accidental inoculation of S19 vaccines and by drinking raw infected milk (FAO, 1964). The spread of the disease from place to place could occur by movement of infected animals or their products.

PATHOGENESIS OF BRUCELLOSIS

All of the species of *Brucella*, except *B . neotomae* which show minimal pathogenicity towards its natural host, produce infections which characteristically localise in the reproductive organs. In the female, the uterus and mammary glands usually become infected, with extension to the placenta and foetal tissues which often results in abortion in pregnant animal. In the male, the testes, epididymes and seminal vesicles are the main sites for localisation. All *Brucella* species grow intracellularly and can survive and multiply inside the mononuclear phagocytes of nonimmune animals. Infection is characterised by a bacteraemic phase, usually soon after initial entry of the organism, followed by extension to the tissues of the reticuloendothelial system. Localisation in the specific target organs occurs eventually, although almost any tissue may become infected. Active lesions may be produced in almost any organ, although in the primary hosts often the only overt manifestations of infection are invasion of the placenta and subsequent abortion.

The periodic search by Castaneda (1947) for the brucellae inoculated into laboratory animals showed that the organisms disappeared from the intercellular spaces after a few hours, only to be found within the cytoplasm of monocytes and polymorphonuclears. Several days later histiocytes, reticulo-endothelial cells, and even parenchymatous cells were seen heavily parasitized. Castaneda further showed that similar microscopic pictures could be produced by the injection of large doses of

killed brucellae with the difference that parenchymatous cells were not involved. The usual process of phagocytosis would hardly explain the over-crowded aspect of monocytes and dust cells from the lungs, but the absence of intracellular organisms in parenchmatous cells shows the true nature of the accumulation of live brucellae within the cytoplasm of these cells. It is of interest that at least two of the known intracellular parasites, when killed, can reproduce certain microscopic aspects seen during the infection. This might help in the understanding of the relationship between the infecting agents and their hosts.

The intracellular parasitism of *Brucella* must be taken into consideration in the interpretation of the symptomatology, histopathology and particularly in the adequate treatment of the disease. The tenacity with which brucellae remain attached to their host, in spite of an active cellular and humoral immunity, depends, obviously, on their facility to continue parasitism within certain cells of the spleen, liver, bone marrow and kidney. As a consequence of the persistent stimulation, the patients become hypersensitive to *Brucella* or its metabolic products.

CLINICAL FINDINGS

In most animals, abortion is the cardinal sign of the disease. The clinical signs are the same in sheep, goats and cattle. In these species of animals incubation period varies (Thomsen, 1950) and also there is fluctuating temperature during septicemia characterized by depression and anorexia. In some cases the first clinical sign of infection is abortion

in late stage of pregnancy. Still-born or weak calf, lamb or kid may also occur. Lameness with swelling of the joints (hygroma) is also common (Thomsen, 1950).

Brucella infection in dogs resulted in infertility in female, or early and undetectable embryonic death or abortion. Abortions most frequently occur around day 50 of gestation. Infection in males also may result only in clinically inapparent infertility (Carmichael and Kenney, 1968, 1970).

The clinical entities of fistulous withers and poll evil are the usual manifestations of *Brucella* infection in horses because of some degree of resistance of this species of animal to the infection. They usually occur in conjunction with a second infecting organism, *Actinomyces bovis*. The species of *Brucella* organism responsible for infection in horses depends on the host of contact (Portugal *et al.*, 1971) but most commonly *B. abortus*.

The clinical signs most commonly observed in pig brucellosis are abortion which may occur at almost any time of gestations. Other clinical signs include sterility, orchitis, lameness, posterior paralysis and occasionally metritis and abscessation of the extremities or other parts of the body (Hutchings, 1950).

The cats though generally are not naturally immune to brucellosis, there is an evidence that they possess a high degree of resistance (Boyd, 1950).

Clinically, brucellosis in human have variable incubation period and low mortality rate of 2 - 3 percent (Burrows 1973). Five varied clinical manifestations also occur namely: Intermittent ambulatory, undulant, malignant and atypical. Detailed descriptions of their manifestation have been well described (Burrows 1973).

PATHOLOGY OF BRUCELLOSIS

In cattle, once infection is established in sexually-mature animals they provoke an acute lymphadenitis. The inflamed glands are enlarged, often much so, hyperplastic with no clear corticomedullary distinction and frequently bear small or large medullary haemorrhages. The sinuses are infiltrated with neutrophils and eosinophils; germinal centres and proliferative activity become obvious; and there is a slow but remarkable accumulation of plasma cells in the medullary sinusoids (Payne, 1959). Localization of the organisms is largely restricted to the spleen, mammary glands, mammary lymph nodes, and pregnant uterus of the female; lymphoid tissues, testis and accessory glands in the male. Localization does occasionally occur in synovial structures to produce a purulent tendovaginitis, arthritis, or bursitis (Smith, 1919).

In sheep the placenta is grossly oedematous. Periarteritis and arteritis are distinctive features as in all forms of placentitis. (Molello *et al.*, 1963). The ammion is adherent to the chorioallantois and thickened in patches. The intercotyledonary placenta has plague-like thickenings which may coalesce to resemble yellow-white chamois leather. The majority oflambs is alive at the commencement of parturition, but

mummification or progressive autolysis are also common. As usual , the foetus tends to be moderately oedematous. (Young, 1953).

In most respects, the Caprine disease resembles bovine brucellosis. In the early bacteraemic phases, goats may suffer severe illness and even die, but many infections are asymptomatic (Jubb and Kennedy, 1970).

In pigs localization of *B . suis* may occur in many organs but especially in the male and female genitalia, the skeleton including the vertebral column, synovial structures, mammary glands, lymph nodes, spleen, liver, kidney, bladder, and even in the brain (Cameron, 1957). *B . suis* can grow and multiply in the phagocytes and the typical granulomatous lesion begins with the accumulation of histocytes and epithelioid cells. Giants cells are absent or scarce. Calcium may be deposited in the necrotic foci. Articular lesions caused by *B . suis* begin as a synovitis and affect chiefly the compound and large joints of the limbs. (Forbus, 1946). The reaction is purulent or fibrino - purulent. osteomyelitis in this disease is typically vertebral (James & Graham, 1930). In the infected uterus and Fallopian tubes there are few or very many yellowish-white nodules seeded in the mucosa. When the nodules are numerous, they may coalesce to form irregular plaques and then are associated with thickening of the uterine wall and structure of the lumen. The same lesion is usually present also in the Fallopian tubes where obstruction results in pyosalpinx. Placenta retention may occur. This placenta is congested with small haemorrhages and patechy oedema. (Thomsen, 1934).

In both male and female dogs infection causes splenitis and generalized lymphoid hyperplasis, particularly in the chronically infected animal. In male dog infection is accompanied by epididymitis, prostatis, scrotaloedema or testicular atrophy alone or in various combination (Moore and Kakuk, 1969.; Camichael and Kenney, 1968, 1970).

Cats are resistant to natural infection with *Brucella*.

In horses *Brucella* organisms localize in connective tissues such as muscles, tendons and joints with evidence of inflammatory changes (Portugal *et al.*, 1974).

The pathological findings in human affected by brucellosis are those of acute, septicemia. The organism may be localized to cause vegetative endocarditis, orchitis, osteitis, meningitis or subacute arthritis. The chronic lymphogranulomatous form of brucellosis characterized by enlargement and lymph nodes (Forbus, 1943).

LABORATORY DIAGNOSIS OF BRUCELLOSIS

The disease, brucellosis not only deserves a greater importance as a major and widespreading disease of animals resulting in severe economic loss, but also it occupies a significant place among zoonotic diseases, since it can be transmitted to human beings either through ingestion of contaminated milk or by contact with infected animals. Hence attention of many Scientists was directed to finding an easy to perform, simple, accurate, sensitive and specific method for the diagnosis of brucellosis. As a result of that, lot of literature has been accumulated on various serological tests for the diagnosis of

brucella infection.

They are:

(i) Blood serum tube agglutination test by Wright and Smith (1897)

(ii) Blood serum plate agglutination test by Huddleson (1920)

(iii) Flocculation test by Sachweh (1935)

(iv) Coombs' test by Coombs *et al.*, (1945)

(v) Blocking test by Griffiths (1947)

(vi) Surface fixation test by Castaneda (1953)

(vii) Capillary tube stained antigen test by King (1951)

(viii) Whole milk plate test by Blake *et al.*, (1952)

(ix) Milk ring agglutination test by Gregory (1953)

(x) Vaginal mucus test by Kerr (1955)

(xi) Precipitin test by Burki (1957)

(xii) Gel diffusion precipitin test by Bruce and Jones (1958)

(xiii) Whey tube agglutination test, Cited by Stableforth and Galloway (1959)

(civ) Seminal plasma agglutination test cited by Stableforth and Galloway (1959)

(xv) Heat inactivated serum test by Amerault *et al.*, (1961)

(xvi) Whey plate agglutination test, cited by Krishna Murthy and Kaushik (1963)

(xvii) Whole blood plate agglutination test by Krishnamurthy and Kaushik (1963) and

(xviii) Complement fixation test (Mc'Fadyean, 1909)

Most of the tests depends on antigen-antibody reactions, presence of IgM, IgG and pH. The most popular of the rapid methods for diagnosis of human brucellosis is the plate test proposed by Huddleson (1943). The specificity and sensitivity of this test has been studied by many workers. In setting up the plate test (Huddleson test), the practice in the USSR is to use a single brucellosis diagnostic preparation made with a 120g/litre sodium chloride solution with the addition of gentian violet and brilliant green. In the U.S.A., a new rapid method called the card test has been developed for the diagnosis of bovine brucellosis. This method has been in use there since 1966 in screening herds for brucellosis. In other countries (France and United Kingdom), the test has been called the Rose bengal plate test (RBPT). The antigen is prepared from the smooth form of *Br. abortus* strains 99 or 1119. A suspension of heat-killed brucellae is stained with Rose bengal and suspended in a lactic acid buffer of pH 3.6. RBPT has been studied principally as a test for animal (cattle, sheep and reindeer) sera (Nicoletti, 1967, Nicoletti, 1969; Davies, 1971; Morgan, 1971; Corbell, 1972).

