PREVALENCE OF BRUCELLOSIS IN SHEEP AND GOATS KEPT IN HOMES AND ASSESSMENT OF OWNERS' KNOWLEDGE AND PREVENTIVE PRACTICES IN ZARIA, NIGERIA

By

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DECLARATION

I declare that the work in this dissertation entitled "PREVALENCE OF BRUCELLOSIS IN SHEEP AND GOATS KEPT IN HOMES AND ASSESSMENT OF OWNERS' KNOWLEDGE AND PREVENTIVE PRACTICES IN ZARIA, NIGERIA" was carried out by me in Diagnostic Department, National Veterinary Research Institute, Vom and the Department of Community Medicine under the supervision of Prof. A.T. Olayinka of Medical Microbiology Department, Ahmadu Bello University, Zaria.

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

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Samuel Yohanna Badung **Date**

CERTIFICATION

This dissertation entitled 'PREVALENCE OF BRUCELLOSIS IN SHEEP AND GOATS KEPT IN HOMES AND ASSESSMENT OF OWNERS' KNOWLEDGE AND PREVENTIVE PRACTICES IN ZARIA, NIGERIA' by Samuel Yohanna Badung, meets the regulations governing the award of the Degree of Master of Field Epidemiology of Ahmadu Bello university, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to my Lord and Saviour Jesus Christ who gave me the opportunity to undertake the Nigeria Field Epidemiology and Laboratory Training Programme (NFELTP) and also to my beloved wife Gloria S. Badung and the children who were there for me all through.

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SUMMARY

Brucellosis is a zoonotic disease associated with significant morbidity leading to increased rates of spontaneous abortions in livestock and also poses a serious human health hazard. This study sought to determine the prevalence of brucellosis in sheep and goats kept in homes in Zaria town and also to assess the knowledge and practice, of the animal owners, on prevention and control of brucellosis. A cross-sectional survey was carried out in Zaria town, Kaduna State, Northern Nigeria, between February, 2013 and April, 2013. The study population consisted primarily of sheep and goats kept in homes and secondarily of the animal owners in Zaria town. A multi-stage sampling technique was used to select animals for the study. A total of 52 houses that reared sheep and or goats were selected. From each selected house, a systematic random sampling technique was used to select the animal for the survey. Structured questionnaires were used to capture all information related to the animals and bio-data, knowledge and practice of animal owners. The 208 sampled animals were screened for Brucella using the Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT). All data from the survey were double-entered into and analysed using Epi-Info 3.5.3 and Microsoft excel 2007. Frequencies, proportions, prevalence rates, prevalence ratio and Chi square test at significance level of $P < 0.05$ were also determined. Out of the 208 animals sampled, 171 (82.2%) were females and 156 (57.2%) were sheep. Of all animals screened, 35 (16.8%) were sero-positive for Brucella, 27 (77.1%) of them being females. All animals that were positive were those reared under the free range system of farming. Of the two species, the sero-prevalence of Brucella was higher in goats (17.9%) and also higher among the age groups > 24months to 36months . Out of the 52 animal owners, 80.7% of them were males while majority (55.8%) of them were between 30 to 40 years of age and 32.7% had no formal

education. On their occupation, 92.4% of them reared animals for income, while 52.0% practiced free range farming system. Fifty-five percent (55%) of the owners do not know what zoonosis is, 27% and 18% know what brucellosis and tuberculosis are respectively. Also 54% believed that transmission of diseases from animal to humans is by contact, 14% do not know the mode of transmission. The study indicated that Brucella infection exists among sheep and goats kept in homes in Zaria town. Its prevalence is higher in free range animals. There is also a relatively low awareness of brucellosis among the animal owners. There is therefore the need to embark on control and prevention of Brucellosis among small ruminants in Zaria town in order to reduce risky zoonotic transmission to humans.

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LIST OF ACRONYMS

AI	-	Avian Influenza
BEAP	-	Brucellosis Emergency Action Plan
CO2	-	Carbon dioxide
CFT	-	Complement Fixation Test
DNA	-	Deoxyribonuclic Acid
ELISA	-	Enzyme Linked Immunosorbent Assay
FAO	-	Food and Agriculture Organisation
H2S	-	Hydrogen Sulphide
IM	-	Intramuscular
IV	-	Intravenous
KAPs	-	Knowledge, Attitude and Practices
MERS-CoV	-	Middle East Respiratory Syndrome Corona Virus
MRT	-	Milk Ring Test
NPV	-	Negative Predictive Value
PCR	-	Polymerase Chain Reaction
RNA	-	Ribonuclic Acid
RBPT	-	Rose Bengal Plate Test
SAT	-	Serum Agglutination Test
US	-	United States of America
vCJD	-	variant Creutzfeldt-Jakob Disease
WHO	-	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Brucellosis is a global zoonotic disease associated with significant morbidity that can lead to increased rates of spontaneous abortions in livestock and also poses a serious human health hazard.[1] Brucellosis is a disease of mainly cattle, swine, goats, sheep and dogs. The disease is widely distributed throughout the developing world, considered to be a serious problem in at least 86 countries.[2] It is a severe zoonosis in North African countries[3] and the Near East[4] causing economic and livestock losses and affecting industrial production.[5]

The infection is transmitted to humans by animals through direct contact with infected materials like afterbirth or indirectly by ingestion of animal products and by inhalation of airborne agents. Consumption of raw milk and cheese made from raw milk (fresh cheese) is the major source of infection in man. Most of the fresh cheeses are sheep and goat cheese. Next to this it is considered to be an occupational disease for people who work in the livestock sector. Human-to-human transmission is however very rare.[6]

Infections are caused by various bacteria of the genus *Brucella*, which tend to be host-specific. However, most species of *Brucella* are able to infect other animal species as well and some of them have zoonotic potential.[7] In humans, brucellosis can be caused by *B. abortus*, *B. melitensis*, *B. suis* biovars 1-4 and, rarely, *B. canis* or marine mammal *Brucella*. *B. ovis*, *B. neotomae*, and *B. suis* biovar 5 have not been associated with human disease. The disease affects cattle, swine, sheep and goats, camels, equines, and dogs. It may also infect other ruminants and marine mammals. The disease in animals is characterized by abortions or

reproductive failure. While animals typically recover, and will be able to have live offspring following the initial abortion, they may continue to shed the bacteria. Brucellosis in cattle (*B. abortus*) in sheep and goats (*B. melitensis*) and in swine (*B. suis*) are diseases listed in the World Organization for Animal Health (OIE) Terrestrial Animal Health Code.[8]

In endemic areas, human brucellosis has serious public health consequences. Worldwide, *Brucella melitensis* is the most prevalent species causing human brucellosis, owing in part to difficulties in immunizing free-ranging goats and sheep. In countries where eradication in animals (through vaccination and/or elimination of infected animals) is not feasible, prevention of human infection is primarily based on raising awareness, food-safety measures, occupational hygiene and laboratory safety. In most countries, brucellosis is a notifiable disease.[2]

1.2 STATEMENT OF THE PROBLEM

Brucellosis is a zoonosis of both public health and economic significance in most developing countries. In many developed countries, the animal disease has been brought under control, which has led to a subsequent decrease in the number of human cases. The occurrence of the disease in humans is largely dependent on the animal reservoir. Where brucellosis exists in sheep and goats, it causes the greatest incidence of infection in humans.[10]

Brucellosis, especially caused by *Brucella melitensis,* remains one of the most common zoonotic diseases worldwide with more than 500,000 human cases reported annually[9]. In the U.S., there are up to 200 new cases of human brucellosis annually.[10]

Investigations and reports have shown that brucellosis is endemic in Nigeria and evidence of infection as well as frank outbreaks have occurred both in cattle[11, 12]and in human beings.[13] An investigation also showed serological evidence of infection in goats in certain parts of the country.[14] Brucellosis in small ruminants (sheep and goats) has been reported in northern Nigeria.[13]

Previous work on isolation of brucella in Nigerian livestock has indicated that *Brucella abortus* has been the major species isolated from cattle[13,14,15] and from sheep and goats.[16-18]

However, *Brucella melitensis* had been isolated from sheep and goat milk in Nigeria.[19] There were also serological reports of brucellosis in small ruminants in various parts of Nigeria.[20-22]

1.3 JUSTIFICATION

Brucellosis is endemic in Nigeria; it causes severe economic losses to livestock farmers and ranchers, and is a serious risk to human health.[23]

Of all reproductive diseases of livestock, not only in Nigeria but all over the world, brucellosis is of major economic significance[24] because it causes considerable losses through abortion, infertility, neonatal death, reduced milk yield, dystocia and uterine prolapse in animals.[25]Studies suggest an increasing trend in the prevalence of the disease.[19]

In humans, consumption of contaminated food and occupational contact are the major risks of infection. The main routes of infection are consumption of unpasteurized dairy products, small ruminants, camel milk and milk products like cheese and sour milk. It has also been shown that the organism can survive pickling and inadequate smoking.[26-28] Contact with infected materials such as aborted foetuses, placentas, urine, manure, carcass and salvaged

animals has been reported in some countries to cause human brucellosis in 60 - 70% of cases.[29]Infection by contact has been reported to be common among veterinarians, abattoir workers, farmers, rendering-plant workers, packing-house employees, animal handlers and others who work with animals and their products.[29]

The major farming practice in this area is the free range and seasonal confinement system. The animals are allowed to fend for themselves during the dry season. The keeping of sheep and goats together with cattle, provides an opportunity for the spread of brucella infection from cattle to small ruminants.[18]

In the rainy season the animals are taken out for grazing or tethered during the day while in the evenings they are being brought into the house.[22] Usually these animals are allowed to roam around in the compounds mixing up with humans and sometimes the animals sleep with the owners in the same room.[9] This farming practice poses a risk to the animals as well as the human population.

Furthermore, Zaria has recorded cases of Brucella outbreaks among sheep and goats in farms.[30]

Zaria is a fast growing suburban town as well as a center of learning, yet animals are being kept in homes for either personal consumption or income. This informed this study to enable provision of answers to the following questions;

1.4 RESEARCH QUESTIONS

1. How adequate is the knowledge of animal owners on brucellosis in Zaria town?
2. What infection prevention Practices against brucellosis do animal owners do in Zaria town?
3. What is the Prevalence of Brucellosis in sheep kept in homes in Zaria town?
4. What is the Prevalence of Brucellosis in goats kept in homes in Zaria town?

1.5 GENERAL OBJECTIVE

To determine the prevalence of brucellosis in sheep and goats kept in homes and assess owners' knowledge and practice of its prevention, in Zaria town.

1.5.1 Specific objectives

a. To determine prevalence of Brucellosis in sheep and goats kept in homes in Zaria town.

b. To assess the knowledge of the animal owners, on brucellosis and its prevention, in Zaria town.

c. To assess the practice of the animal owners on brucellosis prevention, in Zaria town.

CHAPTER TWO

LITERATURE REVIEW

2.0. BRUCELLOSIS AS A ZOONOTIC DISEASE

Brucellosis is a zoonotic disease occurring in humans and various species of domesticated and feral (wild) animals.[36] The three species of *Brucella* of major concern here are: *Brucella abortus* (biovars 1–6), affecting primarily cattle, other bovidae, and cervidae; *Brucella suis* (biovars 1–5), affecting primarily swine; and *Brucella melitensis* (biovars 1–3), affecting primarily sheep and goats. All the above *Brucella* spp. are not host-specific, and may transmit to other animal species under appropriate conditions. [36]

Initial infection in the reservoir species is often followed by abortion and subsequent delayed or permanent infertility. Infection is usually chronic in animals, and treatment is rarely undertaken. Infected animals shed the organisms in uterine discharges following abortion and subsequent parturition, and also in the colostrum and milk.

Brucellosis is a herd or flock problem. It is spread within the herd primarily by ingestion of contaminated material. Venereal infections can also occur, but this is mainly seen with *B. suis* infections.[37] Congenital (*in utero*) or perinatal infections may also occur, with the ensuing development of latent infections. Spread between herds usually occurs by the introduction of asymptomatic chronically-infected animals.

Human infections are characterized by a variable incubation period (from several days up to several months), and clinical signs and symptoms of continued, intermittent or irregular fever of variable duration, with headaches, weakness, profuse sweating, chills, depression and

weight loss. Localized suppurative infections may also occur. The course of the disease can be variable, especially in persons either not or inadequately treated.

Diagnosis of clinical brucellosis in humans and animals is initially made by use of appropriate serological or other immunological tests, and confirmed by bacteriological isolation and identification of the agent.[15]

Transmission of infection to humans occurs through breaks in the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted foetuses or placentas.[38] Food-borne infection occurs following ingestion of raw milk and other dairy products, but rarely from eating raw meat from infected animals. Occupational airborne infection in laboratories and abattoirs has also been documented. Accidental inoculation of live vaccines (such as *B. abortus* Strain 19 and *B. melitensis (*Rev.1) can also occur, resulting in human infections. [39] There are also case reports of venereal and congenital infection in humans.

