A HOSPITAL-BASED SURVEY ON HUMAN BRUCELLOSIS AND RISK FACTORS IN KANO METROPOLIS

BY

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DECLARATION

I declare that the work in the dissertation entitled "A Hospital-based survey and sensitivity of clinical diagnosis of Human Brucellosis and risk factors in Kano Metropolis" has been performed by me in the Department of Veterinary Public Health and Preventive Medicine under the supervision of Drs E. C. Okolocha and J. Kabir.

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

Isa Aisha Nasiru _____ _____

Signature Date

CERTIFICATION

This dissertation entitled "A HOSPITAL-BASED SURVEY AND SENSITIVITY OF CLINICAL DIAGNOSIS OF HUMAN BRUCELLOSIS AND RISK FACTORS IN KANO METROPOLIS" by Isa, Aisha Nasiru meets the regulations governing the award of the degree of Master of Public Health of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To the Nasiru Isas and Kabiru Kazaures for the love and support.

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ABSTRACT

Introduction/Background: Brucellosis is a zoonosis of major public health significance that occurs worldwide in both man and animals.

Human brucellosis is a severe debilitating disease that requires prolonged treatment, if untreated can result in permanent disability and loss of productivity. Human brucellosis presents a great variety of clinical manifestations making it difficult to diagnose clinically. In some endemic areas every case of human fever of unknown origin is assumed to be due to brucellosis. Therefore, the diagnosis must be confirmed by laboratory tests.

A study was conducted in Kano State, North-western Nigeria to determine the sensitivity of clinical diagnosis of human brucellosis and determine its risk factors of among hospital patients presenting to some selected hospitals with febrile illness.

Methods: We conducted a cross-sectional descriptive study from September to December 2011. One major hospital was selected from each of the five local government areas within Kano metropolis.

Two hundred and fifty participants were enrolled in the study. Data was obtained using a standardized questionnaire and blood samples were collected, screened with Rose Bengal Plate Test (RBPT) and confirmed for anti-Brucella immunoglobulin G (IgG) and anti-Brucella immunoglobulin M (IgM) using Enzyme-linked immunosorbent assay (ELISA).

Results: There were 50 samples that tested positive to RBPT and were confirmed by ELISA.

Of the 50 positive cases, 36 (64%) were positive by IgG where as 14(28%) were positive by IgM.

Independent risk factors for human brucellosis were consumption of fresh milk (p 0.02) and kindirmo (p 0.00); keeping goats (p 0.00), assisting animal parturition (p 0.01), processing raw meat (p 0.03) and eating raw meat (p 0.00). Clinical signs that had strong association with human brucellosis were recurrent fever (p 0.04), cough/sneeze (p 0.02) and osteomyelitis (p 0.00).

Conclusion: Significant risk factors for Human Brucellosis in Kano were consumption of fresh milk and kindirmo(local yoghurt), processing and consumption of raw meat, keeping goats and assisting animals' parturition. The government should sensitize the populace on the risks and importance of this disease and physicians should raise the index

for diagnosis of human brucellosis by including it in their differential diagnosis in patients that present with signs suggestive of it such as recurrent fever, cough/sneezing, joint pains, and osteomyelitis.

Key words: Brucellosis, Risk factors, Hospital, Kano.

CHAPTER ONE

INTRODUCTION

1.1 Background

Brucellosis is a bacterial disease caused by species of the genus Brucella [as] an important zoonosis and a significant cause of reproductive losses in animals. [The disease] usually caused by *Brucella abortus* in cattle, *B. melitensis* or *B. ovis* in small ruminants, *B. suis* in pigs and *B. canis* in dogs [has also been discovered] in sea mammals, in many species of pinnipeds and cetaceans.[i] [This] discovery of the Brucella in marine mammals changed the concept of a land-based distribution of brucellosis and associated control measures.[ii] Brucella species are also maintained in wildlife populations such as feral pigs, bisons, elks and European hares.[iii]

Brucellosis is an infectious zoonotic disease that is associated with chronic debilitating infections in humans and reproductive failure in domestic animals.[iv] Brucellosis is also considered as an occupational, laboratory and travel-acquired disease.[v]

The risk of disease and its severity is to a significant extent determined by the specie of Brucella to which an individual is exposed.[vi] Food animals are the major reservoirs for the disease. Nigeria's food animals' population is estimated at 15.2 million cattle, 23 million sheep and 28 million goats.[vii]

Human brucellosis presents a great variety of clinical manifestations making it difficult to diagnose clinically. In some endemic areas every case of human fever of unknown origin is assumed to be due to brucellosis. Therefore, the diagnosis must be confirmed by laboratory tests.