The serum agglutination test (S.A.T.) using standardized antigen has been used extensively for the diagnosis of brucellosis in man and domestic animals. However, it suffers from the disadvantage that false positive reactions sometimes occur. These may be the result of cross-reactions between antigens of *Brucella spp.* and unrelated organisms, such as *Yersinia entercocolitica* (Ahvonen, Hansson and Aho, 1969; Corbel and

Cullen, 1970) or they may result from the presence of nonspecific agglutinins distinct from antibodies, which are present in certain bovine sera (Hess, 1953). In an attempt to differentiate specific brucella agglutinins from those non-specific factors, Rose and Roepke (1957) introduced a modification of the plate-agglutination test in which the antigen was buffered at pH 4.0 immediately before use. They observed that at this pH, agglutination of bovine serum was inhibited, whereas the activity of specific brucella antibodies showed that the acid plate-agglutination test was of value as a supplemental test in screening field samples of serum for brucella-specific agglutinins (Lambert and Amerault, 1962).

The application of the complement fixation and agglutination test for the diagnosis of bovine brucellosis was realised as early as 1909 by Mc'Fadyean and Stockman in England and Hölth (1909-10) in Denmark. Even today, most of the workers believe that the conventional agglutination test alone is not reliable. The simultaneous performance of both these tests may be utilized in the differentiation of infection and vaccinal titres.

The complement fixation test has been used for the diagnosis of *Br. ovis* infection in sheep by several workers (Clapp, 1955, 1961; Biberstein and McGowan, 1958; Biberstein *et al.*, 1964; Ryan, 1964; Gorrie, 1964, 1968; Hughes and Claxton, 1968).

Conglutinating complement absorption test was originally described by Bordet and Strenge (1909); Stoker and Marmion (1950; Gulrajani, 1951)

employed this test in the sero-diagnosis of influenza, vaccinia and swine influenza viruses respectively. Thereafter Englehard and Carlise (1955), Millian and Englehard (1961), and Sharma *et al.*,(1966) employed this test in sero-diagnosis of brucellosis.

The possible use of heat for inactivating nonspecific agglutinins had been recognized earlier. Jones (1927) reported that antibody destruction progressed as temperature increased, and that heating at 65^{oc} for 20 minutes appreciably affected antibody activity. Hoerlein (1953) reported that serum of *Brucella-free* swine, which reacted to the standard tube test, had negative or much lower titres when incubated at 5^{oc} for 16 hours. Morse *et al.*, (1955) obtained similar results using bovine serum. Rose (1955) found two non-specific brucella agglutinins in bovine serum by using physico—chemical methods; one agglutinin was heat-stable and the other was heat-labile at 56^{oc}. Hess (1951) observed that the non-specific agglutinins of bovine origin, associated with suspect brucella agglutinins of bovine origin, associated with suspect brucella titres, were inactivated when heated at 70^{oc} for 10 minutes.

The milk ring test was first introduced by Fleischhauer in 1937. Investigators in Denmark and Sweden succeeded in developing the test to its present level of sensitivity. It has been used in those countries for routine checking of certified brucella-free herds and areas as well as for detecting infected herds in the eradication programme. Bendtsen and Wood (1950) developed Vital-stained antigens for milk ring test.

Roepke and associates (1949) adapted the test to cream samples and in this way increased its range of usefulness for field examinations.

Extensive use of the antiglobulin test using a polyvalent antiglobulin reagent, has been made by Cunningham (1967, 1968, 1971) when investigating serological responses to *B . abortus* strain 19 and 45/20 vaccines. In man the antiglobulin test using monospecific anti-immunoglobulin sera was used by Kerr *et al.*, (1967) in the diagnosis of chronic cases of human brucellosis. These workers found non-agglutinating antibody activity associated with IgG and IgA.

Serum antibodies to *B . ovis* in sheep can be detected by serum agglutination of bacterial suspensions (Buddle, 1954), indirect haemagglutination (Ris and Te Punga, 1963), immunodiffusion(Matthews and Trueblood, 1967; Meyers and Siniuk, 1970), and complement fixation using a whole cell antigen (Clapp, 1955) or soluble antigen prepared by sonication (Riberstein and McGowan, 1958) or refluxing of *B . ovis*. suspension (Clapp, 1961).

Cultural diagnosis

The use of media containing antibiotics for the selective isolation of brucellae from suspected contaminated materials has been one of the valuable recent advances in the diagnosis of brucellosis.

Bang (1897) first isolated *B. abortus* from an aborted bovine foetus using a semi-solid gelatin agar containing 33 percent serum. Schroeder and Cotton (1911) also isolated *B . abortus* from the guinea pig spleen

inoculated with cow's milk sample. Stafseth (1920) discussed the wide variety of media used for the isolation of *Bacterium abortus*. These included media containing extracts of the bovine uterine wall, foetal membrane and foetuses, and in addition to amniotic fluid agar, blood clot agar and glycerine agar.

Stafseth (1920) improved the basal medium by addition of one percent glucose or starch to beef infusion agar.

The development of spleen and liver infusion by Stafseth (1920), which was described by Holth (1911) resulted in a much improved medium, the spleen infusion being further fortified by the addition of glucose. The efficacy of liver infusion agars was confirmed by Huddleson (1920) and they soon gained wide acceptance for the isolation of *B . abortus*, subsequently forming the basis of the selective agars which in-corporate aniline dyes and antibiotics. The disadvantage of liver infusion broth is that each batch has to be subjected to rigorous quality control.

The variability in quality of commercial peptones and meat-infusion broths resulted into the use of peptic and tryptic digest media in both the United States (Stickel and Meyer, 1918) and the United Kingdom (Cole and Onslow, 1916). Meyer and Shaw (1920) found that the peptic digest agars prepared from either pig or bovine liver were suitable for the primary isolation of *B . abortus* from contaminated sources. Kuzdas and Morse (1953) described a selective medium, for the culture of *Brucella*, including fastidious types, and this was modified by Renoux

(1954). Castaneda (1947) described method of brucella isolation from blood sample. In 1975 Brodie and Sinton developed a new liquid medium for enrichment of *Brucella* when attempting to isolate the organism from contaminated materials. Farrell (1977) modified serum dextrose agar for isolation from contaminated materials such as milk, placenta, semen and vaginal discharge.

Laboratory animals such as mice or guinea pigs could be used for an indirect isolation of the organisms.

IMMNITY

Immunity to *Brucella* infections depends essentially on the intracellular destruction of these organisms in phagocytes. Despite the fact that brucellae are subject to extracellular lysis with antibody and complement, antibody probably plays little part in protection, since immunity cannot be transferred passively with serum, and dead vaccines which produce high titres of antibody to the surface somatic antigens are ineffective (McDiarmid, 1949).

Vaccines

The living, attenuated (Rev.1) vaccine developed by Elberg and faunce in 1957 has been used and shown to produce immunity against *B . melitensis* infection in maltese goats by Alton (1959), but this vaccine has certain limitations. It is known to cause abortion in pregnant animals and at least one month must elapse between vaccination and mating.

Pilet, Bonneau and Valette (1970) reported on a new type of brucella vaccine, PB19, prepared from S19 strain treated with heat and formol saline and suspended in antibrucella hyperimmune bovine serum and was tested in cattle and goats. Studies of its antigenic properties carried out in 50 cattle and 3 goats, showed that the vaccine did not produce antibodies in diagnostically significant titres, according to the results of serology tests.

B . abortus, strain 19 which has been widely used for vaccination of cattle has also been used in Russia in sheep (Popov and Abakin, 1956: Sofronov and Novaev, 1958).

PUBLIC HEALTH SIGNIFICANCE OF BRUCELLOSIS

Brucellosis is a zoonotic disease. The species of *Brucella* infect not only animals; it also readily infects human.

Man contracts brucellosis mainly through direct contact with infected animals or their tissues or by ingestion of unpasteurized dairy products derived from infected animals. The organisms can penetrate the apparently unbroken surfaces like skin and mucosa and where pasteurization is practised widely brucellosis has become largely an occupational disease of those who raise and care for livestock, or are employed in the slaughter of meat animals and processing of the product. A minor epidemiological pathway is by inhalation of aerosols, produced in abattoirs, or the laboratory centrifuges, or dust laden with the organisms.

Sabbaghian (1975) found that of 1220 fresh white cheese samples made from raw goats and sheep milk and collected in the Isfahan area of Central Iran 87 (7%) were found to be infected with *B . melitensis* biotype 1. The average number of *B . melitensis* per gram of infected cheese was 2120 after collection which decreased until the nineth week when no Brucella could be isolated. About 40% of infected cheeses became free of *Brucella* one week after collection. The seasonal fluctuation in infected cheese corresponds to that of human brucellosis in which most cases are seen from April to August.