The disease occurs worldwide, except in those countries where bovine brucellosis (*B. abortus*) has been eradicated. This is usually defined as the absence of any reported cases for at least five years. These countries include Australia, Canada, Cyprus, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden and the United Kingdom.[40] The Mediterranean countries of Europe, northern and eastern Africa, Near East countries, India, Central Asia, Mexico and Central and South America are especially affected. While *B. melitensis* has never been detected in some countries, there are no reliable reports that it has ever been eradicated from small ruminants.

The sources of infection for humans and the species of *Brucella* spp. found vary according to geographical region. It is usually either an occupational or a food-borne infection. Both sporadic cases and epidemics occur in humans, but often the disease or infection is either unrecognized, or, if diagnosed, not reported to the public health authorities.[41]

Methods of prevention include health education to reduce occupational and food-borne risks, including pasteurization of all dairy products. However, education campaigns have never resulted in fully eliminating the risks of infection, and the ultimate prevention of human infection remains elimination of the infection among animals. This can be achieved by a combination of vaccination of all breeding animals to reduce the risks of abortion and raise herd immunity, followed by elimination of infected animals or herds by segregation and slaughter.

2.1.0. ZOONOSIS

Any disease or infection that is naturally transmissible from vertebrate animals to humans and vice-versa is classified as a zoonosis according to the PAHO publication "Zoonoses and communicable diseases common to man and animals". Zoonoses have been recognized for many centuries, and over 200 have been described. They are caused by all types of pathogenic agents, including bacteria, parasites, fungi, and viruses.[31]

Reducing public health risks from zoonoses and other health threats at the human-animal-ecosystems interface (such as antimicrobial resistance) is not straightforward. Management and reduction of these risks must consider the complexity of interactions among humans, animals, and the various environments they live in, requiring communication and

collaboration among the sectors responsible for human health, animal health, and the environment.[8]

Some examples of zoonoses, classified according to the type of causative agent, are given hereafter.

2.1.1 Bacteria

Every year millions of people get sick because of food-borne zoonoses such as Salmonellosis and Campylobacteriosis which cause fever, diarrhoea, abdominal pain, malaise and nausea. Other bacterial zoonoses are anthrax, brucellosis, infection by verotoxigenic *Escherichia coli*, leptospirosis, plague, Q fever, shigellosis and tularaemia.[32]

2.1.2 Parasites

Cysticercosis / Taeniasis is caused by a parasite which infects swine and can cause seizures, headache and many other symptoms in humans. In Latin America for example, 100 out of 100 000 inhabitants suffer from this disease (estimation). Other parasitic zoonoses are trematodosis, echinococcosis/hydatidosis, toxoplasmosis and trichinellosis.[31, 32]

2.1.3 Viruses

Rabies is a disease of carnivores and bats mainly transmissible to humans by bites. Almost all persons infected by rabid animals will die if not treated. An estimated number of 55 000 persons, mainly children, die of this disease in the world every year. Dogs are responsible for most human deaths. Other viral zoonoses are avian influenza, Crimean-Congo hemorrhagic fever, Ebola and Rift Valley fever.

2.1.4 Fungi

Dermatophytoses are superficial mycoses that may be acquired from infected animals and affect the skin, hair and nails of humans, causing itching, redness, scaling and hair loss. Another mycotic infection that can be zoonotic is sporotrichosis.[32]

2.1.5 Unconventional agents

The agent of Bovine Spongiform Encephalopathy is thought to be the cause of variant Creutzfeldt-Jakob Disease (vCJD) which is a degenerative neurological disease different from CJD, at present inevitably lethal in humans.

Zoonoses still represent significant public health threats, but many of them are neglected, i.e. they are not prioritized by health systems at national and international levels. They affect hundreds of thousands of people especially in developing countries, although most of them can be prevented.[32]

2.2.0 Interesting facts about zoonotic diseases

a. About 75% of recently emerging infectious diseases affecting humans are diseases of animal origin, and approximately 60% of all human pathogens are zoonotic.
b. Tick-borne diseases, including Lyme disease and Rocky Mountain spotted fever, are serious public health problems, infecting tens of thousands in the United States each year. CDC is working closely with local communities, developing innovative control approaches and researching improved diagnostics.
c. Almost all persons infected by rabid animals will die if not treated appropriately. Dogs are responsible for most human rabies deaths worldwide, but the public health threat of canine rabies has been virtually eliminated in the United States.

d. There have been 1.5 million West Nile virus infections since 1999. Two and half (2.5) billion people are at risk for dengue in more than 100 endemic countries with 50 million cases of dengue fever each year

2.3.0 ONE HEALTH, ONE WORLD, ONE MEDICINE CONCEPT

The One Health concept recognizes that the health of humans is connected to the health of animals and the environment.

There are many examples that show how the health of people is related to the health of animals and the environment. For instance, animals can pass some diseases on to humans. These diseases are known as zoonotic diseases. Examples include: Rabies, Salmonella infection, West Nile virus fever etc.[33]

Animals also share our susceptibility to some diseases and environmental hazards. Because of this, they can serve as early warning signs of potential human illness. For example, birds often die of West Nile virus before humans get sick with West Nile virus fever.

One Health is not a new concept, but it has become more important in recent years because many factors have changed the interactions among humans, animals, and the environment. These changes have caused the emergence and reemergence of many diseases.

Sixty percent of the pathogens that cause diseases in humans are of animal origin. These diseases, known as zoonoses, can be transmitted by domestic or wild animals. Animal diseases that are transmissible to humans, such as avian influenza, rabies, brucellosis and bovine spongiform encephalopathy, present a public health risk worldwide and it is imperative to prevent or combat them at every level, including the global one. The most effective and

economical solution to protect humans is to combat all zoonotic pathogens by controlling them at their animal source.[33]

The 'One Health' concept is founded on an awareness of the major opportunities that exist to protect public health through policies aimed at preventing and controlling pathogens within animal populations, at the interface between humans, animals and the environment.

Director of Agriculture and Rural Development, The World Bank writes the following: "A global surveillance and control system that is established primarily for emerging infectious zoonotic diseases with pandemic potential can be readily improvised to address the endemic diseases that are a priority in many developing countries, few of which have the capacity or resources necessary to monitor or control them effectively". In other words, to date there is no "One Health" surveillance and control system for endemic (i.e., enzootic) diseases like brucellosis, particularly - but not exclusively – in developing countries. Such a strong statement contradicts our intuitive feeling that there are benefits for public health and society to be gained by implementing sound control and eradication brucellosis programs for livestock, although such benefits need to be demonstrated, particularly in countries with scarce resources.[34]

It is fair to say that there are many unknowns and misconceptions that may lead to the implementation of improvised control measures for endemic diseases as written by Juergen Voegele. It is important to stress that there is an inherent risk that improvised measures might beat best not justified and would not help in providing a sustainable solution or worse, improvisation may be counter-productive or even detrimental. In order to be successful, a "One Health" approach has to be truly multidisciplinary and every component of a

global/holistic approach has to be addressed proficiently in its own right. More, given the changes in the livestock sector, its contact with wildlife and the resurgence and emergence of zoonotic diseases linked to it, a new "One Health" research and policy-generation strategy has to be defined.[124] It is in this context that the World Animal Health Organization (OIE) is endorsing a "One Health" approach which will result in a deeper and sustainable political support for the coordinated prevention of high public health and animal impact diseases at the human-animal interface.[35]

Why is the Human-Animal-Ecosystem Interface important to public health and WHO? Zoonoses (diseases moving between animals and people) and other health threats at the hu-man-animal-ecosystem interface pose ongoing and increasing risks to public health and global health security. The human-animal-ecosystem interface (HAEI) encompasses all direct and indirect hu-man exposure to animals and animal products and to the various environments and ecosystems we all share.

Health threats at this interface include those existing and emerging pathogens transmitted through contact with animals, food, water, and contaminated environments. Examples include:

A. Antimicrobial resistance in pathogens.

B. Avian influenza H5N1 and H7N9, and pandemic A (H1N1) 2009 influenza.

C. Bovine spongiform encephalopathy and Var-iant Creutzfeldt-Jacob disease.

D. Food-borne E. coli and salmonella infections.

E. Middle East respiratory syndrome corona-virus (MERS-CoV).

F. Rabies.

G. Rift Valley fever.

H. Brucellosis etc.

2.4.0 History of brucellosis

Military medicine has played a major role in studying and describing brucellosis in humans.[42] In 1751 G Cleghorn, a British army surgeon stationed on the Mediterranean island of Minorca, described cases of chronic, relapsing febrile illness and cited Hippocrates' description of a similar disease more than 2,000 years earlier.[43] Three additional British army surgeons working on the island of Malta during the 1800s were responsible for important observations of the disease. JA Marston described clinical characteristics of his own infection in 1861.[44] In 1887 David Bruce, for whom the genus *Brucella* is named, isolated the causative organism from the spleens of five patients who died from the disease and placed the microorganism within the genus *Micrococcus.*[45] Ten years later, ML Hughes, who coined the name "undulant fever," published a monograph that detailed clinical and pathological findings in 844 patients.[46]

That same year, Danish investigator B Bang identified an organism, which he called the "bacillus of abortion," in the placentas and fetuses of cattle suffering from contagious abortion.[47] In 1917 AC Evans recognized that Bang's organism was identical to that described by Bruce as the causative agent of human brucellosis. The organism infects mainly cattle, sheep, goats, and other ruminants, in which it causes abortion, fetal death, and genital infections.[48,49] Humans, who are usually infected incidentally by contact with infected animals or ingestion of dairy foods, may develop numerous symptoms in addition to the usual ones of fever, malaise, and muscle pain. Because of the worldwide distribution of brucellosis,

international travel and military deployments increase the risk of exposure.[50] The disease frequently becomes chronic and may relapse, even with treatment. Laboratory-acquired infections have been documented as awareness of this disease has increased.[51–54] Laboratory accidents may become more frequent and significant as bio-defense research expands in the academic and biotechnology industries. Strict adherence to proper engineering controls, good laboratory and microbiology techniques, and personal protective equipment, in addition to vaccination (when possible), significantly reduce the incidence of laboratory-acquired infections.[55,56] However, no human brucellosis vaccine is available for laboratory workers.

The first human blood culture of *B. abortus* (rather than *B. melitensis*) was obtained in Baltimore in 1924.[57] Infection of cattle became recognized as far more wide spread than that of goats or swine. In the mid-1930s 13-16% of all U.S. cattle in 70% of the herds were estimated to be infected, as well as 10% of the milk goats in Texas.[39] In 1926, 46 human cases were reported in the U.S.[58] The first cases to be recognized in Iowa or Minnesota, where Brucellosis became a major endemic problem, occurred in 1927.[39] The prevalence of the disease increased rapidly, but the extent to which this merely indicated better diagnosis is uncertain. In the 14 years (1930-1943) 36,513 cases of Brucellosis were reported in the U.S., 59% of these during the latter seven years.[39,59]

Brucellosis has been difficult to diagnose conclusively because blood cultures in chronic cases usually are negative and, conversely, the agglutination reaction may be falsely negative and a positive reaction may not indicate currently active disease or cross-reaction with another pathogen.[39] These factors, as well as the marked variability of symptoms have made treatments difficult to evaluate. The disease may be self-limited. The best diagnostic clue has

been the occupational history. Since most milk has been pasteurized most at risk have been slaughterhouse workers and farmers.

The greatest reliance in the 1930s was placed on various therapeutic vaccines[60] although they "appear to be very uncertain in their effect". Nevertheless, there were small trials of numerous drugs. There appears to have been particular interest in metallic drugs which were available for the treatment of other diseases: e.g., I.V. Neoarsphenamine (arsenical), I.V. Mercurochrome (mercurial).[61] I.M. Enesol (bismuth-mercury), I.M. Fouadin (antimonial).[62] However, the first drug to be tried widely that appeared to hold promise for a rapid effect was sulfanilamide. The first of numerous English language reports appeared in 1938[63,64] but failures already began to be reported in 1939.[66] Subsequent sulfonamides as mono-therapy were equally ineffective.