Brucellosis is a multisystemic, acute to chronic, disease characterized by fever, headache, joint pains, musculo-skeletal pains, sweating, malaise and body wasting. Because of these rather non-specific signs, this has caused tremendous problems with the clinical

diagnosis of brucellosis in sub-Saharan Africa, where it is constantly mis-diagnosed as malaria, which is very prevalent.[viii,ix] The incubation is normally 1–3 weeks, but it can be several months before showing signs of infection.[x]

Brucellosis occurs worldwide in both man and animals. Brucellosis-free status has been granted by the European Union (EU) to Sweden, Denmark, Finland, Germany, the UK (excluding Northern Ireland), Austria, Netherlands, Belgium, Luxembourg, Norway and Switzerland. [xi] Mediterranean region and Arabian Peninsula are acknowledged endemic regions of human brucellosis.[xii]

Several studies[xiii,xiv,xv,xvi,xvii,xviii,xix,xx] confirmed that;

Brucellosis exists in the whole of Africa in both human and animals; however very little is known about its prevalence especially in sub-Saharan Africa. Available evidence, from sub-Saharan Africa, supports the hypothesis that transmission to humans is primarily from livestock reservoirs of brucellosis. Thus, the risk to humans is a function of the risk in livestock and the human–livestock effective contact rate.

Human brucellosis is a severely debilitating disease that requires prolonged treatment with a combination of antibiotics leaving permanent and disabling sequel, and results in considerable medical expenses in addition to loss of income due to loss of working hours.[xxi,xxii,xxiii]

The interest in brucellosis has been increasing because of the growing phenomena of international tourism and migration, in addition to the potential use of Brucella as a biological weapon. The global burden of human brucellosis remains enormous; it causes more than 500,000 infections per year worldwide.[xxiv]

Brucellosis is a serious public health problem in many developing countries and in regions of high endemicity such as Africa, the Mediterranean, the Middle East, parts of Asia and Latin America.[xxv]

In Africa, brucellosis remains an uncontrollable public health problem due to the absence of national surveillance programs, diagnostic facilities and reliable data.

In Nigeria, evidence of human Brucellosis has been documented from South west, North East and North Central regions.[xxvi] In Kano, some studies have been carried out on the prevalence of Human Brucellosis but no available evidence on the study of risk factors associated with the disease.

1.2 Statement of the problem

Cases of brucellosis very often remain unrecognized and are treated as other diseases often labeled "fever of unknown causes".[25]

Despite the fact that brucellosis is being identified from several reports in various species of livestock in Nigeria with Serological prevalence rate of between 0.20% and 79.70%,[xxvii,xxviii, xxix] there are few studies to investigate the occurrence of the disease in humans.

In Nigeria, like in several other developing countries there is an intimidate relationship between livestock, households and livelihoods. Often people live with their livestock including cattle, sheep and goats in their homes. It is common for some of these animals to freely roam around and thus contaminate the environment with zoonotic pathogens when infected increasing the chances of transmission to humans particularly in places of high human population density like Kano metropolis.

There is no deliberate brucellosis control program in animals in Nigeria, so the extent of its occurrence in animals in also unknown, so also the rate of infection in humans due to incidental and occupational exposure is not properly quantified.

The relative risks due to foods of animal origin (such as milk and meat) and occupational exposure is not fully understood, therefore control strategies may not be risk-based. Similarly, the impact of HIV/AIDs on the epidemiology of the disease in Nigeria has not been demonstrated in view of the fact that it is a chronic disease due to an intracellular bacterium. Brucellosis may be misdiagnosed as Malaria/Typhoid fever, thus the disease is under reported.

1.3 Justification

Investigating human brucellosis in the hospital will assist in bridging the gap in knowledge on the risk factors associated with human brucellosis and the extent to which it is misdiagnosed in hospitals. It is desirable to provide baseline data that will assist in raising the awareness of the health sector to prioritize and develop a mechanism for control and prevention of the disease.