Guerra (1957) found that *B . abortus* survived for 6 weeks in cream stored at 4^{oc}, while *B . melitensis* survived for only 4 weeks. King (1957) reported that *B . abortus* survived for 30 days in ice-cream kept at 32^{oF} and for 142 days in butter at 46^{oF}. Fulton (1941) reported that some strains of *B . abortus* survived for 13 months in unsalted butter and for 6 months in butter containing 2.1% salt. Grosso and Bergamini (1959) isolated brucellae from 1.8% of the 142 commercial butter samples they examined.

Several authors have reported the results of their investigations into infection by *Brucella* of those in frequent contact with animal reservoirs of infection, such as slaughterers, butchers, shepherds, veterinarians, stablemen and dairy farm workers.

Henderson (1967) and Henderson and Hill (1972) carried out a survey among Veterinarian dairy farm workers and slaughtermen in a rural area in England where bovine brucellosis was common.

Giuliani (1949) studied the problem in Santeramo, Bari province, where notifications for human brucellosis in the five years 1936-1940 were 374.64 per 10,000 and of 1,617 goats tested 42, or 2.6 percent were positive. Among 27 persons examined, engaged as above stated, the sera of ten agglutinated (37%); 4 at 1:20, 3 each at 1:40 and 1:80. Three agglutinated *B . melitensis*, one *B . abortus*, two *B . paramelitensis*, two all in equal titre but higher for abortus. They had shown no symptoms and are consequently regarded as "Latent" cases, infected by frequent inoculation of small doses of the organism.

Randhawa *et al.*, (1970) on examination of 700 fresh samples of organs and tissues from 100 slaughtered goats revealed *B . melitensis* in lymph mode, testicle and muscle from two carcasses stored in the refrigerator and none from abattoir equipment. Agglutination tests done before slaughter were negative.

Wilson (1957) suggested that contact with infected animals is more dangerous than consuming contaminated foodstuffs. Veterinarians, animal owners and workers, especially on dairy farms, are exposed to brucellosis in aborted fetuses. Uterine contents and discharges, contaminated floors, utensils and dust are sources of infection. Infection may be conveyed to man through the mouth by the hands or may enter via the conjunctiva, dust inhalation, abrasions or even intact skin. Abattoir workers are exposed to carcass which may be in the bacteramic phase of the disease, when all parts including skins, may be contaminated to some extent, but most dangerous are infected uteri and their content and udders.

All parts of the abattoir may become contaminated, including chutes and machinery.

The Veterinarians and his lay assistants run the additional risk of accidental self-vaccination when using the living *B . abortus* strain 19 vaccine (Gulasekharam 1970).

Brucellosis is one of the most easily acquired laboratory infections; *B . melitensis* and *B . suis* are more dangerous in this respect than *B . abortus* (Spink, 1969).

In France, Berthet (1963) found that 88% of human cases were attributable to direct contact with cattle, and only 0.5% to consumption of milk. In Italy, Brizio (1952) and Nucciotti (1956) also found that most cases probably arose through direct contact with infected animals. Epidemiological studies in Australia from 1936 to 1956 suggested that brucellosis was often acquired through handling infected cows (Wilson, 1957).

In other countries, many cases have been traced to consumption of raw milk and its products. In Britain, most cases of human brucellosis appear to be due to drinking raw, infected milk (Bothwell, 1963). Wynns (1944) considered that two thirds of all human brucellosis cases in California arose through consumption of raw milk or raw milk products. An outbreak of brucellosis in America was traced to cheese made from unpasteurized goats' milk (Stiles, 1945).

The incidence of brucellosis in man depends upon the incidence of brucellosis in cattle, sheep, goats and other domesticated animals, and the extent to which raw milk and its products are consumed.

There is not at the present time any definite proof that man can transmit the disease to animals excepting by mechanical means; for example, by the medium of footwear, soiled with infected manure from a cowshed where the disease exists in animals to another cowshed or a farmshed which is uninfected. Inter-human contagion is rare and would not really play a part in the epidemiology of brucellosis, though Burnet showed in 1926 that human milk could contain *Brucella*. The same fact was verified in the case of *Brucella* infection in a baby aged five months (Renoux, 1952). This is only a curiosity and it is the animal infection which remains the source of human infection.

ECONOMIC IMPORTANCE OF BRUCELLOSIS

Brucellosis in animals and man has major significance for both the livestock industry and human health. Economic losses in animal industry arise from abortions, stillborn, early death, infertility in both sexes, unthriftiness and rarely death of adult animals. The abortion factor is important economically especially to the livestock owners. Following abortion there is generally much reduced milk yield. Also frequent occurrence of abortion causes a loss of young animals needed for sale or for replacement. Infertility may take the form of failure to conceive, or lengthen the calving interval and so a smaller proportion of animals is in milk at anytime.

Abortion occurs in late pregnancy. Other domestic animals like dogs, sheep, goats, man and pigs can get infection from infected cattle.

Esuruoso (1979) has calculated the probable total losses per annum due to bovine brucellosis in Nigeria to be ₦140.80m.

There are an estimated 8 million heads of sheep and 28 million goats in Nigeria. Following Esuruoso's (1979) method of analysis it is estimated that total losses per annum due to brucellosis in sheep and goat industry in Nigeria will be close to half of the total loss in cattle. The actual total losses per annum is difficult to calculate because the actual total number of sheep and goats are not known precisely. The cost of losses in case of infection in human is a difficult task to perform.

Abortion in mare is very rare. Abortions infact may occur in mares with alarming frequency but only rarely is one of the *Brucella spp.* responsible (Boyd, 1950).

Swine brucellosis, although not of great economic significance to the swine producer, is often a serious economic problem to the professional swine breeder.

Canine brucellosis poses the greatest problem to breeders of dogs, especially to those engaged in the production of animals for research purposes and to owners of pure bred animals. It affects dogs of all breeds (Carmichael, 1968).

The presence of brucellosis in farm animals exposes farmers and often animal handlers to brucella infection. Prolonged misery,

incapacitation and nervous disorder result from Brucella infection. Majority of livestock owners in Nigeria are usually peasant farmers and least able to support the loss of revenue caused by brucellosis in their flocks or herds of animals and are least able to afford the long and expensive treatment for themselves and their families in case they contract brucellosis.

CHAPTER II

SEROLOGICAL STUDY OF OVINE AND CAPRINE BRUCELLOSIS IN NORTHERN NIGERIA

INTRODUCTION

The serological evidence of brucellosis in sheep and goats in Nigeria have been recognized as far back as 1934 (Eze, 1977). Adam and McKay (1966) in their survey of government-owned farms in Eastern Nigeria found no detectable agglutinating antibodies to brucella organisms in 110 goats sera examined.

Kramer, Nduaka and Uzoukwu, (1967) in a serological survey of diseases of cattle, sheep and goats in the eastern provinces of Nigeria found that one of the 115 ovine sera examined was positive and these animals were from the unimproved farms around the University of Nigeria's Nsukka campus. One of the 163 caprine sera examined was positive, and the positive goat was one of the 40 goats obtained from the northern provinces of Nigeria.

Falade, Ojo and Sellers (1974) showed that the incidence of brucellosis in Western Nigeria was 4.3 percent in goats but could be as high as 8.9 percent in some areas.

Morrison, Bullock and Kenward, (1975) in a survey of certain part of Northern Nigeria found the incidence of brucellosis as 1.8 percent in sheep and 1.5 percent in goats.

Many abortions in sheep, goats and cattle have been reported from all parts of Northern States although serological tests for brucellosis were not carried out.

In 1963, at Bulassa Livestock Breeding Centre, sera from 60 goats out of which 9 goats had aborted were tested serologically, all sera were negative (Nuru, 1974). Eid (1972), found that out of the 44 goats sera from Birnin Kebbi, 17 (39%) were positive.

Previous serological surveys have either been restricted to certain geographical locations (Adam and MacKay, 1966, Kramer *et al.*, 1967; Falade, Ojo and Sellers, 1974; Morrison *et al.*, 1975) or involved abattoir and slaughter slab animals. Both have never involved sampling on a wider geographical distribution or animals resident in the locations since abattoir animals are usually trade animals from far and near including neighbouring countries.

The investigations reported here therefore attempt to determine the prevelence by serological survey of brucellosis in sheep and goats of Northern Nigeria by sampling resident and transit animals and evaluate the relative efficiency of the three different serological methods most commonly used for the diagnosis of brucellosis in sheep and goats in the area survey.

MATERIALS AND METHODS

The goats tested were mostly Sokoto Red goats, Kano Brown and crosses between the two while the sheep were mostly Yankasa, Udah, Balouchi, Balami, crosses between these four breeds and a few fat tailed Sudanese sheep. These animals were occurring in largest number in the areas surveyed.

The survey areas were broadly divided into three groups consisting of (a) animals from abattoirs and slaughter slabs obtained from far and near including neighbouring countries and they were regarded as transient animals; (b) animals from Government farms or institutional farms were regarded as Institutional resident animals and (c) animals from local markets places, Fulani men , private farms and individual households were regarded as local resident animals.

Blood samples for serum were collected from sheep and goats during slaughter at slaughter houses while blood samples were collected from the jugular vein from flock of resident animals. The samples were transported to the laboratory in an ice-box.

Blood samples were allowed to clot at 4°c and serum decanted from clotted blood and centrifuged at 3,000 rpm for 15 minutes. All sera were stored at -20°c until they were tested. Sera were tested for presence of antibodies by three serological methods; Rose Bengal plate test (RBPT), tube serum agglutination test (S.A.T.) and complement fixation test (CFT).

The methods used for all the three tests were as described by Morgan, Mackinnon, Gill, Gower and Norris (1978). The results were converted to international units (IU) as set out by Morgan *et al.*, (1978).