Between 1947 and 1951 antibiotic therapy became established, although also without complete reliability. The first hope was accorded to I.M. streptomycin[66] although another report deemed it worthless as mono-therapy. The latter report was the first to find streptomycin combined with oral sulfadiazine to possibly be effective.[67] This was rapidly confirmed.[58] However, the parenteral administration of streptomycin made the treatment frequently impractical. Next, in 1948, the first trial of chlortetracycline (Aureomycin) was made in Mexico, initially combined with sulfadiazine, but soon by itself. This drug had the practical advantage over streptomycin of oral administration and lack of oto-toxicity. Some cases who had failed streptomycin – sulfadiazine were cured.[68] As with previous drugs, in 1950 cases in which Aureomycin failed were reported.[69] Spink *et al*., in 1951 adhered to the superiority of Aureomycin mono-therapy.[69]

2.5.0 EPIDEMIOLOGY OF BRUCELLOSIS

Worldwide, brucellosis remains a major source of disease in humans and domesticated animals. Although reported incidence and prevalence of the disease vary widely from country to country, bovine brucellosis caused mainly by *B. abortus* is still the most widespread form. In humans, ovine/caprine brucellosis caused by *B. melitensis* is by far the most important clinically apparent disease. The disease has a limited geographic distribution, but remains a major problem in the Mediterranean region, western Asia, and parts of Africa and Latin America.[70]

The geographical distribution of brucellosis is constantly changing, with new foci emerging or re-emerging. The epidemiology of human brucellosis has drastically changed over the past few years because of various sanitary, socioeconomic, and political reasons, together with increased international travel.[9] New foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening.[41] The disease occurs worldwide, except in those countries where bovine brucellosis (*B. abortus*) has been eradicated. This is defined as the absence of any reported cases for at least five years. These countries include Australia, Canada, Cyprus, Denmark, Finland, The Netherlands, New Zealand, Norway, Sweden and the United Kingdom.[9] The Mediterranean Countries of Europe, northern and eastern Africa, Near East countries, India, Central Asia, Mexico and Central and South America are still not brucellosis free. While *B. melitensis* has never been detected in some countries, there are no reliable reports that it has ever been eradicated from small ruminants in any country.[70] Although in most countries brucellosis is a nationally notifiable disease and reportable to the local health authority, it is under reported and official numbers constitute only a fraction of true incidence of the disease. Thus the true incidence of human

brucellosis is unknown and the estimated burden of the disease varies widely, from <0.03 to >160 per 100,000 population.[41] Recent re-emergence in Malta and Oman indicates the difficulty of eradicating this infection.[71] Sheep and goats and their products remain the main source of infection, but *B. melitensis* in cattle has emerged as an important problem in some southern European countries, Israel, Kuwait, and Saudi Arabia. *B. melitensis* infection is particularly problematic because *B. abortus* vaccines do not protect effectively against *B. melitensis* infection; the *B. melitensis* Rev.1 vaccine has not been fully evaluated for use in cattle. Thus, bovine *B. melitensis* infection is emerging as an increasingly serious public health problem in some countries. A related problem has been noted in some South American countries, particularly Brazil and Colombia, where *B. suis* biovar 1 has become established in cattle.[72] Reported incidence in endemic-disease areas varies widely, from <0.01 to >200 per 100,000 population.[73] While some areas, such as Peru, Kuwait, and parts of Saudi Arabia, have a very high incidence of acute infections, the low incidence reported in other known brucellosis endemic areas may reflect low levels of surveillance and reporting, although other factors such as methods of food preparation, heat treatment of dairy products, and direct contact with animals also influence risk to the population. In North America in the 1990s, a low number of newly affected herds continued to be disclosed every year. Consequently, in 1997, the Brucellosis Emergency Action Plan (BEAP) was implemented. According to the plan, all activities involving brucellosis surveillance and management of new cases were to be conducted as an emergency action. This means that all activities associated with each new case would be dealt with as a top priority. The plan emphasized depopulation of affected herds, enhanced surveillance, epidemiology and herd management, and rapid response. In the year, this plan was implemented, there were 85 cumulative affected herds in the US.[74] In

December 31st, 2000, there were no affected cattle herds in the United States. This was the first time in the history of the brucellosis program that the United States had no known brucellosis affected herds.[74] However, brucellosis has a variable, sometimes quite lengthy incubation period, so it is expected that additional affected herds will be disclosed. Enhanced surveillance efforts have been maintained to ensure the detection of the last remaining affected herds as soon as possible. Indeed, three more affected herds were disclosed during fiscal year 2001, one each in Arkansas, Kansas, and Missouri. Those herds were rapidly depopulated, and the nation again had zero brucellosis affected herds as of November 30, 2001.[74]

2.6.0 TAXONOMY OF THE BRUCELLAE

The debate of whether the brucellae should comprise a single monospecific genus or multiple species has been a source of much controversy. Differences in biochemical capabilities and susceptibilities to dyes and phages together with the differences observed in host preference, led to the division of the brucellae into six species, some of which could be further divided into biovars (this excludes those isolates from marine mammals to which formal names are yet to be approved). However, based on DNA–DNA hybridizations and with the advent of sequence-based approaches using the 16sRNA gene, the remarkable homogeneity among this group was revealed, and in consequence, the suggestion that they should be considered as a single species, *B. melitensis*.[80,81] This has not been universally accepted, with many holding onto the previously used multiple species nomenclature on practical grounds, while others use nomenspecies. Other methods using restriction mapping and cross-hybridization to provide genomic indexing show agreement with the classical taxonomy of this genus.[80] Large-scale sequencing studies are currently underway and should help resolve the taxonomic standing of

the Brucella species and may provide sufficient data to allow a robust phylogeny of the group to be constructed.

2.6.1 Causative agent

Brucellae are small, non-motile, non-sporulating, non-toxigenic, non-fermenting, facultative, intracellular, gram-negative coccobacilli parasites that may, based on DNA homology, represent a single species.[13,19]

2.6.2 Type of species

For convenience these have been classified into nomenspecies that differ from one another in their preferred host animal[82] these include;

a. *B. melitensis* is found mainly in goats and sheep.
b. *B. abortus* is found mainly in cattle.
c. *B. suis* is found mainly in pigs.
d. *B. canis* is found in dogs.
e. *B. neotomae* is found in wood rats.
f. *B. ovis* is found in sheep.

Animals such as camels, horses, wild rodents can also be infected. The main human pathogens *are B. abortus, B. melitensis, B. suis and B. canis*. There are 3 biovars of *B. melitensis*, 9 biovars of *B. abortus* and 4 biovars *of B. suis* (biovars 8 of *B. abortus* is longer recognized.[82]

Classification of brucella can be done also based on CO_2 requirements, sensitivity to dyes (basic fuchsin and thionin), H_2S production, agglutination by non-specific sera, phage typing and oxidative action on carbohydrate.

Table 2.1: Typical Host Specificity of Brucella species.[82]

Brucella species	Animal host	Human Pathogenicity
B. melitensis	Sheep, goat	High
B. suis	Swine	High
B. abortus	Cattle, bison	Intermediate
B. canis	Dogs	Intermediate
Marine species	Marine mammals	Rare
B. ovis	Sheep	None
B. neotomae	Rodents	None

2.7.0 BRUCELLOSIS IN ANIMALS

Brucellosis is a sub-acute or chronic disease which may affect many species of animals. In cattle, sheep, goats, other ruminants and pigs the initial phase following infection is often not apparent. In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female, usually during the last third of pregnancy and epididymitis and orchitis in the male. Clinical signs are not pathognomonic and diagnosis is dependent upon demonstration of the presence of *Brucella* spp. either by isolation of the bacteria or detection of their antigens or genetic material, or by demonstration of specific antibody or cell-mediated immune responses.

Brucellosis is a disease of many animal species but especially of those that produce food: sheep (especially milk-producing), goats, cattle and pigs and, on a more localized scale, camels, buffaloes, yaks and reindeer. Five of the six currently recognized *Brucella* species cause infection and clinical signs [61] in one or more animal hosts. Four of these also cause human disease: *B. melitensis, B. suis, B. abortus* and *B. canis* in descending order of pathogenicity. The recently recognized types associated with marine animalsmay also have the capacity to cause human disease.

The *Brucella*e are somewhat host-specific but cross-species infections occur, especially with *B. melitensis*. Infections in many wildlife species have been reported but those that obviously affect population fecundity and result in human infections are quite rare. *B. melitensis* infections in dairy herds, however, have severe economic and public health implications.

Infections in sheep and goats are highly contagious because of the pathogenicity of *B. melitensis* and because of close contact caused by the density of the flocks or herds, the

commingling of those of different owners and heavy exposure in housing. Animal-to-animal transmission occurs as a result of the large number of organisms shed in the environment.[94]

Humans are often infected due to direct animal contact or ingestion of contaminated dairy products. Human cases may be a useful indicator of the presence of disease in animal populations and may be the only source of information for surveillance. It is important, however, to determine if the infection was acquired locally or elsewhere, and, if food products are implicated, to establish whether these were locally produced or imported.[85]

2.7.1 Clinical Signs of Brucellosis in Animals

Characteristic but not specific signs of brucellosis in most animal hosts are abortion or premature births and retained placenta. In some areas, abortion is relatively uncommon. In some parts of Africa, hygromas and abscesses are the major clinical signs in nomadic or semi-nomadic cattle herds infected with *B. arbortus* biovar. There is lowered milk production due to premature births. Interference with fertility is usually temporary and most infected animals will abort only once and some are unaffected. The udder is often permanently infected, especially in the case of cows and goats. Shedding of organisms in milk is frequent. Localized infections in sheep result in orchitis or epididymitis in the case of *B. melitensis* and *B. ovis.*[9] In goats, cattle, swine and dogs similar complications may follow infection with *B. melitensis, B. abortus, B. suis* and *B. canis* respectively. Arthritis may also be a rare sign in *B. melitensis*-infected sheep and goats. In horses, local abscess formation in bursae may be the only clinical sign and infection in this species is often asymptomatic. Camels infected with *B. melitensis* shed the organisms in milk and in some countries this is a serious public health problem. Clinical signs of brucellosis in camels appear to be very rare. The severity of the disease

depends upon many factors such as previous vaccination, age, sex and management such as herd or flock size and density.[30]

Abortions are more prevalent in unvaccinated animals and numbers of organisms shed are much greater. The bacteria are found in tissues and fluids associated with pregnancy, the udder and the lymph nodes which drain the relevant areas.

2.8.0 TRANSMISSION

Most infections result from ingestion of bacteria either from diseased animals or contaminated feedstuffs. However, infection may also be acquired by respiratory exposure and by contamination of abraded skin and mucosal surfaces. Natural breeding transmits infection in swine and dogs and, to a lesser extent, sheep and goats. Persistent bacteraemias are also more common in the first two species**.** Bacteraemia occurs during the course of infection in other species but is usually intermittent and of short duration. Cross-transmission of brucellosis occurs between cattle, swine, sheep and goats and other species, including dogs, horses, feral swine, bison, reindeer, caribou and camels. In some instances, the infection is dead-end, e.g. dogs eating placentas from infected animals following abortion, while in other situations, such as feral swine, onward transmission to cattle or domestic swine occurs readily. In most developing and underdeveloped countries, brucellosis is endemic and major sources of new or re-infections in cattle, swine, sheep and goats are from contact with dogs and feral species. Therefore, animals that are free range are at risk of exposure to *Brucella* infections.[78]

Table 2.2: Animals affected by *Brucella* spp

HOST	***B. abortus***	***B. melitensis***	***B. suis***	***B. canis***	***B. ovis***
Cattle	+	+	+ (rare)	-	-
Buffaloes	+	+	-	-	-
Sheep	+ (rare)	+	+ (rare)	-	+
Goats	+ (rare)	+	-	-	-
Swine	+ (rare)	+ (rare)	+	-	-
Dog	+	+	+ (rare)	+	-
Camel	+ (rare)	+	-	-	-
Horse	+	+(rare)	+(rare)	-	-
Rodent	+(rare)	+(rare)	+(biovar5)	-	-

Keys:

Affects = (+)

Do not Affects = (-)

Rarely affects = (+rare)

2.9.0 BRUCELLOSIS IN HUMANS

Brucellosis is an acute or sub-acute febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia and prostration and which, in the absence of specific treatment, may persist for weeks or months. Typically, few objective signs are apparent but enlargement of the liver, spleen and/or lymph nodes may occur, as may signs referable to almost any other organ system.[88,89] The acute phase may progress to a chronic one with relapse, development of persistent localized infection or a non-specific syndrome resembling the "chronic fatigue syndrome". The disease is always caused by infection with a *Brucella* strain and diagnosis must be supported by laboratory tests which indicate the presence of the organism or a specific immune response to its antigens. Several outbreaks of brucellosis have been associated with a history of exposure to a known or probable source of *Brucella* spp. This includes common host species, especially cattle, sheep, goats, pigs, camels, yaks, buffaloes or dogs; consumption of raw or inadequately cooked milk or milk products, and, to a lesser extent, meat and offal derived from these animals.[90,91] In addition, the resistance of the organism and its high infectivity make environmental contamination a probable hazard, although this is always difficult to prove. Occupational exposure and/or residence in an area in which the infection is prevalent, also raise the probability of the diagnosis.