Brucellosis is reportedly a wide spread disease in Nigeria.[xxx,xxxi,xxxii,xxxiii]

Apart from the grave zoonotic consequences, the economic losses associated with the disease are enormous.' It is recognized as a one of the 7 neglected tropical zoonoses by the WHO. It is endemic in Nigeria and evidence of infection as well as frank outbreaks have occurred in cattle,[xxxiv] sheep and goats; [xxxv] and humans.[xxxvi,xxxvii,xxxviii]

Brucellosis in goats has been reported in various parts of Nigeria.[xxxix]

The extent of contact of humans and animals in Nigeria is intimate such as backyard rearing of small ruminants, free grazing, unrestricted movement and trade of animals. General practice of consumption of raw meat amongst butchers, and raw milk amongst larger society is also widespread.

Familiarity with the manifestations of brucellosis and the optimal laboratory studies is essential for physicians to recognize this re-emerging zoonosis.[24]

Long duration of treatment and high treatment failure in human also contributes to lack of attention and priority given to the disease.

1.4 Research Questions

How sensitive is the clinical diagnosis of human brucellosis based on serology in Kano state?

What are the risk factors associated with human brucellosis in Kano state?

1.5 General and Specific Objectives

1.5.1 General Objective

The study aims to determine the extent of missing diagnosis of human brucellosis in hospitals and also identify risk factors associated with the disease.

1.5.2 Specific Objectives

To determine the proportion of human brucellosis in patients with recurrent fever.

To determine the sensitivity of clinical diagnosis of human brucellosis.

To identify the factors associated with brucellosis in humans.

CHAPTER TWO

LITERATURE REVIEW

History/Introduction

Brucellosis is an ancient disease that can possibly be traced back to the 5th plague of Egypt around 1600 BC.[xl] Malta fever, the disease now called Brucellosis was first discovered by the British medical officers in 1850s in Malta during the Crimean war. The causal relationship between the organism and the disease was first established in 1887 by Dr David Bruce.[xli,xlii] Since then, brucellosis has become an emerging disease in many parts of the world.[24]

In 1897, Danish veterinarian Bernhard Bang isolated *Brucella abortus* as the agent; and the additional name Bang's disease was assigned. The popular name undulant fever originates from the characteristic undulance (or "wave-like" nature) of the fever, which rises and falls over weeks in untreated patients.[xliii]

Brucellosis, a bacterial disease caused by members of the genus *Brucella*, is an important zoonosis and a significant cause of reproductive losses in animals.[1] Brucellosis is a contagious disease primarily affecting cattle, swine, sheep goats, dogs and humans. It occasionally affects horses and camels.[xliv]

There has been report of occurrence of Human Brucellosis in pregnant women that had history of abortions.[xlv]

Human brucellosis was once thought to be predominantly transmitted through animal contact. However, it is now being increasingly realized that animal products such as milk and meat products are frequently the source of disease transmission.[xlvi]

It is the second most important zoonotic disease in the world after rabies.[xlvii]

Brucellosis continues to be an important source of morbidity in several countries, particularly among agricultural and pastoral populations. [xlviii]The duration of the human illness and its long convalescence means that brucellosis is an important economic as well as a medical problem for the patient because of time lost from normal activities.[xlix]

Etiology

Brucellosis is a worldwide zoonosis caused by infection with the bacterial genus *Brucella.* These organisms, which are small aerobic intracellular coccobacilli, localize in the reproductive organs of host animals, causing abortions and sterility. They are shed in large numbers in the animal's urine, milk, placental fluid, and other fluids. Exposure to infected animals and animal products causes brucellosis in humans.[24]

***Brucella* species are gram-negative cocco-bacillary organisms, of which four are pathogenic in humans (*Brucella melitensis, B. abortus, B. canis* and *B. suis*)[l]**

Young[li] explains the aetiology of Brucellosis and states that;

There are four species of *Brucella* that are pathogenic for humans and these have a limited number of preferred host animals: *B. abortus* (cattle), *Brucella melitensis* (sheep and goats), *Brucella suis* (swine), and *Brucella canis* (dogs). Identification of the *Brucella* species recovered from Humans can provide a clue to the likely source of infection as well as serve as a barometre of the prevalence of the disease in Animals.'