The Rose bengal plate test (RBPT) was used to screen all the sera using the standard RBPT antigen. Antigen was used at a dilution of 1/16 to detect infected animals. Sera which were

positive by the RBPT were further tested by the S.A.T. using the S.A.T. standardized antigen and by the C.F.T. using CFT standardized antigen*.

For serum agglutination tests (S.A.T.) serial two fold dilutions 1/10 to 1/80 were made in 5 percent phenol saline solution to reduce the prozone effect. Stock concentrated antigen was diluted to 1/10 before being used. The end point of a titration was taken as the highest dilution giving a reading of 50 IU.

The recommendation of the WHO/FAO Expert Committee on Brucellosis was strictly followed. The sera of goats and sheep with serum agglutination test or complement fixation test titres of 50 international units or more were considered as reactors. All the sheep and goats examined were not known to have been vaccinated.

RESULTS

Sera from 885 sheep and 1774 goats were screened by the Rose Bengal plate test (RBPT). Of this, 125 (14.1%) were positive for sheep and 287 (16.1%) were positive for goats (Table 1).

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* *All antigens were supplied by the courtesy of Dr. D.J. Mackinnon, CVL, Weybridge, England.*

Among the 885 sheep, the highest RBPT positive was from the Institutional flocks in which 82 out of 382 (21.4%) were positive while only 17 out of 342 (5.4%) were positive in the local flocks. The trend was the same for goat.

The data on Table 2 show the results of tube serum agglutination test (S.A.T.) for the sheep sera that were RBPT positive. Out of 125 sera that were positive by RBPT 70 were positive by the SAT out of which only two (2.9%) had antibody titre of 50 IU or above, and were classified as reactors.

Table 3 shows the complement fixation test (CFT) results for sheep sera. Of 125 RBPT positive sera thirty two were positive by the CFT but none had antibody titre up to 50 IU or above by this test.

Out of 287 goats sera that were RBPT positive, 166 were positive by the SAT out of which only 29 (17.5%) had antibody titre of 50 IU or above and were regarded as reactors (Table 4). The 287 goats sera that were RBPT positive 121 gave positive results by the CFT. Eight of the 121 positive sera by the CFT had antibody titre 50 IU or above (Table 5).

The data on Table 6 compare positive sera in sheep and goats using SAT and CFT at different antibodies titre levels. The results show that SAT values were higher than the CFT. In sheep there were two reactors by the SAT and none by the CFT. Similarly in goats 29 reactors were detectable by the SAT whereas only eight were detectable by the CFT.

TABLE 1

Sheep and Goats reactors to Brucella by the Rose Bengal Plate Test.

SOURCE OF ANIMALS	SHEEP			GOATS		
	NO. OF SAMPLES TESTED	NO. OF SAMPLES POSITIVE	PER-CENT POSITIVE	NO. OF SAMPLES TESTED	NO. OF SAMPLES POSITIVE	PER-CENT POSITIVE
Slaughter animals	179	26	14.5	1,015	169	16.6
Local flocks	324	17	5.2	261	15	5.7
Institutional flocks	382	82	21.4	498	103	20.6
T O T A L	885	125	14.1	1,774	287	16.1

TABLE 2

Result of Serum Agglutination Test (S.A.T.) in Sheep Sera that were Rose Bengal Plate Test Positive

Source of Animals	RBPT	S.A.T	SERUM AGGLUTINATION TEST TITER				Percent ** Prevalence	
	Positive	Positive	1:10 (13-20.5)*	1:20 (25.5-41)	1:40 (51.5-82)	1:80 (102.5-164)	NO	%
Slaughter animals	26	14	7	5	1	1	2	1.1
Local flocks	17	9	5	4	0	0	0	0
Institutional flocks	82	47	34	13	0	0	0	0
T O T A L	125	70	46	22	1	1	2	0.2

RBPT = Rose bengal plate test

S.A.T = Serum Agglutination test

* = Figures in parentheses are equivalents in International Units per ml (IU)

** = Percentage prevalence calculated with respect to the total number of sera collected.

TABLE 3

Result of Complement Fixation Test in Sheep sera that were Rose Bengal Test Positive

Source of Animals	RBPT	CFT	Complement Fixation Test Titer				Percent Prevalence**	
	Positive	Positive	1:2-(13-20.5)*	1:4-(25.5-41)	1:10-(51.5-82)	1:20-(102.5-164)	NO	%
Slaughter animals	26	10	5	5	0	0	0	0
Local flocks	17	0	0	0	0	0	0	0
Institutional flocks	82	22	14	8	0	0	0	0
T O T A L	125	32	19	13	0	0	0	0

RBPT = Rose Bengal plate test

C F T = Complement fixation test

* = Figures in parentheses are equivalents in International Units per ml

** = Percentage Prevalence calculated with respect to the total number of sera collected.

TABLE 4

Result of Serum Agglutination Test in Goats Sera that were Rose Bengal Plate Test Positive

Source of Animals	RBPT	S.A.T	SERUM AGGLUTINATION TEST TITER				Percent Prevalence **	
	Positive	Positive	1:10 (13-20.5)*	1:20 (25.5-41)	1:40 (51.5-82)	1:80 (102.5-164)	NO	%
Slaughter animals	169	93	42	35	10	6	16	1.6
Local flocks	15	11	7	3	0	1	1	0.4
Institutional flocks	103	62	29	21	11	1	12	2.4
T O T A L	287	166	78	59	21	8	29	1.6

RBPT = Rose bengal plate test

S.A.T = Serum Agglutination test

* = Figures in parentheses are equivalents in International Units per ml

** = Percentage Prevalence calculated with respect to the total number of sera collected.

TABLE 5

Result of Complement Fixation Test in Goats Sera that were Rose Bengal Test Positive

Source of Animals	RBPT	CFT	COMPLEMENT FIXATION TEST TITER				Percent ** Prevalence	
	Positive	Positive	1:2-(13-20.5)	1:4-(25.5-41)	1:10-(51.5-82)	1:20 (102.5-164)	NO	%
Slaughter animals	169	78	34	39	3	2	5	0.5
Local flocks	15	7	5	2	0	0	0	0
Institutional flocks	103	36	27	6	3	0	3	0.6
T O T A L	287	121	66	47	6	2	8	0.5

RBPT = Rose Bengal plate test

C.F.T = Complement fixation test

* = Figures in parentheses are equivalents in International Units per ml.

** = Percentage Prevalence calculated with respect- to the total number of sera collected.

TABLE 6

A Comparison of the Serum Agglutination Test and Complement Fixation Test in 125 Sheep and 287 Goats Sera Positive by the Rose Bengal Plate test

TITRE (IU) International Units	NUMBER OF SERA POSITIVE IN EACH TEST AT TITRE IN INTERNATIONAL UNITS SHOWN			
	S H E E P		G O A T S	
	S.A.T.	C.F.T.	S.A.T.	C.F.T.
13-20	46	19	78	66
25.5-41	22	13	59	47
51.5-82	1	0	21	6
102.5-164	1	0	8	2
T O T A L	70	32	166	121

DISCUSSION

There was evidence of brucellosis in sheep and goats from all sample area. On the whole, there seems to be more positive cases in the Institutional animals than those in the local flocks or in abattoirs when tested by the rose bengal plate method (RBPT). Similar trend was observed by the tube serum agglutination test (SAT) among the goats. There were however no reactors in the sheep based on the FAO/WHO (1971) standard 50 IU or above in a nonvaccinated animals. Few reactors were detectable by the complement fixation test (CFT) only in the Institutional and slaughter goats and there was no reactor in all the sheep from all sources by this test.

It is not surprising that there seemed to be an evidence of higher rate of infection in Institutional animals than animals kept by local farmers or those found in abattoirs. The Institutional animals are kept together under intensive husbandary. This type of management tends to encourage spread of any disease within the herd or flock. For example, if an animal aborts because of brucellosis, the chance of infecting other animals is high. On the other hand, the local animals and those brought to abattoirs for slaughter are kept under free range and nomadic type of husbandary, the chance of a group of animals remaining or returning to a contaminated environment for some time is usually remote and this tends to reduce the chances of susceptible animal being infected. This is similar to the findings in cattle by Nuru (1976).

The RBPT in general, gave the highest positive results of the serological test. The SAT results were much higher than the CFT results but less than the RBPT.

It was observed that even by the SAT, it was possible to have titre as high as 50 IU or above while the CFT for the same serum was negative. It has been concluded that no single test detects cases of brucellosis, that is, a single negative test cannot be relied on (Olascoaga, 1976) and the author has suggested that serological tests in various animals be interpreted with caution.

It is generally agreed that the RBPT is a screening test which is highly sensitive but heterospecific (Olascoaga, 1976). Infection due to organisms such as *Vibrio cholerae, Yersinia enterocolitica* type IX, *Pasteurella spp, Salmonella* or some other members of the Brucellaceae family could give false positive results to brucella antigen although antibody titers in these cases are usually low (Olascoaga, 1976). Since RBPT is carried out at only one dilution this heterospecificity becomes a big problem. On the other hand SAT and CFT may help to eliminate the problem in active infection. Both tests use serum diluted to determine antibody titer. It is therefore not suprising that RBPT gave much higher positive results than SAT and CFT.