Susceptibility to brucellosis in humans depends on various factors, including the immune status, routes of infection, size of the inoculum and, to some extent, the species of *Brucella.*[85]In general, *B. melitensis* and *B. suis* are more virulent for humans than *B. abortus* and *B. canis,* although serious complications can occur with any species of *Brucella.* Common routes of infection include direct inoculation through cuts and abrasions in the skin,

inoculation via the conjunctival sac of the eyes, inhalation of infectious aerosols, and ingestion of infectious unpasteurized milk or other. The disease is acute in about half the cases, with an incubation period of two to three weeks. In the other half, the onset is insidious, with signs and symptoms developing over a period of weeks to months from the infection.[92]

2.9.1 Clinical Signs of Brucellosis in Human

The clinical manifestations are varied and nonspecific. They include fever, sweats, fatigue, malaise, anorexia, weight loss, headache, arthralgia and back pain. Commonly, patients feel better in the morning, with symptoms worsening as the day progresses. The desire to rest can be profound, and depression is pervasive.[1] If untreated, the pattern of the fever waxes and wanes over several days ("undulant fever").

Other complications include cutaneous, ophthalmic, cardiovascular, pregnancy and breastfeeding, genitourinary, respiratory tract, hepatobiliary and gastrointestinal.[7]

2.10 STATUS OF BRUCELLOSIS IN NIGERIA

Some investigations and reports have shown that brucellosis is endemic in Nigeria and evidence of infection as well as frank outbreaks has occurred both in animals and humans. An investigation by Falade.[15] also showed serological evidence of infection in goats in certain parts of the country. Evidence from various sources indicates that of sheep and goats are carrier of *brucella* organism, although, brucellosis has been diagnosed in other species of animals and human beings. Cattle, sheep and goats are the most important livestock source, reservoir and sufferer of brucellosis in the country. Even when outbreaks in humans have been detected, there have been close associations with these animals.

Brucellosis is endemic in Nigeria and, as elsewhere, causes severe economic losses to livestock farmers and ranchers, and is a serious risk to human health.[23,75] Studies in various parts of the country indicate that the disease is widespread among animal populations ranging from cattle, sheep, goats, camel and dogs particularly in ranches, livestock breeding centers and dairy farms. In these locations, the prevalence of the disease in cattle ranges between 3.7% and 48.8%[12,76,77] while in the traditional nomadic Fulani cattle herds the prevalence is between 0.4% and 26%.[13,38,78,79] All these figures are based on serological surveys; there are few available reports based on the isolation of *Brucella* from cattle.[13,19] Field experience in Nigeria has shown that abortion is common in cattle, sheep and goats that are usually herded together, although the causes of such abortions have not always been investigated in detail in the laboratory.[20]

Brucellosis in sheep and goats is usually caused by *B. melitensis.* Infection with *B. abortus* is rare, although the association of *B. abortus* with abortion in sheep has been demonstrated in several countries through isolation of the organisms.[21,39]

In Nigeria, serological surveys for *Brucella* antibodies in sheep indicate a prevalence of 1.4% to 14.5%.[20,79] Little effort has been made to isolate *Brucella* from cases of abortion in sheep. The only available report in literature of the isolation of *Brucella* from such cases in Nigeria was by Okoh,[16] who used milk from nursing ewes; the report did not indicate the biovar involved.

2.11 EXPOSURE OF ANIMAL OWNERS AND WORKERS TO ZOONOTIC DISEASE

Brucellosis is an occupational disease; farmers, veterinarians, inseminators etc are at higher risk of contracting it. There is an even stronger association with poverty; poor people live closer to their animals, are more likely to consume unpasteurized milk products and meat from infected animals, and are less prone to protect themselves when dealing with foetal fluids and vaginal discharges after abortion or full-term parturition. Furthermore, as with other conditions; poor people, especially in rural areas, are less likely to get proper diagnosis and treatment, and since brucellosis is a zoonosis it is a double burden – i.e. it affects both people and their animals - in poor households.[29]

The prevalence of 14.3% of occupational diseases support the fact that humans are exposed to biological hazards in the work environment largely due to exposure to live animals or animal products (blood, tissue, milk, eggs) and other biologically sensitive and infectious materials especially in the laboratory.[108] The risk of acquiring occupational infections can be high for animal related occupations because of their close contact with animals,[108] Bacteria, dermatophytes, viruses and parasites, carried by these animals or in the surrounding environment, can infect these workers. Prevalence of skin diseases, respiratory diseases and lesser extent salmonellosis recorded in this study suggested that some of the workers were exposed to these organisms possibly through abraded skin, mucosal tissues, ingestion, inhalation, and injection while a case of vertebra disc slip observed may have resulted from over exertion and wrong postures during lifting and moving of animals and other heavy equipment.[99]

2.12 KNOWLEDGE OF ANIMAL OWNERS AND WORKERS ON BRUCELLOSIS

Considering livestock owners' awareness about the zoonosis' multiple routes of transmission, together with reducing the prevalence in their animals, are the most efficient ways to reduce the prevalence of human brucellosis cases. The level of knowledge on zoonoses, work-related zoonoses and associated implications by these workers is adequate compared to findings of Swai et al. 2010 who reported a low level of knowledge on zoonoses among animal health workers, livestock keepers and veterinary staffs in Tanzania.[99] He found that less than 50% of the workers were aware of various preventive measures against work related zoonotic diseases.

Traditional practices and beliefs may prevent the acceptance of control activities. Therefore, the proper understanding of the cause and risks of brucellosis and of the benefits of preventive measures is essential for the acceptance of such measures. Examples of such practices/beliefs include the consumption of fresh milk and the belief that vaccination causes abortion. People in low-income countries do not always understand the dangers of infectious diseases.

Recently, farmer's attitudes and husbandry practices have received more attention as important factors influencing the spread of animal disease.[112,113] Assessing knowledge, attitudes and practices for other diseases, such as avian influenza (AI), has been helpful for policy makers to develop control strategies and health education campaigns.[114] To date, there is no information on the knowledge, attitude and practices (KAPs) associated with brucellosis in Egypt, however studies in Kenya and Saudi Arabia have suggested that awareness of the disease and its routes of transmission among livestock keepers may, on occasions, be poor.[115,116]

Infection with *Brucella spp*. continues to pose a human health risk globally despite strides in eradicating the disease from domestic animals.[100]

2.13 PRACTICES OF ANIMAL OWNERS AND WORKERS ON BRUCELLOSIS

Veterinary services play a minor role in preventing the introduction of infection, while its role in preventing the spread of the infection inside the farms or herds has a major role. These results are coincide with Crespo,[113] Mainar–Jaine & Vazquez- Boland and AL-Majali,[101] who suggested that the absence of disinfection programs in raising farms considered a risk factor for *Brucella* seropositivity in small ruminants. Proper disposal of aborted materials and highly hygienic procedures are extremely important steps in any successful *Brucella* control program. It is well known that delivering adequate animal health services results in a low incidence of diseases, and especially those diseases that have an infectious nature. In addition, controlling brucellosis in small ruminants (mainly by Rev-1 vaccination) will indirectly reduce the prevalence of this disease in other animal species especially cattle. Poor veterinary service has been identified as a risk factor for brucellosis in Argentina[116] and Mexico.[114]

Intake of raw milk, direct contact with infected animals and animal materials and fluids are the major risks for transmission of brucellosis to the human population. Pasteurisation of milk and use of protective clothing when dealing with infected animals (e.g. when assisting with lambing or handling abortion materials) are highly recommended but may be very difficult to implement under field situations because of a lack of proper supplies or because of traditional beliefs and practices. In general, measures to improve hygiene and sanitation are not popular.[103]

2.14 LABORATORY METHODS FOR DIAGNOSIS OF BRUCELLOSIS

The laboratory methods for diagnosis of brucellosis in animals are divided into three methods including;

2.14.1 Bacteriological Tests

Appropriate facilities are needed to isolate and identify all suspect *Brucella* spp. from abortion materials (foetal stomach contents and cotyledons), milk and vaginal discharges, as well as tissues from slaughtered reactor animals, such as supra-mammary lymph nodes. Ideally, isolates of *Brucella* spp. should be "fingerprinted" by biotyping. Periodically, isolates should be confirmed by submission to a WHO/FAO Collaborating Centre.[83]

As laboratory exposures to *Brucella* spp. have occurred frequently, any laboratory to be used for isolation of brucellae should have a primary biohazard containment facility, to minimize the risk of human infection.[84]

2.14.2 Serological Tests

Many serological tests for brucellosis have been developed, or are under development. However, for serological surveillance to be successful, it is advisable to concentrate on a few tests only, and ensure that these are quality controlled and can be carried out with the facilities available. Given that no test is both 100% sensitive and specific, it is not advisable to use a "battery" test approach in the mistaken belief that if enough tests are done the results will become clear.[85, 86] Rather, what is needed is a simple and clearly defined testing strategy with defined endpoints, and a rigorous approach to borderline cases, which takes into account the epidemiological features of brucellosis. Note that only certain tests for surveillance purposes are recognized as official by the OIE Manual of Standard DiagnosticTests and Vaccines.[87] If

sensitive and specific enough, any country may, however, declare other tests used for diagnosis, control and surveillance purposes in their programmes.

When individual animals are tested to ascertain if the herd is infected, the number of animals tested and the critical number of reactors used to decide the health status of the herd becomes very important in influencing the herd level sensitivity and specificity. If the test specificity is less than 100%, then as the number of animals tested increases the probability of at least one false-positive animal increases, and so the herd specificity decreases. The herd sensitivity, herd negative predictive value and herd apparent prevalence increase directly with the number of animals tested, but the herd positive predictive value decreases. Herd sensitivity can be increased by using a test that is less than 100% specific. These features should be borne in mind when interpreting the natural history of brucellosis, and particularly as it is recognized that larger herds are more likely to be infected than smaller herds.[88]

2.14.2.1 Serological tests are divided broadly into two groups:

Screening tests used in the field clinics or in regional laboratories, such as the Rose Bengal or buffered plate agglutination. The Rose Bengal test has a very high sensitivity to ensure that infected animals are not missed. The milk ring test is also an excellent screening test for dairy cattle. Indirect ELISA tests are also being used to screen milk and serum.

Confirmatory tests used in a central or regional laboratory, such as competitive ELISA,

Immune-diffusion or complement fixation tests[86] are very useful in distinguishing vaccine antibody responses from those induced by field infections.

It is important to note that, during the course of a brucellosis programme, testing strategies will change. For example, when the prevalence of infection is high, a test of adequate sensitivity but high specificity is desirable to detect most of the truly diseased animals and herds, and to minimize the number of false-positive reactors. In contrast, as the prevalence decreases, a sufficiently specific but highly sensitive test is recommended. It is thus important to decide how to classify positive animals, such as single reactors in an otherwise negative herd. This may be the first sign of a herd breakdown, or it may be a false positive of no importance. In some countries, the problem of false-positive serological reactions due to cross-reactive bacteria (e.g. *Yersinia enterocolitica* 0:9) has complicated the eradication of brucellosis.[36, 86]

A number of commercial kit tests are also available and may be particularly useful for confirmatory purposes, but their costs often preclude their use in large surveillance programmes. Automation of some tests may also allow for economies of scale. 'Banking' of a representative sample of sera in a deep freeze is strongly recommended for retrospective investigations of problem herds.

2.14.3 Diagnosis in animals

Diagnosis and control of the disease in animals must be carried out on a herd basis. There may be a very long incubation period in some infected animals and individuals may remain serologically negative for a considerable period following infection. The identification of one or more infected animals is sufficient evidence that infection is present in the herd, [76,95] and that other serologically negative animals may be incubating the disease and present a risk.

Diagnostic tests fall into two categories: those that demonstrate the presence of the organisms and those that detect an immune response to its antigens. The isolation of *Brucella* is definitive proof that the animal is infected, but not all infected animals give a positive culture and the methods and facilities that must be employed are not always readily available. The detection of antibody or a hypersensitivity reaction provides only a provisional diagnosis, but in practice is the most feasible and economic means of diagnosis. False positive reactions to serological tests can occur through a number of factors, including vaccination, and this must be borne in mind when interpreting results. Similarly, dermal hypersensitivity only indicates previous exposure to the organism, not necessarily active infection, and may also result from vaccination.[19]

Vaccination is an extremely important and effective facet of most control strategies but has the disadvantage that its use may confuse diagnosis by stimulating the production of hypersensitivity or antibodies detectable by serological tests. Antibody titres may persist for a prolonged period in a small proportion of vaccinated animals and this proportion increases with age at vaccination. To reduce this problem, in cattle vaccination is usually employed in young animals below the age of six months, but may be used in adults if a reduced dose is given, especially[41, 96] by the intra-conjunctival route. There is currently no widely available test that is able to distinguish vaccinated from infected animals, although some tests are under evaluation.