There are conflicting reports in the literature as regards these three commonly used tests and the type of immunoglobulins measured by them. Waghela (1978) showed that in adult goats

experimentally infected with *B . melitensis* agglutinating antibodies by SAT appeared first followed by those of agar gel immmnodiffusion test (AGIT), RBPT and CFT in that order. CFT, RBPT and AGIT persisted longer while SAT became negative after a while. Corbel (1972) and Olascoaga (1976) reported that the RBPT measured IgG_1, SAT measures IgM and IgG and in CFT IgG_1 is the major component of immunoglobulin fixed by complement. Allan *et al.*, (1976) on the other hand reported that IgM reacted more efficiently than IgG_1 and IgG_2 in both the RBPT and SAT and even the CFT was also found to be mostly more sensitive to IgM than to IgG_1, a conclusion which contrasts those of Corbel (1972) and Olascoaga (1976).

Other reasons could account for the discrepancies in the results of the three serological tests. It has been found that *B . abortus* and *B . suis* antigen are less sensitive when tested with sera from *B . melitensis* infection (Olascoaga, 1976). The same author concluded that SAT may be negative in chronic infection because IgM is no longer present. It is possible that in such cases the low level of antibody due to IgG could still be picked up by the RBPT if we are to go by the finding of Corbel (1972) and Olascoaga (1976).

The stage of infection and other physiological factors have been shown to affect the serological results. For instance in animals exposed for the first time during gestation, it is quite frequent for agglutinins to appear several days and even up to two weeks after abortion or parturition. During gestation, a small proportion of the

animals may show a transitory decrease in the agglutinin rate. This happening has been explained by the movement of immunoglobulins from the general circulation to the mammary glands.

It is concluded that reactor rates in Northern Nigerian sheep and goats are about 0.0 to 0.2 percent for sheep and 0.5 to 1.6 percent for goats based on the standard CFT or SAT (FAO/WHO, 1971, Morgan, *et al.*, 1978). It is however, possible that the infection rate could be higher based on the facts and limitations already discussed above.

The results of this survey compare favourably with those of previous workers. Falade, Ojo and Sellers (1974) reported a reactor rate of 4.31 percent in goats with a range of 0 - 8.9 percent depending on location using the SAT. Morrison Bullock and Kenward (1975) on the other hand reported a 1.8 percent reactor rate in sheep and 1.5 percent in goats. The authors observed that all the positive sheep came from four premises, suggesting that infection was limited to certain foci in Northern Nigeria, a finding which could also account for variation in results obtained at different times and places by different workers. The hypothesis of close correlation between SAT and RBPT reported previously (Falade, 1978) appear not to be so in this study because RBPT gave the highest figures.

SUMMARY

A serological survey for ovine and caprine brucellosis was conducted in 885 sheep and 1774 goats sera collected from various parts of the Northern Nigeria.

Sera were grouped according to sources of animals as Institutional flocks, from (Government and quasi-government farms), abattoirs animals, at the time of slaughter and local household animals which include Fulani flocks and animal from markets.

Sera were screened by the rose bengal plate test (RBPT) and if positive were further tested by the serum agglutination test (SAT) and complement fixation test (CFT).

Of the total animals tested 125 (14.1%) sheep and 287 (16.1%) goats were positive by the RBPT. Only two sheep had 50 IU or above by the SAT and none by the CFT. In goats 29 had antibodies 50 IU or above by SAT and eight by the CFT. Based on antibody titre of 50 international units or over, the prevalence is between 0.0 to 0.2 percent in sheep as determined by the CFT or SAT respectively. In goats the prevalence is between 0.5 to 1.6 percent again by CFT or SAT respectively.

The sensitivity and limitations of the three serological tests are discussed.

CHAPTER III

BACTERIOLOGICAL INVESTIGATION OF OVINE AND CAPRINE BRUCELLOSIS IN GOVERNMENT FARMS IN NORTHERN NIGERIA

INTRODUCTION

Brucellosis is widespread in all domestic animals. The clinical manifestations however are mostly frequent and economically important in cattle, sheep, goats and swine. In these animals it is a disease causing abortion, infertility and orchitis (Bruce, 1930; Boyd, 1950; Allsup, 1969).

Brucella melitensis and *B .abortus* are the etiological agents of brucellosis in sheep and goats (Nilakantan and Pande, 1948; Stoenner, 1951; Bruce, 1930; Meurou and Pineau, 1937). Recently *B . ovis* was described as a specific species of *Brucella* whose host is sheep (Buddle, 1956; Philpott and Auko, 1971).

The route of infection of brucellosis include the ingestion of contaminated feed or water; inhalation of dust contaminated by droplets or sometimes through unbroken skin. The spread of the organism could occur by movement of infected animals or their products (FAO, 1964).

The route of excretion of *Brucella* from sheep and goats include; milk (Nilakantan and Pande, 1948; Stoenner, 1951), semen (Anon, 1961; Cameron *et al.*, 1971), vaginal discharge (Polydorou, 1974), urine and other body fluids (FAO, 1964)

Specimens from which *Brucella* can be isolated include: milk, urine, faeces, vaginal discharge, from lymph nodes such as: supramammary, submaxilliary or retropharyngeal and internal iliac. Other organs include: uterus, spleen, aborted fetus and aborted

fetal membranes (Alton *et al.*, 1975).

Abortions, together with contagious caprine pleuropheumonia((CCPP), pneumoenteritis complex (CEP), helminthiasis and nutritional problems, accounts for serious economic loss in sheep and goats population in Nigeria (Beaton, 1928 ; Beaton, 1931, Ojo, 1971, Schillhorn Van Veen, 1973. Akerejola *et al.*, 1979).

The presence of brucellosis in sheep and goats have been recognised since 1934 (Eze, 1977). Recent focal serological surveys showed that the incidence in sheep in Eastern Nigeria was 0.9% and in goats was 0.6 percent (Kramer *et al.*, 1967). In Ibarapa division of Western State of Nigeria Falade *et al.*, (1974) reported that the incidence of brucellosis in goats was 8.9 percent. Also in parts of Northern Nigeria Morrison *et al.*, (1975) showed that 1.8 percent of sheep and 1.5 percent goats surveyed had brucellosis.

Northern Nigeria has long been the scene of animal movements between seasons of the year and as a source of animals to the Southern parts of the country. Cultural study of brucella organisms from sheep has not been undertaken. However, in a study of caprine brucellosis in Southern part of Nigeria *B. abortus* was isolated from one of 590 milk samples examined (Falade, 1978).

It has not been possible so far to find out the distribution of brucellosis in sheep and goats since screening for the disease among the privates farms, villages and the Fulanis covering the whole

country is a gigantic task to perform.

B . melitensis has three biotypes which differ from one another only in their behaviour with monospecific sera. *B . abortus* has nine biotypes differing from one another in biochemical and serological reactions.

There is paucity of literature on brucellosis in Northern Nigeria where over 80 percent of the country's sheep and goats reside. There are no reports of cultural studies to determine species and biotypes of *Brucella* in these animals.

In view of this lack of reports in the literature regarding the isolation and biotypes, it becomes necessary to investigate in order to fill these existing gap.

The investigations reported here are on the isolation and biotyping of *Brucella* sheep and goats in the government farms in some parts of Northern Nigeria.

Background to the Livestock Improvement and Breeding Centre (L.I.B.C)

Flock No. 1 belongs to National Animal Production Research Institute, Shika, Zaria. The breeds of sheep on the farm are Yankasa, Udah, Balami and crosses of the three. The breeds of goats are Sokoto Red , Kano Brown and their crosses. The sheep and goats are for research purposes and they graze together.

Flock No. II is a Goat Improvement Centre, Kukan Aljana. The goats are bred for meat and milk production. Sokoto Red goats are predominant though there are few exotic breeds. History showed that there were no cases of abortions in this flock nor was any sheep grazing with the goats. The goat milk is sold to the people living around the farm including the Semi-Nomadic Fulanis in the area. Milk is for consumption purposes and for making cheese. Breeding is throughout the year. Crossbreeding with exotic breeds is also practised.

Flock No. III is a Mutton Improvement Centre, Katsina. Thirty sheep aborted in 1978 and had since been culled. Different breeds of sheep are reared and these include: Balouchi, Yankasa, Udah, Balami and crosses between the four. There are also exotic breeds of sheep crossed with the local sheep. There were no goats within the establishment and sheep are fed on hays and feed supplements.

Flock No. IV: This is a Goat Improvement Sub-Centre, Rimi which is an extension of Herd No. II. The flock consists of 130 goats which were made up of Sokoto Red and Anglo-Nubian goats. There were crossbreeding between local and the Anglo-Nubian goats.

Flock No. V is a Federal Government sheep meat production Project Centre, Tuma. The flock consists of 950 sheep consisting of Yankasa, Udah, Balami and crosses of the three different breeds bought locally mostly from Borno State. There is a quarantine system at Ladanbawa where all the sheep stay for 3-6 months before

being brought to Tuma. There is a high standard of sheep management and husbandry and the surrounding is very neat.

Flock No. VI: This Rano farm stocks exotic breed of sheep - Fat tailed Sudanese sheep from Sudan. There were previous cases of abortion storms in 1978 and the sheep involved were culled after abortion.

Flock No. VII: This is another Goat Improvement Centre in Dangora. There are two breeds of goats stock in the farm namely: Kano Red and Anglo-Nubian goats. There are crosses of the two breeds also. Breeding is throughout the year.

MATERIALS AND METHODS

Sources of specimens:

The milk samples used for cultural examination were obtained from sheep and goats in the following government farms: NAPRI, Shika; Kukan Aljanan; Mutton Improvement Centre, Katsina; Tuma and Rano; Goat improvement sub-Centre, Rimi and Dangora. In each Government farm, milk samples were collected from lactating animals at various stages of lactation. Problems inherent in getting samples from the abattoir and other sources have left us with one alternative, that is milk sample at Government farms which is readily available as long as the animal is kidding or lambing.