It is of utmost importance that the use of vaccination is strictly controlled, that it is used at the correct age, that vaccine of sufficient quality is used and that vaccinated animals are correctly identified. If this is not the case, correct serological diagnosis is confused. The vaccination

programme can be suspended when the prevalence of the disease reaches a very low level, when the disadvantages of vaccination outweigh any benefit that it may bring on the basis of cost-benefit and cost-effectiveness analysis.

2.14.4 Bacteriological methods

The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis and may be useful for epidemiological purposes and to monitor the progress of a vaccination programme. It should be noted that all infected materials present a serious hazard, and they must be handled with adequate precautions during collection, transport and processing.

Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Ziehl-Neelsen (Stamp) or Kosters' methods. The presence of large aggregates of intracellular, weakly acid-fast organisms with *Brucella* morphology is presumptive evidence of brucellosis. Care must be taken as other infectious agents such as *Coxiella burnetii* or *Chlamydia* may superficially resemble *Brucella.*[23]

Brucella may most readily be isolated in the period following an infected abortion or calving, but isolation can also be attempted post-mortem. They are excreted in large numbers at parturition and can be cultured from a range of material including vaginal mucus, placenta, fetal stomach contents and milk using suitable selective culture media. It is of the utmost importance that faecal and environmental contamination of the material is kept to a minimum to give the greatest chance of successfully isolating *Brucella*.[68] If other material is unavailable or grossly contaminated, the contents of the fetal stomach will usually be otherwise sterile and are an excellent source of *Brucella*.

In some circumstances it may be appropriate to attempt the isolation of *Brucella* post-mortem. Suitable material includes supra-mammary, internal iliac and retropharyngeal lymph nodes, udder tissue, testes and gravid uterus. Milk samples should be allowed to stand overnight at 4 °C before lightly centrifuging.[84] The cream and the deposit are spread on to the surface of at least three plates of solid selective medium. Placental samples should be prepared in the field by selecting the least contaminated portion and cutting off pieces of cotyledon. In the laboratory, the portions should be immersed in alcohol which should be flamed off before cutting with scissors or scalpel and smearing the cut surface on three plates of selective medium. Other solid tissues can be treated in a similar manner, or, ideally, they should be macerated mechanically following flaming before plating out. The tissues may be ground manually or homogenised in a blender or stomacher with a small proportion of sterile water. Fetal stomach contents are collected, after opening the abdomen, by searing the surface of the stomach with a hot spatula and aspirating the liquid contents with a Pasteur pipette or syringe.[81,83]

Bacterial colonies may be provisionally identified as *Brucella* on the basis of their cultural properties and appearance, Gram staining, and agglutination with positive antiserum (Fig. 4 and 5). If available, a PCR-based molecular identification method may be used. Definitive identification of suspect colonies can only be made using techniques available at *Brucella* Reference Centers.

2.14.5 Serological Methods

The detection of specific antibody in serum or milk remains the most practical means of diagnosis of brucellosis. The most efficient and cost-effective method is usually screening all

samples using a cheap and rapid test which is sensitive enough to detect a high proportion of infected animals. It is absolutely essential that only internationally recognized tests using antigens standardized against the 2nd International anti-*B. abortus* Serum are used. Appropriate quality control sera should be included with each batch of tests, and tests should be repeated if the quality control criteria are not met.[18]

Serological results must be interpreted against the background of disease incidence, use of vaccination and the occurrence of false positive reactions due to infection with other organisms. As with all laboratory based diagnosis, it is imperative to correctly identify the "audit trail" of individual animal identity, sample number and test result so that there is complete certainty of the linkage between animal and result.

2.14.6 Rose Bengal plate test (RBT)

The RBT is one of a group of tests known as the buffered Brucella antigen tests which rely on the principle that the ability of IgM antibodies to bind to antigen is markedly reduced at a low pH. The RBT and other tests such as the buffered plate agglutination tests and the card test play a major role in the serological diagnosis of brucellosis worldwide. It is a simple spot agglutination test where drops of stained antigen and serum are mixed on a plate and any resulting agglutination signifies a positive reaction. The test is an excellent screening test but may be oversensitive for diagnosis in individual animals, particularly vaccinated ones.[18,37] The procedure can be automated but this requires custom-made equipment.

2.14.7 ELISA tests

The ELISA tests offer excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial

sources in kit form. They are more suitable than the CFT for use in smaller laboratories and ELISA technology is now used for diagnosis of a wide range of animal and human diseases.[19] Although, in principle ELISAs can be used for the tests of serum from all species of animal and man, results may vary between laboratories depending on the exact methodology used. Not all standardization issues have yet been fully addressed. For screening, the test is generally carried out at a single dilution.[87]

It should be noted, however, that although the ELISAs are more sensitive than the RBT, sometimes they do not detect infected animals which are RBT positive. It is also important to note that ELISAs are only marginally more specific than RBT or CFT.[86]

2.14.8 Serum agglutination test (SAT)

The SAT has been used extensively for brucellosis diagnosis and, although simple and cheap to perform, its lack of sensitivity and specificity mean that it should only be used in the absence of alternative techniques.[87]

2.14.9 Complement fixation test (CFT)

The sensitivity and specificity of the CFT is good, but it is a complex method to perform requiring good laboratory facilities and trained staff. If these are available and the test is carried out regularly with good attention to quality assurance, then it can be very satisfactory.

It is essential to titrate each serum sample because of the occurrence of the prozone phenomenon whereby low dilutions of some sera from infected animals do not fix complement. This is due to the presence of high levels of non-complement fixing antibody isotypes competing for binding to the antigen.[73] At higher dilutions these are diluted out and

complement is fixed. Such positive samples will be missed if they are only screened at a single dilution. In other cases, contaminating bacteria or other factors in serum samples fixor destroy complement causing a positive reaction in the test, even in the absence of antigen. Such "anti-complementary" reactions make the test void and a CFT result cannot be obtaine.[73, 94]

2.14.10 Supplementary tests

Many other serological tests have been employed. Some, such as the Rivanol or 2-ME test, are variations of the SAT and, although more specific, share many of its disadvantages. At present, the use of such procedures in the place of the standard test is not advised.[90]

2.14.11 Milk testing

In dairy herds, milk is an ideal medium to test as it is readily and cheaply obtained, tests can be repeated regularly and give a good reflection of serum antibody. Milk from churns or the bulk tank can be screened to detect the presence of infected animals within the herd which can then be identified by blood testing. This method of screening is extremely effective and is usually the method of choice in dairy herds.[18]

2.14.11a Milk ring test

The milk ring test (MRT) is a simple and effective method, but can only be used with cow's milk. A drop of haematoxylin-stained antigen is mixed with a small volume of milk in a glass or plastic tube. If specific antibody is present in the milk it will bind to the antigen and rise with the cream to form a blue ring at the top of the column of milk. The test is reasonably sensitive but may fail to detect a small number of infected animals within a large herd. Non-

specific reactions are common with this test, especially in brucellosis free areas. The milk ELISA is far more specific than the MRT.[98, 99]

2.14.11b Milk ELISA

The ELISA may be used to test bulk milk and is extremely sensitive and specific, enabling the detection of single infected animals in large herds in most circumstances.

2.14.12 Fluorescence polarization assay

This technique, which requires special reagents and reading equipment, is claimed to have advantages in sensitivity and specificity over other methods. Evaluation has been limited however, and the procedure is not widely available.[86] Further information is required before its overall value can be assessed.

2.14.13 Intra-dermal test

This procedure, using a standardized antigen preparation such as Brucellin INRA or brucellergene OCB, can be used for monitoring the status of herds in brucellosis-free areas. It is sensitive and specific but false positive reactions can occur in vaccinated animals.

2.15 PREVENTION, CONTROL AND ERADICATION OF ANIMAL BRUCELLOSIS

The prevention of the introduction of brucellosis into populations of animals are the same as those for the control of the disease in populations which are already infected: economic benefits and the protection of public health.[18]

Brucellosis is a zoonosis with a strong correlation between animal and human diseases. While public health measures such as pasteurisation and education have varying degrees of success,

it remains primarily a veterinary responsibility to control brucellosis, including application of principles of epidemiology and animal husbandry.[18]

2.15.1 Prevention

It is nearly always more economical and practical to prevent diseases than to attempt to control or eliminate them. For brucellosis, the measures of prevention include: Careful selection of replacement animals. These, whether purchased or produced from existing stock, should originate from *Brucella*-free herds or flocks.

Pre-purchase tests are necessary unless the replacements are from populations in geographically circumscribed areas that are known to be free of the disease. Isolation of purchased replacements for at least 30 days. In addition, a serological test prior to commingling is necessary.[77] Prevention of contacts and commingling with herds of flocks of unknown status or those with brucellosis. If possible, laboratory assistance should be utilized to diagnose causation of abortions, premature births, or other clinical signs. Suspect animals should be isolated until a diagnosis can be made.

Herds and flocks should be included in surveillance measures such as periodic milk ring tests in cattle (at least four times per year), and testing of slaughtered animals with simple screening serological procedures such as the RBT. Proper disposal (burial or burning) of placentas and non-viable fetuses. Disinfection of contaminated areas should be performed thoroughly.[4,104]

Cooperation with public health authorities to investigate human cases is necessary in Brucella prevention programme. Animal brucellosis, especially when caused by *B. melitensis*, can often be identified through investigations of cases in humans.

2.15.2 Control

The aim of an animal control programme is to reduce the impact of a disease on human health and the economic consequences. The elimination of the disease from the population is not the objective of a control programme and it is implicit that some "acceptable level" of infection will remain in the population. Control programmes have an indefinite duration and will need to be maintained even after the "acceptable level" of infection has been reached, so that the disease does not re-emerge. In many countries, methods for the control of brucellosis are backed by governmental regulation/legislation.[10] In others, no authorities exist. Therefore, the procedures for management of infected herds and flocks may vary widely. Nevertheless, certain principles apply, namely:

(1) The reduction of exposure to *Brucella* spp.

(2) The increase of the resistance to infection of animals in the populations. These procedures may be further classified under the general categories of test and isolation/slaughter, hygiene, control of animal movement, vaccination.[19,25]

2.15.3 Test and isolation/slaughter

There are no pathognomonic signs of brucellosis in animals at individual level; the occurrence of abortion storms in naive herds/flocks is usually a strong indicator of infection. Therefore, serological (and sometimes allergic) tests are the usual method of identifying possible infected animals. Bacteriological procedures are useful for confirming test results and for

epidemiological studies. The decision about slaughter of test-positive animals is made after regulatory, economic and prevalence factors are considered. In most cases, test and slaughter of positive animals is only successful in reducing the incidence if the herd or flock prevalence is very low (e.g. 2%). Retention of positive animals is less hazardous if the remaining animals have been vaccinated but should only be considered as a last resort. The isolation of test-positive animals is essential, especially during and after parturition.[7, 114]

The immediate slaughter of test-positive animals is expensive and requires animal owner cooperation. Compensation is usually necessary. Furthermore, the application of test and slaughter policies is unlikely to be successful with brucellosis of sheep and goats where the diagnostic tests are less reliable than in cattle. Test and slaughter is also unlikely to be successful in cattle if the remainder of the herd is unvaccinated, especially in large populations. Repeated herd or flock tests are necessary to further reduce the incidence of brucellosis and to confirm elimination.[96]

2.15.4 Hygiene

The goal in the application of hygiene methods to the control of brucellosis is reduction of exposure of susceptible animals to those that are infected, or to their discharges and tissues. This is a classical procedure in disease control. Factors such as the methods of animal husbandry (e.g. commingling of herds or flocks), patterns of commerce, prevalence of clinical signs, type of facilities, and degree of dedication of the owners of animals, will also determine success. Owners are often poorly informed about disease transmission and recommendations, such as separation of parturient animals, can be difficult or impossible to implement.[18]

Antibiotic treatment of known infected animals, or of those which are potentially exposed to them, has not been commonly used and it should be ruled out as an option in the control of brucellosis. A limited number of studies have shown rapid reductions in the incidence of brucellosis when the herd of flock was treated but this procedure is considered to be restricted in practice.

Treatment has been used in animals of special breeding value, but because of the uncertain outcome it is not generally recommended.[3,7]

2.15.5 Control of animal movement

This may be regarded as an aspect of hygiene. However, it is essential in any programme to limit the spread of brucellosis. Animals should be individually identified by brand, tattoo or ear tag. Unauthorized sale or movement of animals from an infected area to other areas should be forbidden. Similarly, importations into clean areas must be restricted to animals that originate from brucellosis-free areas, that have a herd/flock history of freedom from the disease and that have given negative reactions to recently performed diagnostic tests. In practice, it is much more difficult to control the movement of camels and small ruminants kept under nomadic or semi-nomadic conditions than that of beef or dairy cattle kept under intensive conditions. The owners of herds and flocks may be accustomed to seasonal migrations which may cross national boundaries.[11, 78]

2.15.6 Vaccination

There is general agreement that the most successful method for prevention and control of brucellosis in animals is through vaccination. While the ideal vaccine does not exist, the attenuated strains of *B. melitensis* strain Rev.1 for sheep and goats and *B. abortus* strain 19

have proven to be superior to all others. The non-agglutinogenic *B. abortus* strain RB51 has been used in the USA and some Latin American countries, with encouraging results.[64, 73] The source and quality of the vaccines are critical. The dosages and methods of administration, especially with Rev.1, vary and these can affect the results.

Consequently, whole herd or flock vaccination can only be recommended when all other control measures have failed. When applied, the vaccinated animals must be identified by indelible marking and continually monitored for abortions resulting from the vaccine. Positive serological reactors and secretors must be removed from the herd on detection.[18]

It is often recommended that vaccination with strains 19 and Rev.1 should be limited to sexually immature female animals. This is to minimize stimulation of post-vaccinal antibodies which may confuse the interpretation of diagnostic tests and also to prevent possible abortions induced by the vaccines. However, field and laboratory studies have demonstrated that conjunctival administration of these vaccines makes the vaccination of the herd or flock a practical and effective procedure. Rapid herd immunity is developed and application costs are minimized. The lowered dose results in lower antibody titres and these recede rapidly. Several diagnostic tests have been developed which are useful in differentiating antibody classes. Of these, the complement fixation test and ELISA are currently the most widely used.[40]

Vaccination of animals usually results in elimination of clinical disease and the reduction in numbers of organisms excreted by animals which become infected. Furthermore, animal owners are more likely to accept vaccination as a method of control since they are accustomed to this form of disease control. In many countries, vaccination is the only practical and economical means of control of animal brucellosis.

The worldwide trend towards more animal commerce and larger populations, along with limited resources, has made the control of brucellosis very difficult in many countries. Evaluation of the procedures used for the prevention and control of animal brucellosis should be performed. This should include surveillance of animals and humans and investigations of outbreaks.[40]

Procedures, including case definition and diagnostic tests, should be standardized and should be flexible enough to allow modification when new information becomes available.

2.15.7 Eradication

Eradication means the elimination of a pathogenic agent from a country or a zone (i.e. part of the territory of a country with a distinct animal health status). A highly organized effort is needed to reach eradication in either a territory and in a population. Eradication is conceptually very different from control: it is neither a casual nor an automatic consequence of a control programme, no matter how well planned and implemented the control programme is. It is based on sanitary measures and on an organization of activities completely different from those implemented for a control program.[97, 106]

Crucial factors for the success of an eradication programme are the implementation of an effective surveillance system with adequate laboratory support, and the understanding and sharing of objectives for eradication by the decision-makers, farmers, and all other stakeholders. To keep an unaffected population free from an infection, prevention measures must be implemented to segregate an infectious organism from a geographical area and its human and animal populations. Adequate knowledge of the local human and animal populations and of the territory is essential.[40]

On a long-term basis, eradication programmes in general are more economically advantageous compared to control programmes. This advantage, however, cannot always be translated into practice. In fact, an eradication programme involves the mobilization of an amount of resources (financial and human) that may not be available or whose returns for the investment may require a time span longer than any decision-making authority can afford. Cost-benefit and cost-effectiveness analysis can be used to support decisions on control strategies. However, no in-depth analysis is possible in absence of epidemiological surveillance. There is also little doubt that very often failures of control and eradication efforts are due to the absence of an adequate epidemiological surveillance system sustaining both technical and political decision-making.[107]

CHAPTER THREE

METHODOLOGY

3.1 BACKGROUND OF STUDY AREA

The study was conducted in Zaria town, Kaduna state, northern Nigeria. Zaria suburban area is a very large, heterogeneous city whose, 1.490,000 population and 85,626 household with an average household size of 9 persons, comes from different parts of the nation. It is second in size only to the State capital, Kaduna. Zaria is accessible from different parts of the country by air via Kaduna, Kano, and Abuja and by rail and road via Kaduna, Jos, Katsina, Kano and Sokoto. [133]

Zaria town, over the years, developed from two Local Government Areas (LGAs), Sabon-Gari and Zaria LGAs, (i.e. part of the town is in Zaria LGA and the other part in Sabon-Gari LGA).

The major occupation of the people in this area is Pastoralist agriculture (growing of both animals and crops). Many households also keep sheep, goats, and chickens that are usually reared free range within and outside the houses.

Zaria has a tropical continental climate with a pronounced dry season, lasting up to seven months (October - May). During the dry season, a cool period is usually experienced between November and February, and a brief period of hot but dry weather in March and April.[133]

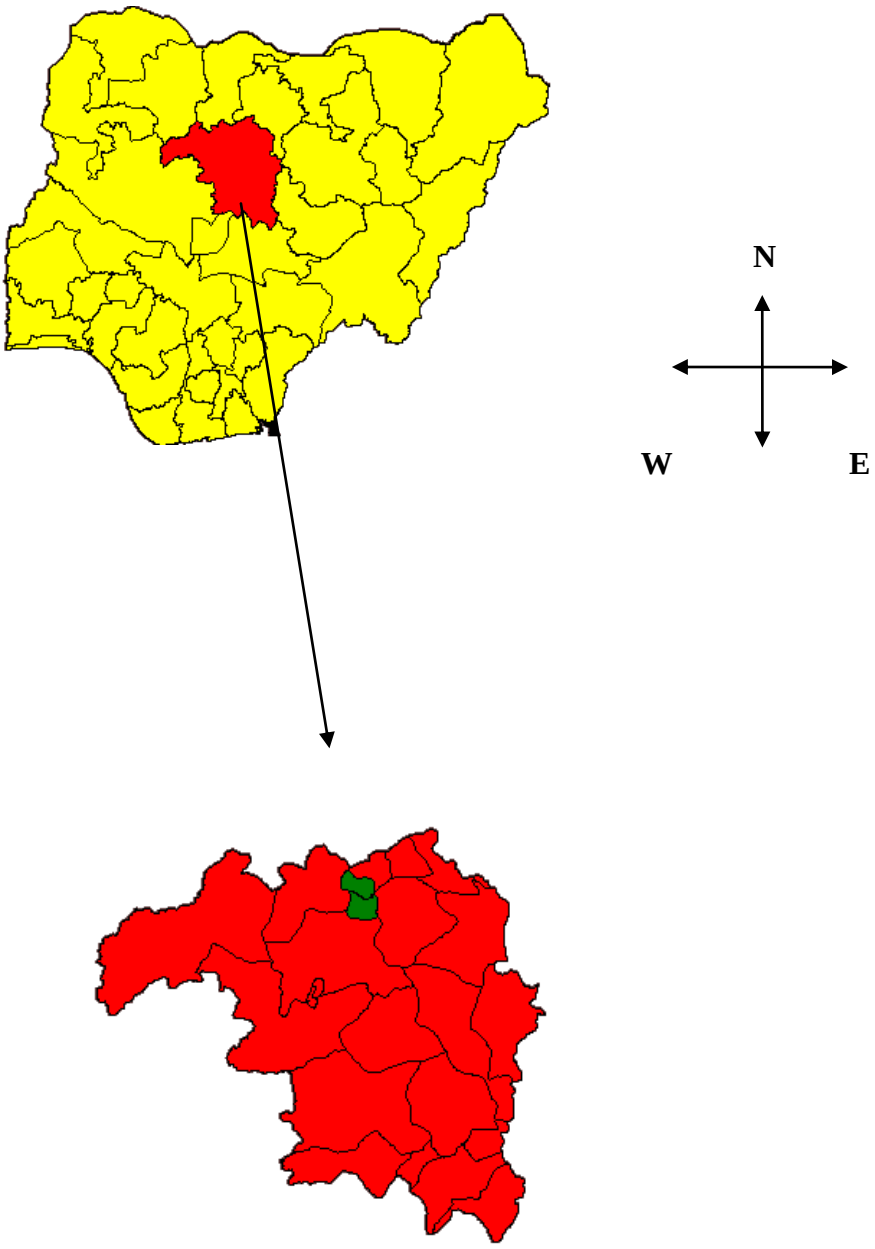

Figure 3.1: Map of Kaduna State highlighting Zaria Urban Town

3.2 STUDY DESIGN

A cross-sectional survey was carried out in Zaria town, Kaduna State, northern Nigeria, between February - April, 2013.

3.3 STUDY POPULATION

i. The study population consisted of sheep and goats drawn from homes that keep small ruminant (sheep and goats) in Zaria town.

ii. The animal owners whose animals were selected for the study in Zaria town.

3.3.1 Inclusion criteria

i. All sheep and goats, greater than 6 months old, kept in homes in selected areas of Zaria town.

ii. Owners of the recruited animals.

3.3.2 Exclusion criteria

i. Sheep and goats, kept in designated farms within Zaria town.

ii. Owners of animals who keep animals in designated farms in Zaria town.

3.4 Definition of some variables used in this study

a. **Ruminant:** Sheep and goats

b. **A house:** Is a building that functions as a home for humans ranging from simple dwellings such as rudimentary huts of nomadic tribes and the improvised shacks in shantytowns to complex, fixed structures of wood, brick, or other materials containing plumbing, ventilation and electrical systems.[132]

c. **Free range:** A system of keeping animals, where the animals are left to roam about looking for food

d. Semi Intensive: A system of keeping animals where the animals are kept with little restriction as they feed during the day (tying the animal at a specific spot to graze) before it is taken in to its housing.

e. Intensive System: This is a system of keeping animal where animals are kept permanently confined in houses or pens.

f. Good knowledge of brucellosis: When a respondent is able to give the name of the disease in a language he / she understands well and also give at least two (2) signs and symptoms (especially abortion, infertility, drop in production, still birth). This is graded as follows;

- Name of disease + two (2) signs and symptoms = 3 points
- Name of disease only = 1 point
- One (1) sign and symptom = 1 point

Therefore a person with three (3) points was said to have **good knowledge**

A person with point </ = 1 was said to have **Poor knowledge**

g. Good knowledge of zoonosis: When a respondent is able to describe zoonosis, as a disease that can be transmitted from animals to humans, with at least one (1) example.

3.5 SAMPLE SIZE

This was calculated using the formula for cross sectional studies[108]

Formula:

$$n = \frac{Z^2 \times p\,(1-p)}{d^2}$$

n = required sample size

z = confidence level at 95% (standard value of 1.96)

p = estimated prevalence of brucellosis 16.1% (0.161).[95]

d = margin of error at 5% (standard value of 0.05)

n = 206 samples

3.6 SAMPLING METHOD

A 3 level multi -stage sampling method was used to select animals for the study.

The four districts that make up Zaria town are; Birni da kewaye (Zaria walled city), Tudun Wada, Sabon Gari and Samaru. Each of these was considered as a stratum.

Stage 1: From a list of settlements (obtained from the Local Government Area profile) in each of the stratum, a sampling area (settlement) was randomly selected.

Stage 2: From each of the sampling area using the systematic random sampling technique, thirteen (13) houses were sampled from the list of houses that keep sheep and or goats (obtained from list developed from the census conducted by the study team prior - to the commencement of the study) for the household survey. A total of 52 households that reared sheep and or goats were selected.

Stage 3: From each selected house that reared sheep and goats; a systematic random sampling technique was used to select the animal. A maximum of four animals

were selected per house. The selection of sheep or goat was based on proportionate to size principle. Where only one breed was available, the sampling was carried out on that breed alone.

Selection of Animal Owners

For the assessment of knowledge and practice of animal owners, one animal owner was selected from each household and administered a questionnaire. Informed consent was taken from study participants (animal owners) before they were enrolled; refusal to participate was accepted.

3.7 Instrument for Data collection

A standardized questionnaire was used to obtain information on animal's exposure variables, knowledge of brucellosis, farm management and sanitary practices from the animal owners.