Sampling technique:

Milk samples were taken from an individual female sheep or goats on survey farms. Before taking a milk sample from an individual sheep or goat, the hands of the milker and the teats of the lactating animal were cleansed with clean warm water and dried with paper towel. The first few jets of milk from the animals were discarded, and a sample from both two halves was collected into a sterile 15-ml Universal bottle, 0.5 ml of 1% $^{w}/_{v}$ boric acid solution was added to each milk sample and samples packed inside ice-box and brought back to the laboratory on the same day. All milk samples were identified, marked and stored overnight at 4^{oc} when the tests were carried out.

Laboratory examination of samples

Milk ring test

The milk ring test (MRT) was carried out on each sample as follows: A milk sample was gently mixed by inverting the universal bottle used for collection. One millilitre of milk was placed in a narrow kahn test tubes to give a column of milk of about two centimetres high and 0.03 ml of haematoxylin-stained brucella antigen batch 149/50* was added. The contents of the tubes were gently mixed within one minute of adding the antigen and read after incubating for three hours at 37^{oc}. Positive reaction was indicated

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** Supplied by the Courtesy of the Director, CVL, Weybridge, London*

by a blue-coloured cream layer or button formation at the bottom of the tube.

Reading of the milk ring test was according to standard technique (Appendix A; Morgan *et al.*, 1978).

Isolation and identification

Five millititres of individual milk samples were centrifuged in screw capped centrifuge tubes at 3000 r.p.m. for 15 minutes. The milk under the cream layer was poured off using a sterile applicator stick to pierce the cream layer. The cream was mixed with the sediment thoroughly and approximately 0.2 ml of the cream-sediment mixture was inoculated into Brodie and Sinton's liquid medium (BS). All samples in BS liquid medium were numbered and incubated at 37^{oc} in an atmosphere of 5-10 percent added CO_2 (as described in Appendix B) for 5 days. About 0.2 ml from the broth was then plated onto Farrell's solid medium (FSM) and serum dextrose agar (SDA) and incubated under the same conditions. Two sheep blood agar plates and two MacConkey agar plates were plated and one of each incubated aerobically and the other in micro-aerophilic jars.

Isolated bacteria on FSM and SDA were studied for typical colonies of *Brucella*.

Colonies selected for further investigation include a, the typical smooth colonies, convex in shape and often glistening surface. b, opaque, off-white and granular colonies typical of

the rough strains.

Suspected brucellae were restreaked on fresh FSM and SDA for isolation in pure culture and maximum growth. Isolates were stained by Gram's and modified Ziehl-Neelsen stain and examined under microscope for staining reaction and cell morphology.

Biochemical identification were carried out according to standard techniques (FAO/WHO, 1975). Tests included catalase, oxidase, indole, nitrate, citrate, Voges-Proskauer, H_2S production, dye sensitivity and CO_2 requirements.

Phage typing

Phage typing procedure in accordance to Corbel *et al.*, (1978).

RESULTS

A total of 418 samples of milk consisting of 277 from sheep and 141 from goats were tested with MRT antigen for brucella agglutinin. The same samples were cultured for brucellae. The data on Table 1 show the results of the MRT. Forty four (15.88%) of the sheep and 49 (34.75%) of the goats were positive by the MRT.

Table 2 shows the relationship between the degree of MRT reaction and the presence of brucellae in the milk. Four

brucellae were isolated from the sheep samples, three of these were from 24 samples which were strongly positive, and one from 14 moderately positive by MRT. No isolates were obtained from weakly positive, suspicious or negative samples. In goats, six brucella isolates were obtained, five from strongly positive and one from moderately positive samples. No isolates were obtained from weakly positive, suspicious or negative samples.

Table 3 shows the flock strength and type of animals in the flock and the results of the MRT and cultural examination in the livestock improvement and breeding centres.

The MRT results show that all the flocks were infected. However, the 10 isolates were obtained from only four of the seven centres and only two of these four centres had reported abortions in their animals previously.

The brucellae were confirmed by biochemical and serological tests according to standard techniques (Alton *et al.*, 1975; Morgan *et al.*, 1978). All the isolates were serologically typed as *B. melitensis.* (Table 4).

The isolates were similar to those described by Henry (1933). In oblique light appeared dry, granular and reddish-yellow to yellowish-white. They agglutinated in 0.1 percent acriflavine, in 0.15M Na Cl at 80-100°C and by anti-rough monospecific antiserum. They were thus described as rough strain of *B. melitensis.*

Their biotypes could not be determined by phage typing because they were rough strains of *Brucella.*

Table 1

Results of Milk Ring Test On Sheep and Goats Milk obtained from seven Governmental Farms

Source of milk	Total No. examined	Number Positive	Percent Positive *	MRT Reading ** 3+	2+	1+	+ -	-
Sheep	277	44	15.9	24	14	6	0	233
Goats	141	49	34.8	31	6	6	6	92

* Positive test from $\pm$ to +++

** MRT Reading according to standard technique (Morgan et al., 1978)

TABLE 2

The relationship between the degree of milk ring test reaction and cultural isolation of brucellae.

Source of milk	Total Number examined	MRT Positive	MRT reading and culture positive									
			3+	Culture Positive	2+	Culture Positive	+	Culture Positive	+-	Culture Positive	-	Culture Positive
Sheep	277	44	24	3	14	1	6	0	0	0	233	0
Goats	141	49	31	5	6	1	6	0	6	0	92	0

TABLE 3

Survey of brucellosis in sheep and goats milk from various Establishments using the milk Ring Test Screening Method

No. of Flock	L.I.B.C. Location	Type of Animal	Flock * Strength	Cases of abortion	Number goats sampled	Number sheep sampled	MRT Positive	% MRT Positive	No. of milk culture Positive
I	NAPRI Shika, Zaria	Sheep	275	None	N/A	94	14	14.9	0
		Goats	75	None	60	N/A	24	40.0	0
II	Kukan ALJANAN	Goats	321	None	59	N/A	17	28.8	3
III	MIC KATSINA	Sheep	391	30	N/A	48	6	12.5	2
IV	GISC RIMI	Goats	130	None	10	N/A	1	10.0	0
V	TUMA	Sheep	950	None	N/A	76	4	5.3	2
VI	RANO	Sheep	208	5	N/A	59	20	33.9	0
VII	DANGORA	Goats	116	None	12	N/A	4	33.3	3
TAL 7		Sheep/Goats	2,466	35	141	277	90	21.5	10

* The flock strength refers to the total animals on the farms which includes: Rams, ewes, kids, lambs, nannies and doe.

N/A - Not applicable

TABLE 4

Biochemical Reactions and Typing of Brucellae isolated from milk

STRAIN LABORATORY NUMBER	MILK SOURCE	CATALASE	OXIDASE	INDOLE	METHYL RED	VOGES PROSKAVER	NITRATE	MODIFIED ZIEHL NEELSEN STAIN	CO2 REQUIREMENT	H_2S REQUIREMENT	AGGLUTINATION IN 0.1 PERCENT ACRIFLAVINE	Agglutination with Mono-specific anti sera			Tbilisi Phage Lysis		UREASE ACTIVITY	Growth on dyes					
																		Basic fuchsin			Thionin		
												A	M	R	RTD	10^4RTD		1/25000	1/50000	1/100000	1/25000	1/500000	1/100000
DANGORA 5	Goat	+	+	-	-	-	-	Gram nega-tive coccobacilli	-	trace	+	-	-	+	NL	NL	-	-	+	+	-	+	+
DANGORA 6	Goat	+	+	-	-	-	-	gm-ve coccobacilli	-	trace	+	-	-	+	NL	NL	-	-	+	+	-	+	+
DANGORA 9	Goat	+	+	-	-	-	-	gm-ve coccobacilli	-	trace	+	-	-	+	NL	NL	++	-	+	+	-	+	+
MIC 18	Sheep	+	+	-	-	-	-	gm-ve coccobacilli	-	-	+	-	-	+	NL	NL	-	-	+	+	-	+	+
TUMA 18	Sheep	+	+	-	-	-	-	gm-ve coccobacilli	-	-	+	-	-	+	NL	NL	-	-	+	+	-	+	+
TUMA 39	Sheep	+	+	-	-	-	-	gm-ve coccobacilli	-	-	+	-	-	+	NL	NL	-	-	+	+	-	+	+
KUKAN ALJANAN 24	Goat	+	+	-	-	-	-	gm-ve coccobacilli	-	-	+	-	-	+	NL	NL	trace	-	+	+	-	+	+
KUKAN ALJANAN 48	Goat	+	+	-	-	-	-	gm-ve coccobacilli	-	-	+	-	-	+	NL	NL	-	-	+	+	-	+	+

Negative = -; Positive = +; Strongly positive = ++; No Lysis = NL.

DISCUSSION

All the isolates were serologically, biochemically proved to be *B. melitensis* but were the rough strains. Previous work by Alton (1960) on goats milk showed that there was a frequent occurrence of dissociated strains of *B. melitensis* in the milk of goats in Malta. It was further observed that the crystal violet method of staining colonies does not reveal any differences in the degree of dissociation, and it is likely that many of the dissociated strains, were, in fact, intermediate forms. Polding (1939) also recorded some information about dissociated strains of *B. melitensis* encountered in Malta. Milk from lactating animals is generally easier to obtain than blood specimens and in addition it can be used for cultural examinations from which *Brucella* can be obtained more easily than blood because of tissue predilection of the organism. The MRT is particularly valuable as a screening test on a herd or flock basis and in individual animals. Isolation of brucellae are more probable in strongly MRT-positive specimens as shown by this work.