The questionnaire was pilot tested for its validity and reliability, among animal owners who rear sheep and goats in their house in Kaduna north area (outside of the study area). Changes were made to the final instrument following the comments from the animal owners. The initial part of the questionnaire comprised demographic information of animal owners such as occupation, age, gender, residence and educational level. The second part of the questionnaire comprised questions regarding animal characteristics such as species, breed, sex, age, number of animals. The third part comprised animal management, biosecurity and hygienic practice and other exposure factors such as contact with other animal, aborted material and proximity to waste disposal, while the fourth part assessed the knowledge, hygiene and sanitary practices related to brucellosis. Before administration of the questionnaire, the purpose of the study was explained to each respondent and confidentiality of the information was assured.

3.8 TRAINING OF RESEARCH ASSISTANTS:

Research assistants, from the Zaria zonal office (Livestock Department) of the State Ministry of Agriculture were given orientation on sample collection and administration of questionnaire for data collection.

3.9 COLLECTION AND ANALYSIS OF SAMPLES

Five (5) mls of blood sample was collected from each animal. A new needle and blood tube was used for each animal. The samples were centrifuged at 1000rpm for 20 minute to separate the cells from the serum. The serum samples were transported under cold chain (using Ice packs and clod boxes). The serum samples were stored, frozen at - 20^0C until required for analysis.

3.9.1 Laboratory Analysis

The laboratory analysis was conducted at the National Veterinary Research Institute, Vom. This included; the Rose Bengal Plate Test and the Serum Agglutination Test.

The 208 samples collected were run first using the Rose Bengal Plate Test (RBPT), then secondly all was run using the Serum Agglutination Test (SAT). The results were recorded accordingly.

3.9.1.1 Rose Bengal Plate Test (RBPT)

Principle

It uses the principle of agglutination on plates in a buffered acidified medium (pH 3.6) which makes it possible to eliminate non-specific agglutinations. The use of a stain

(Rose Bengal) makes it easier to demonstrate the presence of agglutinates (testing procedure in appendix 4).

Result Validation

Results of the controls were checked to ensure that results are in accordance with the standard.

Result Interpretation

Where there is no agglutination, it indicates absence of antibodies (Negative result) and where there is any agglutination, it indicates the presence of antibodies (Positive result)

3.9.1.2 Serum Agglutination Test (SAT)

Principle

The tube agglutination test is usually used in the semi-quantitative test. Known particle antigen is mixed directly with serial dilution of diagnostic serum in the tubes. Observe the agglutinates in the tubes after certain time. The result was then read and agglutination titers determined (testing procedure in appendix 4).

Result Interpretation:

Titter of 1:40 (50 IU/ml) and above were taken as diagnostic for brucellosis.[107]

NOTE:

i. Positive and negative controls were also run alongside the samples to validate the results.
ii. For this study a sample is recorded positive if positive by RBPT and SAT.

3.10 DATA MANAGEMENT

Data were collected using pre-tested structured questionnaires and laboratory result sheets.

All data from the survey were double-entered into Epi-Info 3.5.3 and Excel Microsoft spread sheet to ensure accuracy in data entry.

3.11 DATA ANALYSIS

Descriptive statistics was used for means, frequency, proportion of both animal owners information and animal characteristic such as age, sex, occupation, etc. Analytical analysis was used to compare the outcome (dependent) variable and independent variables. All variables were dichotomized. Univariate analysis was used to compare sero-positive and sero-negative animals for demographic, environmental, management, and animal exposure variables. The risk factors were subjected to bivariate analysis, Fisher exact test was used to measure the association between the risk factors and the laboratory result.

3.12 ETHICAL CONSIDERATIONS

Ethical clearance was obtained from the Kaduna State Ministry of Agriculture and permission from each animal owner, before bleeding the animals.

All individuals identified to have infection(s) were referred to see a Veterinary doctor for appropriate treatment and management.

3.13 Limitation

This study could not look at the prevalence of the disease among other animals and humans. It also did not isolate or speciate the Brucella organism.

CHAPTER FOUR

RESULTS

A total of 52 structured questionnaires were administered to the animal owners that kept sheep and goats in their home in Zaria town, Kaduna State. Out of the 52 respondents, 80.7% of them were males, 55.8% were between 30 to 40 years of age and 32.7% had no formal education. Of the respondents sampled 44.2% were farmers while 72.3% of them practiced the free range system of farming (Table 4.1).

Table 4.1: Socio - demographic characteristics of animal owners who keep sheep and goat at homes in Zaria town (n=52)

Characteristics		Freq. (n= 52)	% of Respondents
Sex			
	MALE	42	80.7
	FEMALE	10	19.3
Age (Years)			
	<10	0	0
	10 – 20	2	3.8
	>20 – 30	10	19.2
	>30 – 40	29	55.8
	>40	11	21.2
Educational Status			
	No Formal Education	17	32.7
	Primary	7	13.5
	Secondary	21	40.3
	Tertiary	7	13.5
Occupation			
	Farmer	23	44.2
	Trader	9	17.3
	Civil Servant	9	17.3
	Others	11	21.2
Management system			
	Free Range	27	52
	Semi Intensive	23	44.2
	Intensive	2	3.8

Of the 52 participants interviewed only 11 (21.2%) had good knowledge of zoonosis while only 5 (9.6%) had good knowledge of causes of zoonosis. Furthermore, 45 (88.5%) of the respondents had good knowledge of brucellosis while 42 (80.8%) had poor knowledge of transmission of *Brucella* from animal to humans. Thirty – four (65.9%) of the respondents said transmission of diseases from animal to humans was by contact while 9 (17.3%) didn't know the mode of transmission of Brucella (Table 4.2). Of the total participants 42 (80.8%) believed transmission of Brucellosis from animals to humans could be prevented through animal vaccination.

Table 4.2: Table on knowledge of animal owners with regards to zoonosis (n=52)

Variable	Freq.	Percent response (%)
Knowledge of Brucellosis		
Good Knowledge	46	88.5
Poor knowledge	6	11.5
Knowledge of transmission of Brucella from animals to Humans		
Yes	10	19.2
No	42	80.8
Knowledge of mode of transmission of animal diseases		
Contact	34	65.9
Drinking contaminated water	7	13
Bite	2	3.8
I don't know	9	17.3
Transmission of Brucellosis from animals to humans can be prevented		
Yes	42	80.8
No	10	19.2
Animal vaccination is important in the prevention of brucellosis		
Yes	42	80.8
No	10	19.2
Knowledge of Zoonosis		
Good Knowledge	11	21.2
Poor knowledge	41	78.8
Causes of Zoonotic diseases		
Good Knowledge	5	9.6
Poor knowledge	47	90.4

Of the total respondents, 48 (92.4%) keep the animals for income, 30 (57.7%) keep sheep only while 38 (73.0%) sourced their animals from the market. In housekeeping practices, 46 (88.5%) carried out only physical cleaning of their animal house, 1 (1.9%) washed with water and disinfectant. Less than half 24 (46.2%) do the housekeeping weekly (Table 4.3). When an animal is sick 22 (42.3%) of the respondents invite veterinary officers, 22 (42.3%) carry out self-medication and 8 (15.4%) cull and sell the sick animal. On disposal of animal abortus, 40 (76.9%) dispose of it in open waste bins while 12 (23.1%) dispose by burial.

Table 4.3a **Practice of animal owners with regards to animal keeping (n=52)**

Characteristics		Freq (n=52)	% of respondents
Purpose of keeping animal			
	Income	48	92.4
	Food	2	3.8
	Festive	2	3.8
Animal reared			
	sheep only	30	57.7
	Goat only	8	15.4
	Both	14	26.9
Source of animal			
	Market	38	73
	Farm	11	21.2
	Others	3	5.8
Housekeeping practices			
	Physical cleaning	46	88.5
	Washing with water only	4	7.7
	Washing with water and soap	1	1.9
	Washing with water and disinfectant	1	1.9
Frequency of housekeeping			
	Daily	15	28.8
	Weekly	24	46.2
	Monthly	1	1.9
	Anytime	12	23.1
Practices when animals are sick			
	Invite a Vet	22	42.3
	Self-medication	22	42.3
	Cull/ Sell	8	15.4
Disposal of animal abortus			
	In open waste bin	40	76.9
	Burial of abortus	12	23.1
Distance of house to waste bins			
	Closer (<200m)	29	55.8
	Far away (>200m)	23	44.2

Out of the 208 animals sampled, 171 (82.2%) were females and 156 (57.2%) of all animals were sheep. Age group 25 – 36mths had the highest frequency 79 (37.9%) while the age group >48mths had the least frequency 5 (2.5%), as shown in table 4.4 below.

Table 4.4: Demographic distribution of animals sampled in Zaria

Variable		Goat (n=89)	Sheep (n=119)	Total
Sex	Female animals	73 (82.0%)	98 (82.4%)	171
	Male animals	16(18.0%)	21 (17.6%)	37
Age	6mths – 12mths	6 (6.7%)	12 (10.1%)	18
	13mths – 24mths	30 (33.7%)	44 (37.0%)	74
	25mths – 36mths	35 (39.3%)	44 (37.0%)	79
	37mths – 48mths	15 (16.9%)	17 (14.3%)	32
	>48mths	3 (3.4%	2 (1.7%)	5
Sampling Areas	Sabon-Gari	22 (24.7%)	30 (25.2%)	52
	Samaru	35 (39.3%)	17 (14.3%)	52
	Wusasa	12 (13.5%)	40 (33.6%)	52
	Zaria City	20 (22.5%)	32 (26.9%)	52

Of the two species, the sero-prevalence of *Brucella* was higher in goats (17.9%) than in sheep (15.9%). Of the total 208 animals sampled, 35 (16.8%) were sero-positive for Brucella and 27 (77.1%) of those positive, were females. Samaru area had the highest sero-prevalence of 40.0% while Sabon Gari had the lowest sero – prevalence of 8.6%. The sero-prevalence of the disease among animals differed across various age groups studied (Table 4.5). Of the four (4) breeds sampled, 81 (39%) were Yankasa, 79 (38%) were Red Sokoto, 36 (17%) were Balami and 12 (6%) were Uda.

Table 4.5: Sero-prevalence of Brucellosis in sheep and Goats kept at homes in Zaria. (n= 208)

Variable		Number + VE	Number - VE	Sero - Positivity (%)
Specie	Goat	16 (45.7%)	73 (42.2%)	**17.9**
	Sheep	19 (54.3%)	100 (57.8%)	15.9
Breed	Yankasa	10 (28.6%)	70 (40.5%)	12.5
	Red Sokoto	16 (45.7%)	64 (37.0%)	20.0
	Balami	17 (20.0%)	29 (16.8%)	47.2
	Uda	2 (5.7%)	10 (5.8%)	16.6
Age	6mnths – 12mths	4 (1.4%)	14 (8.1%)	22.2
	13mths - 24mths	12 (34.3%)	62 (35.8%)	16.2
	25mths – 36mths	14 (40.0%)	65 (37.6%)	17.7
	37mths – 48mths	4 (11.4%)	28 (16.2%)	12.5
	48mths	1 (2.9%)	4 (2.3%)	20.0
Sex	Male	8 (22.9%)	29 (16.8%)	21.6
	Female	27 (77.1%)	144 (83.2%)	15.7
Sampling Areas	Sabon-Gari	3 (8.6%)	49 (28.3%)	5.7
	Samaru	14 (40.0%)	38 (22.0%)	26.9
	Wusasa	6 (17.1%)	46 (26.6%)	11.5
	Zaria City	12 (34.3%)	40 (23.1%)	21.1

On the bivariate analysis, separating sick animals from the healthy ones was the only factor significantly associated with brucellosis infection (P-value <0.05).

Furthermore, 26 (74.2%) of the animals that were sero-positive had contact with other animals but the association between contact with other animals and brucellosis infection was not statistically significant (P-value >0.05) as in Table 4.6.

Table 4.6: Analysis of Risk Factors associated with transmission of Brucellosis in sheep and goats kept at homes in Zaria

Variable	Sero Positive	Sero Negative	Odds Ratio	95% C.I	P-Value
Management					
Free Range	35	171	0.81	0.01 – 0.43	0.44
Semi Intensive	0	2			
Contact with other animals					
There is Contact	26	150	0.44	0.18 -1.06	0.06
No Contact	9	23			
Specie					
Goat	16	73	1.15	0.55 - 2.39	0.84
Sheep	19	100			
Sex					
Male	8	29	1.47	0.61 - 3.56	0.39
Female	27	144			
Source of Animal					
Market	29	130	1.59	0.62 - 4.11	0.33
Farm	6	43			
Separated from sick ones					
Separation from sick	30	115	3.03	1.12 - 8.21	0.02
No Separation	5	38			

***** Zero point five (0.5) was added to the cell with zero (0) to enable us calculate the level of association involved with the management system being practiced.

CHAPTER FIVE

5.1 DISCUSSION

This study shows that the animal owners have good knowledge of zoonosis and causes of zoonosis. Furthermore, though they have good knowledge of the disease, brucellosis, their knowledge of prevention of transmission of brucella from animals to humans is poor, this is similar to the findings of Bertu,[95] Unger,[110] Bale,[95] Okoh [16] and also similar to the findings in Tajikistan.[112] Furthermore, the study shows that sick animals are usually separated from the entire herd, this differs to the findings of Ocholi ,[111] Unger [107] in four West African countries.

Of the small ruminant animal species (sheep, goats, rabbit and antelope) reared in homes in Zaria, sheep and goats are the majority, however, there were more sheep than goats as at the time of this study. This may be due to season of festivity and the traditional value attached to sheep in northern Nigeria. Majority of the sheep and goats being reared were female, most likely for reproductive purpose. Most of the houses practice free-range system of farming, where animals freely roam in search of feed because it is cheaper. This is common among the poor communities where majority are low income earners. Many keep the animals for income. Only few animal owners seek veterinary services, this may be as a result of poor knowledge of animal keeping.

Seventy-six point four percent (76.4%) of the farmers source for their animals from the market, this is also a predisposing risk factor for the transmission of brucellosis, this may constitute the reason why 35 (85.4%) of the sero-positive animals are from the market.

In Nigeria brucellosis is an endemic disease and occurrence of infection in humans and animals has been documented by several workers.[23,38,95,96,107] Although, the public health

implication of the disease has been highlighted by earlier workers, cases continue to occur in our communities particularly in individuals working closely with livestock. There is evidence that the disease is on the increase[23,25] despite the effort of the government to control it.

From this study, the prevalence of brucellosis among small ruminants (sheep and goats) reared in homes in Zaria was 16.8%. This compares with studies in Plateau State, Nigeria, where they had a prevalence of 16.1%. The study also shows that all the animals that were sero-positive are from those reared under the free range system of farming and this may constitute risk in the transmission of zoonotic disease in the area. It is also similar to the findings of Bertu et al.[98] Sero -positivity is higher in goats than in sheep but it is not statistically significant (>0.05). This is contrary to that found in Sokoto which shows that prevalence is higher in sheep (22.9%) by Junaidu,[130] in Ibadan (0.9%) by Cadmus [128] and in Portugal (8.9%) by Coelho.[129] This could be as result of difference in management system in use.

Furthermore, the study shows that the sero-positivity of brucellosis among sheep (15.9%) and goats (17.9%), this is similar to that found in Plateau Sate.[20]

The study shows that the people who keep small ruminants were mostly farmers with low education background. This low education may directly or indirectly contribute to the risk of transmission especially people that share the same dwelling space with their animals. There are more male (80.7%) that takes care of the animal which is similar to the findings of Breu.[101]

5.2 CONCLUSION

In conclusion, this study shows that there is *Brucella* infection among sheep and goats kept in homes in Zaria town with a prevalence of 16.8%. Its prevalence is higher in free ranged animals.

Furthermore, the study reveals that there is a relatively poor knowledge of mode of transmission and prevention of brucellosis among the farmers. The animal owners may be at risk of the infection.

5.3 RECOMMENDATIONS

These recommendations are made, based on the findings of this study:

5.3.1 The department of Agriculture at LGA, state level and Non-Governmental organizations (NGOs) should;

1. Create awareness among animal owners, especially those who keep animals at home, on zoonosis. This can be done through sensitization meetings, radio, hand bills, worship centers, and other social gatherings.
2. Create awareness on good hygiene practices that are necessary for prevention and control of zoonosis.
3. Increase awareness on the need to vaccinate animals for the prevention and control of Brucella.
4. Veterinary extension workers should play a major role by sensitizing the animal owners on the need to apply sanitary procedures and measures as they manage their animals.

5.3.2 The State and Federal Ministry of Agriculture should;

1. Discourage free range system of farming to reduce the risk of transmission of Brucella and other zoonosis.
2. Collaborate with other stakeholders should ensure the availability of vaccine against brucellosis to farmers.
3. Conduct survey in the state using serology, cultural isolation, and molecular characterization methods. Information from this may help in the production of vaccine and the control of brucellosis in the state and country at large.

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APPENDIX 1

INFORMED CONSENT FORM [*English Language*]

Good day Sir/ Ma, My name is Badung Samuel Yohanna, a student of Ahmadu Bello University, Zaria.

I am conducting a study that requires me asking animal owner questions about their sheep and goats reared in their houses. This is mainly for research purpose.

I assure you that all your responses will be treated with utmost confidentiality. Your participation in the study is voluntary and you may decide not to participate if you wish. However, your participation in the study will be highly appreciated.

Do you consent to participate in the study? Yes ☐ No ☐

Signature/Thumbprint of Respondent…….

Date of consent…….

Thank you.

NEMAN SANI *[Hausa Language]*

Salammu Allekum. Sunana Badung Samuel Yohanna. Ni Dalibi ne na Jami'ar Ahmadu Bello, Zaria.

In na gudanar da binchike ne wadda yake neman amsar tambayoyi daga masu kiwon dabobi . Ina neman sanin yadda kake aiwatarda kiwon awaki da tumaki a gadan ka ne. Wannan binciken domin neman sani ne kawai, kuma nayi alkawalin boye dukan wani siri da aka gaya ma ni. Kasancewar ka a wannan binchike ba dole ba ne, sai dai muna fatar goyan bayanka domin zai taimaka kwarai.

Ka yarda ka kasance a wanan bincike? I ☐ A a ☐

Sa hanun wanda aka tambaya ……. Rana……

Mungode.

APPENDIX 2

QUESTIONNAIRE

Section A: Animal owner information

1. Name …… …… 2. Phone No……

3. Address/District……

4. Gender a. Male b. Female

6. What is your educational level? a. Primary b. Secondary c. Tertiary d. No formal education

7. Type of housing a. Cemented house b. Mud house c. others (specify)……

8. What type of floor surface? a. cemented b. non cemented

9. How old are you ? a. Less than 20yr b. >21-30 yr c. 30-40yr d. >40yrs

10. What is your primary occupation a. Farmer b. Civil Servant c. Trader

d. Others (specify)……..

11. Which of the animal do you keep? a. Sheep only .b. Goat only c. both

d. others (specify)…….

Section B: Animal profile

12. What type of breeds of sheep do you have? a. Yankasa b. Balami c. Uda.

d. others (specify)……

13. What type of breeds of goats do you have?

a. Red sokoto b. West African dewarf c. Red kano d. others (specify)……

Section C: Management, Biosecurity and Hygiene Practices

14. What type of management do you practice? a. Free range b. Semi intensive

c. Extensive

15. What is your purpose of keeping the animal? a. Meat b. Income c. Breeding

d. Festive d. Others……..

16. Where do you keep the animals? a. Unroofed housing b. Roofed housing

c. (Other specify)……

17. What are your sources of water for the animal?

a. Well b. River c. Pipe borne water d. Others (specify)…….

18. Do you separate sick animal from healthy? Yes /No

19.Do you separate new animal from old animals? Yes/No

20. Do you allow the animal to search for feed? YES/No

If Yes a. Eating/drinking out in waste disposal site

b. Eating/drinking out near abattoir area c. Eating /drinking out near poultry house

21. Do you have other animals in contact with the sheep and goat? YES/No

22. What is the usual source of your animals? A. market b. village c. farm. d. friends e. other (specify)……

23. How regular do you carry out the sanitary practice where you keep the animals?

24. Kindly tick the options below

a. Physical clean e.g. sweeping

I. Daily ii weekly iii. Monthly iv. Anytime v. not often

b. Wash with water only

I. Daily ii weekly iii. Monthly iv. Anytime v. not often

C. Wash with disinfectant

I. Daily ii weekly iii. Monthly iv. Anytime v. not often

Section D: Health History

25. In this present flock What disease symptoms do you often see in the animals?

a. Abortion b. Still birth c. Red urine d .infertility e. Others (specify)……

26. How do you dispose abortion/ still birth materials?

A.dispose in open waste bins b. By burying c. By both a and b d. Others (specify) .....

27. What is the distance of your house to the waste disposal site?

A. Closer to house (<200meters) B. Far away from house (>200meters)

28. When you see any of the symptoms above what do you do?.

a. Consult a Vet. b. Self medicated c. Cull/sold d .Slaughter d. Do nothing e. Other specify.....

Knowledge of zoonotic disease

29. What is a zoonotic disease?

a. Disease of animal only b. Disease of animal that can be transmitted to humans c. Disease that affect humans only d. Others (specify) .....

30. What are cause(s) of the zoonotic diseases?

a. Bacteria b. Viruses c. Parasite d. I don't known e. others (specify).....

29. What are the symptoms of Zoonotic diseases in humans?

a. Death b. Abortion c. Fever d. Diarrhea e. Vomiting e. I don't know

30. Which of these diseases can be transmitted from sheep/ goats to human?

a. Brucellosis b. Tuberculosis c. Rabies d. I don't know e. Others (specify).....

31. What is brucellosis?

32. What are the signs and symptoms of brucellosis?

a. still birth b. Abortion c. Drop in production d. Infertility e. I don't know f. Others (specify) .....

33. What is the mode of transmission of brucellosis from sheep/goat to human?

a. contacted with infected animal b. Bite c. Through drink un-boiled milk d. I don't know e. Other (specify).....

34. Do you think the brucellosis can prevented. a. Agree b. Disagree c . I don't know.

35. Do you think it necessary to consult Vet doctor to examine you animals for any diseases? Yes/No

36. Do you think it is necessary to visit a hospital if you have fever that fails to respond to treatment ? Yes /No.

37. Is personal protection very important in the prevention of zoonotic diseases? Yes/No

38. If Yes why? .....

APPENDIX 3

Specimen Collection using Vacutainer

1. Remove the clear plastic cover from the bottom of the needle. Insert the bottom end of the needle through the small, threaded opening of the needle holder and twist to lock in place. The tip of the needle (with colored cover) should be outside the holder as shown in the image, at right.
2. Restrain the animal. Locate the jugular vein in the neck. With an alcohol pad, disinfect a blood collection site. Note: Shaving the area, applying pressure 3/4th of the way down the neck and turning the head to one side may help in locating the vein.
3. Remove the plastic cover from the needle tip. Insert the exposed needle into

   the tail vein.

4. With the needle in the vein, push the blood tube inside the needle holder and onto the bottom end of the needle with the heel of your hand. If the needle has punctured the vein, blood should flow into the tube.
5. Fill the tube until blood stops flowing near the top of the tube. At least 2-cc of blood must be collected to complete the test.
6. If it is necessary to remove the needle from the animal to re-position, first remove the blood tube from the end of the needle in the holder. If not, the blood tube will lose vacuum making it impossible to draw blood into the tube.
7. After collection, remove the needle from the animal. Dispose of it properly by covering and discarding in a biohazard container approved for disposal according to local, regional and national regulations.

   Label the tube immediately with the tube number and the animal's identification number.

APPENDIX 4

ROSE BENGAL PLATE TEST (RBPT)

PROCEDURE

i. The antigen and sera were kept 30 to 60 minutes at room temperature 21^{o}C before the beginning of the tests.

ii. On a plate, 30µl of each serum to be tested was placed.

iii. The antigen vial was mixed gently and 30µl was placed besides each serum.

iv. The antigen and the serum were carefully mixed together using an applicator stick.

v. The plate was rocked gently for exactly 4 min and read immediately.

***Note**: In order to standardise the reading, it is better to introduce as control for each series of analysis a positive control serum for Brucellosis and a known-negative serum sample.*

SERUM AGGLUTINATION TEST (SAT)

PROCEDURE:

The stock concentrated *Brucella abortus* S19 antigen to be used was diluted 1:10 before use.

I. Ten (10) sterile test tubes were arranged and labeled accordingly on a test tube rack.

II. 0.8ml of the diluent (0.5% Phenol saline) was dispensed in to the first (1st) test tube. Then 0.5ml of the diluents was dispensed into the rest of the tubes.

III. 0.2ml of the serum was dispensed into the first tube and mixed thoroughly with the diluents. 0.5ml of the mixture in the first tube was then transferred in to the 2nd tube and it was mixed. Again 0.5ml of the mixture was transferred into the 3rd tube. This doubling

dilution continued until the last tube where the final 0.5ml taken from it was discarded. This resulted in dilution titers of 1/5, 1/10, 1/20, 1/40, 1/80, 1/160, 1/320 and so on.

IV. 0.5ml of the diluted Brucella antigen (1:10) was added to all the tubes and rocked to ensure thorough mixing.

V. The tubes were then covered and incubated at 37^0C for 24 hours.