Mathur (1967) showed that abortions and a positive MRT test were two very important indicators of brucellosis in a herd or flock. The "false-positive" reaction of MRT, that is, where MRT-positive failed to yield *Brucella* was most likely due to

non-specific gammaglobulin but in which the antibody titre is usually low. Another situation where an animal could be MRT positive but culture negative is where there could be infection but without excretion of the organism in the milk at the time of examination, as *Brucella* is known to be excreted intermittently (Alton, 1960). The stage of lactation of many of the sheep and goats under study could not be determined precisely and this could have contributed to the high incidence of MRT-positive but culture negative samples in this study.

The figure obtained here however, still appears much higher than previous report in which 590 goat milk were examined and 288 (48.9%) were positive by MRT but only one isolate of *B. abortus* was obtained from among the MRT positive milk samples (Blade, 1978). This work also indicates that *B. melitensis* is the more common species of *Brucella* in sheep and goats in Northern Nigeria.

SUMMARY

Bacteriological investigations of ovine and caprine brucellosis were carried out in Northern Nigeria. Seven government or quasi-government farms were investigated.

A total of 418 sheep and goats milk samples were examined culturally. Investigation revealed a 34.75 percent and 15.88 percent rate of infection in goats and sheep by milk ring test.

Of 277 sheep and 141 goats milk samples examined culturally, brucellae were isolated from four sheep and six goats giving a total of ten isolates. Biochemical and serological studies of the isolates revealed that they were *B. melitensis* indicating *B. melitensis* as probably the common cause of brucellosis in sheep and goats.

The results show that there was greater chance of isolating *Brucella* from milk which were strongly positive than from those which were weakly positive.

The economic importance and public health significance of brucellosis in sheep and goats are discussed.

OVINE AND CAPRINE BRUCELLOSIS IN NORTHERN NIGERIA

GENERAL DISCUSSION

The population of small ruminants in Nigeria is approximately between 8 - 21 million sheep and between 27 - 30 million goats. The prevalence of brucellosis in these animals is unknown. This study was therefore design to investigate the disease serologically and bacteriologically.

This study has shown that brucellosis is present in our sheep and goats.

It has not been possible so far to find out the actual incidence of brucellosis and the role of the disease in abortion and infertility in sheep and goats in Nigeria since screening for the disease among the private farms, Fulani tribesmen and among the households covering the whole country is rather difficult to perform. In addition, cases of abortion in sheep and goats are not reported by private farms, households generally and the Fulanis. Obtaining blood for tests, especially in villages and private farms is very difficult.

Infected goats are particularly dangerous source of brucellosis to man and other animals as the disease in these animals often assumes a chronic form without obvious symptoms. Infected sheep on the other hand do not constitute much threat as disease in sheep is often self-limiting. Brucellosis among goats and sheep in Nigeria has not received the attention that it deserves. They are important not from the point of human infection

with *B . melitensis* by drinking unpasteurised infected animal milk and eating cheeses, but also from the point of view of a possible danger of infection to cattle and as an occupational hazard to man.

Control of the infection is definitely desirable in any infected area where practicable, but unfortunately in many area such as the private farms, households and among the nomadic Fulanis, if present control will be extremely difficult to carry out. Sanitary measures to prevent spread of infection from animal to animal and animal to man are of the greatest importance but, not easy to put into practice. Ideally all sheep and goats which are reactors should be slaughtered, but this is impossible with local people and the Fulanis. Where possible such as Governmental and institutional farms, control of spread of infection should aim at a high level of environmental sanitation, separate room facilities for kids/lambs and early weaning of kids/lambs to a Brucella-free area. All government farms where they breed sheep and goats should screen all the animals against brucellosis twice every year with six monthly intervals. Sero-survey and milk culture with one month intervals except where there are no positive and no suspicious cases or from the animals of already infected farms. All reactor animals should be slaughtered and consumed after cooking except for the internal and genital organs which should be incinerated in cases of strong reactors. Where possible Rev. 1 Vaccine should be given to the animals.

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

All livestock farmers must be enlighted about disease reporting especially cases of abortions in order for investigation of abortions due to *Brucella* and other infectious agents.

They must be encouraged to utilize the diagnostic services provided at the government veterinary diagnostic centres and the Universities or Institutes.

Area for future investigation should include cultural and serological studies in sheep and goats of brucellosis on a wider coverage of the country. The role of sheep and goats as a source of infection of human either through contact or their by-products should be investigated. This area will require a team work consisting of medical and veterinary personnel.

Mode of transmission of infection between large and small ruminants need to be study. We should also investigate brucellosis serologically and bacteriologically in other animals with particular emphasis on pigs, dogs, horses and camels.

APPENDIX

A

Livestock Populations of Nigeria

There are relatively more livestock animals in the Northern Region than in the Southern Region (Eastern Region and Western Region).

The following "Livestock Unit Conversion factors" are given in the FAO Production Yearbook, 1965.

Class of Livestock	Factor
Camel	1.1
Buffalo, horse, mule	1.0
Cattle, Asses	0.8
Pigs	0.2
Goats, Sheep	0.1

Table 1 shows the livestock populations of Nigeria converted to livestock Units, using the above conversion factors

Table 1: Livestock Populations of Nigeria Converted to Livestock Units. (Adapted from Agricultural Development in Nigeria 1965-80 FAO (Rome) 1966).

	Northern Region		Eastern Region		Western Region		TOTAL
	Units (000's)		(Units (000's)		Units (000's)		Units(000's)
Cattle	8204.6	(94.5%)	347.3	(4.0%)	135.1	(1.6%)	8687.0
Goats	1431.7	(67.5%)	468.4	(22.1%)	220.4	(10.4%)	2120.5
Sheep	444.8	(61.5)	175.2	(24.2%)	103.5	(14.3%)	723.5
Pigs	26.6	(17.3)	32.2	(23.7%)	80.2	(58.9%)	139.0
Total	10107.7	86.6%	1023.1	8.8%	539.2	4.6%	11670.0

Table 2: Estimated Livestock Populations of Nigeria (000's). From Agricultural Development in Nigeria 1965-80 (FAO).

	Northern Region		Eastern Region		Western Region		TOTAL
Cattle	10255.7	(94%)	434.1	(4%)	168.8	(2%)	10858.6
Goats	14317	(68%)	4684.3	(22%)	2204.4	(10%)	21205.7
Sheep	4448	(61%)	1751.4	(24%)	1035.4	(15%)	7234.8
Pigs	118.1	(17%)	161.4	(24%)	400.9	(59%)	680.4
Poultry	41716.3	(63%)	14263.6	(22%)	10059	(15%)	66039

Cattle	11.6 million
Goats	23.5 million
Sheep	8.1 million
Pigs	0.84 million
Poultry	83.0 million

B

CULTURE MEDIA AND BIOCHEMICAL USED IN THE ISOLATION AND IDENTIFICATION OF BRUCELLA ORGANISM

CULTURE MEDIA

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

Oxoid tryptone soya broth	30g
Distilled water	1000ml
Equine Serum, inactivated at 56^{oc} 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^{oc} for 15 minutes. After cooling the broth to 50^{oc} the sterile horse serum is added aseptically, followed by the antibiotics. 3ml of the medium is dispensed into 5-ml bijoux bottles and stored at 4^{oc}.

(ii) Brucella medium Base (BSM)

This dehydrated medium contains:

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

45g were added to 1 litre distilled water and soaked for 15 min. Sterilised by autoclaving at 121°C for 15 min. $_pH$ 7.5 (approx.)

(iii) 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 autoclaved at 109°C/10 minutes	40ml

Brucella medium base was boiled to dissolve completely and sterilised by autoclaving at 121°C for 15 minutes. After cooling the medium to 50°C the sterile horse serum was added aseptically. Plates and slopes are then poured.

(iv) Glycerol dextrose agar (GDA)

Brucella medium base (oxoid)	45g
Distilled water	1000ml
25% w/v Dextrose autoclaved at 109°C/10 minutes	40ml
50% v/v Glycerol autoclaved at 109°C/10 minutes	20ml

Sterilised as in (ii) above and poured into plates at between 15-20 ml each.

(v) Dye Sensitivity test media

These are prepared by the addition of the relevant dye to a serum dextrose agar basal medium. 0.1g dye was dissolved in 100ml distilled water to produce stock solutions. These are 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.

	Dye	Stock Solution	Volume added/1 litre basal medium	Final Concentration
a	Basic fuchsin/ Thionin	0.1% w/v	41.60 ml	1/25,000
b	Basic fuchsin/ Thionin	0.1% w/v	22.20 ml	1/50,000
c	Basic fuchsin/ Thionin	0.1% w/v	10.53 ml	1/100,000

(vi) Farrell's modified serum dextrose agar

To SDA basal medium the following were added per litre basal medium.

Antibiotic	Volume added	Approximate final concentration
Bacitracin	12.5 ml	25 units/ml
Polymyxin B sulphate	1.0 ml	5 units/ml=
Cycloheximide	10.0 ml	100 µg/ml
Vancomycin hydrochloride	0.4 ml	20 µg/ml
Nalidixic acid	1.0 ml	5 µg/ml
Nystatin	2.0 ml	100 units/ml

The medium was dispensed in 90 mm petri dishes and stored at 4^{oc}.

The various antibiotics used in Farrell's medium are prepared as follows:

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^{oc} and incorporated in the basal medium at a concentration of 25000 units per litre (25 units/ml).

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

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^{oc} and used in a concentration of 100 mg per litre (100 microgram/ml).

Vancomycin hydrochloride: 0.5g was dissolved in 10 ml of sterile distilled water and stored at -20^{oc} and used in a concentration of 20 microgram per ml.

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

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

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.

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.

Growth on dye plates

Medium: SDA + Basic fuchsin; SDA + Thionin.

Dye plates were incubated overnight as a sterility check and are quartered permitting four samples to be tested on one plate. One loopful of suspension was used to inoculate each quarter with five consecutive streaks without recharging the loop. The loop was sterilised between each sample. After drying, the plates are incubated for 48 hours in air containing 5 - 10 percent CO2. The results of the dye plates are recorded as negative or 1+ to 5+ depending on the growth of each streak.

CO_2 requirement

Medium: SDA Slopes

A typical colony was picked off and sub-cultured on two SDA slopes. One of the slopes was incubated at 37^{oc} in air and the other in air + 5 - 10% CO_2. After 72 hours incubation slope incubated in air was examined and any growth recorded as a negative CO_2 requirement. Absence of growth on this slope but growth on the slope incubated in the CO_2 supplemented atmosphere indicated a positive CO_2 requirement.

Tests for Dissociation

a. Medium: SDA + 50% (v/v) glycerol - glycerol dextrose agar (GDA)

After incubation: The ability of isolates to grow in the presence of GDA medium was observed. Any growth that occurred was examined, under the obliquely transmitted light using a stereoscopic microscope,

for smooth or rough strains and further for purity by staining with crystal violet diluted 1:40.

b. Agglutinability in acriflavine – as described under stains.

c. Agglutination by antiserum A, M and R*

Medium: Farrell's medium

After incubation for five days: A colony was picked with a straight wire and emulsified in the different antisera. Agglutination with the antiserum but not with the control serum indicated that the organism was probably a rough *Brucella*. Agglutination with acriflavine but not with anti -R serum indicated that it probably is not *Brucella*.

d. Thermoagglutination test

The test consisted of heating a suspension of the organism in 0.15 mol/litre sodium chloride at 80°c or 100°c for two hours. *Brucella* strains which have undergone dissociation auto-agglutinate under these conditions.

e. Phage Lysis

Medium: SDA Slopes

After 48 hours incubation of the cultures, suspension of the growth were made in sterile saline. An SDA plate was inoculated with

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** Supplied by CVL, Weybridge, London*

loopfuls of suspension made so as to produce an area of confluent growth. One drop of phage suspension was placed onto inoculated area. The brucella isolates were examined for their sensitivity to phage Tbilisi at Routine Test Dilution (RTD) and 10^4 x RTD. The plates were kept undisturbed on a flat surface for one hour to allow the drops of phage suspension to diffuse and dry before incubating at 37°C for 48 hours. The plates were then examined after 24 hours.

H_2S Test strips - sheets of Whatman filter paper are soaked in a ten percent w/v solution of lead acetate and hung up to dry at 37°c in air free of H_2S. When dry, the sheets are cut into strips of 4 x 7 mm, placed in clean bottles and autoclaved at 121°c for 15 minutes. They were stored away from cultures and other sources of hydrogen sulphide.

CO_2 culture containers 0.4gm of Sodium hydrogen carbonate was placed in 25-ml pyrex conical flask which was placed inside an empty wide-mouth clean Nido tin* which could easily be clamped down to provide an efficient seal for the production of 5 - 10% v/v CO_2 when ready for use 0.5 ml of concentrated hydrochloric acid was added to the sodium bicarbonate and the nido tin close immediately. The volume of CO_2 produced in each tin were determined by Bromthymol blue indicator solution. The reagent consists of a 0.1% w/v aqueous solution of sodium bicarbonate with one or more drops of 0.5% bromthmol blue added, sufficient to give a definite blue colour in a normal atmosphere.

STAINS

a. *Modified Ziehl Neelsen stain*

A heat fixed film of bacteria on a glass slide was stained for 10 minutes with 1/10 diluted carbol fuchsin. The excess stain was

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* *Nido - Nestle's Products Ltd. (Nigerian Food specialities), IKEJA, LAGOS.*

was poured off and the slide washed thoroughly in tap water. The smear was decolorised with 0.5 percent v/v acetic acid for up to 30 seconds care was taken not to over-differentiate. The slide washed again thoroughly in tap water, counterstained with one percent w/v methylene blue in distilled water for 30 seconds and then blotted dry before examination by phase contrast light microscope.

Carbol fuchsin

i. Basic fuchsin 10 g

Ethanol (95% v/v) 100 ml

The dye was dissolved in the ethanol and stored at 37^{oc} overnight

ii. phenol 5% w/v in distilled water 1000 ml solution i and ii are mixed together and filtered.

Methylene blue counterstain

Methylene blue one percent w/v in distilled water.

Brucella organism appear as red cocci or coccobacilli against a blue background. Other organisms are stained blue.

b. Crystal Violet

The stain was prepared as follows:

Solution A 2g crystal Violet in 20 ml of 95% v/v ethanol,

Solution B 0.8g ammonium oxalate in 80 ml of distilled water.

The two solutions are mixed to form a stock solution which was then diluted 1/40 in distilled water for use.

The plate was flooded with the stain and allowed to act for 15 to 20 seconds, after which it was drained into diluted izal. The plate was examined under stereoscopic microscope with a blue filter using obliquely transmitted light (Henry 1933). Smooth colonies appear pale yellow, rough colonies red with a coarse, granular appearance and other dissociated colonies stained with various shades of purple and blue.

c. Acriflavine Solution 0.1 percent w/v acriflavine in distilled water. Agglutinability in acriflavine was done by placing a drop of acriflavine solution on a clean slide. A colony was then touched with a straight wire and emulsified in the acriflavine. Smooth colonies emulsified easily, white rough colonies are agglutinated immediately, mucoid colonies form thread.

Brucella antibodies in sheep and goats in various locations in the Northern states of Nigeria

	SHEEP				GOATS			
	No.of Samples Taken	NO POSITIVE *			No.of Samples Taken	NO POSITIVE *		
		RBPT	SAT	CFT		RBPT	SAT	CFT
(a) Location of Abattoirs								
Funtua	50	14	6	7	200	53	28	9
Jimeta	17	1	0	0	14	0	0	0
Kano	57	1	1	0	469	38	35	33
Sokoto	29	9	6	3	30	10	7	1
Bauchi	19	1	1	0	18	1	1	0
Zaria	0	0	0	0	217	68	22	34
Jos	7	0	0	0	67	0	0	0
T O T A L	179	26	14	10	1,015	170	93	77
(b) Location of Local Animals								
Sabon-Gari/Hanwa	41	8	4	0	15	2	2	1
Dutse-Abba/Soba	69	8	5	0	20	2	2	1
Wusasa/Zuntu	10	0	0	0	13	4	1	1
Yola	46	1	0	0	27	0	0	0
Birnin Gwari	32	0	0	0	33	0	0	0
Maigatari	46	0	0	0	56	0	0	0
Wudil/Gumel	80	0	0	0	97	7	6	4
T O T A L	324	17	9	0	261	15	11	7
(c) Location of Institutional Animals								
Vet. Med., Samaru	25	6	5	1	33	11	8	9
N.A.P.R.I., Shika	105	9	7	3	65	10	8	2
Kukan Aljana	0	0	0	0	245	75	39	22
M.I.C. Katsina	215	67	35	19	0	0	0	0
Dangora	0	0	0	0	116	7	7	5
Kotorkoshi	37	0	0	0	39	0	0	0
	382	82	47	23	498	103	62	38

* Positive but not necessarily a reactor

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CULTURAL AND SEROLOGICAL STUDY OF OVINE AND CAPRINE BRUCELLOSIS IN NORTHERN NIGERIA

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Cultural and serological studies of ovine and caprine brucellosis in various part of Northern Nigeria were undertaken.

In a serological survey, 885 sheep and 1774 goats sera were collected from various parts of the Northern Nigeria, sera were screened by the Rose bengal plate test (RBPT) and if positive were further tested by the serum agglutination test (SAT) and complement fixation test (CFT). The RBPT gave a 14.1 percent and 16.1 percent rate of positive reaction in sheep and goats respectively. Among the animals that were RBPT positive, seventy sheep and 166 goats had agglutinins at various level of antibody titers by the SAT, but only two sheep and 29 goats had antibody level 50 IU or above by this test. The CFT gave a much lower result than the SAT out of the RBPT positive animals. Only 32 sheep and 121 goats seemed to have complement fixing antibodies at any level. Based on antibody titer of 50 international units or over, prevalence rates were between zero to 0.2 percent in sheep as determined by the CFT and SAT respectively. In goats, prevalence rates were 0.5 percent and 1.6 percent again by CFT and SAT respectively. The limitations of the three serological tests arc discussed.

A total of 418 sheep and goats milk samples were tested by the milk ring test and examined culturally for *Brucella*. Investigation revealed a 15.88 percent and 34.75 percent rate of

infection in sheep and goats by the milk ring test.

Of 277 sheep and 141 goats milk samples examined culturally, brucellae were isolated from four sheep and six goats giving a total of ten isolates. Biochemical and serological studies of the isolates revealed that they were *Brucella melitensis* indicating *Br. melitensis* as probably the common cause of brucellosis in sheep and goats. There was good correlation between the degree of milk ring reaction and presence of bacteria in milk as most of the isolates of *Brucella* came from milk samples which were strongly positive. The economic importance and public health significance of brucellosis in sheep and goats are discussed.