Genetic and immunological evidence indicates that all members of the *Brucella* genus are closely related.[lii]

***B. abortus*, *B. melitensis*, *B. suis, B.neotomae, B. ovis*, and *B. canis* have been known for a long time and are traditionally distinguished according to preferential host, biochemical tests and cell surface characteristics.[liii]**

There have been serological reports of brucellosis in small ruminants in various parts of Nigeria.[liv,lv,lvi]

Five out of the nine known *Brucella* species can infect humans and the most pathogenic and invasive species for human is *B. melitensis*, followed in descending order by *B. suis*, *B. abortus* and *B. canis*. [lvii] B melitensis is considered to be most pathogenic.[lviii]

Brucellosis is a highly contagious zoonosis caused by the Gram negative bacteria of the genus Brucella. *B. abortus, B. suis* and *B. melitensis*, three of the so-called smooth (S) The Centers for Disease Control and Prevention in the USA listed *B. melitensis*, *B. suis* and *B. abortus* as potential bio-weapons due to their high infectious nature as they can be readily aerosolized brucellae, preferentially infect cattle, swine and sheep and goats, respectively.[lix]

The Center for food security and public health[1] classifies Brucella species thus;

Each *Brucella* species is associated most often with certain hosts. *B. abortus* usually causes brucellosis in cattle, bison and buffalo. *B. melitensis* is the most important species in sheep and goats, but *B. ovis* can also cause infertility in rams. *B. canis* causes disease almost exclusively in dogs. *B. neotomae* is found in rodents, but has not been linked to disease. *B. suis* contains more diverse isolates than other *Brucella* species, and these isolates have broader host specificity.*B. abortus*, *B. melitensis*, *B. suis*, *B. canis* and marine mammal *Brucella* species are human pathogens.

It is strange that despite the high prevalence of Brucella infection of man, and it being endemic in Nigeria, it is not considered for routine laboratory referrals in cases of acute febrile illness.[lx]

Transmission

The World Health Organization (WHO) laboratory biosafety manual classifies *Brucella* in Risk group III.[lxi]

Brucellosis is a highly contagious fever caused by ingestion of unsterilized milk or meat from infected animals or close contact with their secretions. [47] Infection in man is often both a food-borne as well as an occupational-related disease in addition to environmental factors.[lxii] .

Transmission through consumption of contaminated dairy products is the route that has been well documented in many parts of the world.[lxiii] Contacts are both direct, often through contamination with infected animals, particularly when they are aborting, and indirect, mainly through the consumption of unpasteurized milk, a common practice in sub-Saharan Africa.[lxiv] Airborne transmission of bacteria to humans has also been documented in clinical laboratories and abattoirs.[lxv]

The importance of this highly contagious disease is due to its severe hazards to human health, through either direct contact with infected animals or through consumption of contaminated milk and dairy products, and its economic impact on the animal industry, causing an adverse effect on animal health[lxvi] Transmission of brucellosis through contaminated dairy products have been documented in many parts of the world[lxvii]

***B. abortus, B. melitensis*, *B. suis* and *B. canis* are usually transmitted between animals by contact with the placenta, fetus, fetal fluids and vaginal discharges from an infected animal.[1]**

Humans are infected through occupational or recreational exposure to infected animals, inhalation of infectious aerosols, laboratory exposure.[lxviii] [Also] consumption of contaminated unpasteurized dairy products or consumption of inadequately cooked contaminated meat.[22]

Endemicity of brucellosis especially bovine brucellosis among slaughtered cattle at the abattoir makes it a source of occupational hazard to workers who are directly involved in cattle meat processing.[lxix]

When direct contact with animals was considered, a very high risk of contacting human brucellosis was associated with assisting in animal parturition, but no significant risk associated with other direct (unspecified) animal contact.[22] Hunters are at an elevated risk of Brucellosis for handling carcasses or meat of infected animals.

Humans are generally infected in one of three ways: ingestion of contaminated food/water, inhalation, or contamination of skin wounds. Inhalation of *Brucella* organisms is not a common route of infection, but it can be a significant hazard for people in certain occupations, such as those working in laboratories where the organism is cultured.[lxx]

***Brucella* can be spread on fomites including feed and water.[1]**

Human brucellosis was once thought to be predominantly transmitted through animal contact. However, it is now being increasingly realized that animal products such as milk and meat products are frequently the source of disease transmission.[53]

Dairy products prepared from unpasteurized milk such as soft cheeses, yoghurts, and ice-cream may contain a high concentration of the bacteria and consumption of these is an important cause of brucellosis. It is the commonest mode of transmission in case of *B.melitensis* and *B.abortus* infections in the general population.[10,lxxi]

For the general population which does not have direct contact with animals, the greatest potential source of brucellosis is through consumption of unpasteurized milk and dairy products. Meat may also be a significant source of infection, especially in cultures where the consumption of raw or undercooked meat products is favored.[56]

The general public can be infected by the ingestion of contaminated milk and milk products, but certain occupations are considered carrying high risk such as abattoir workers, veterinarians, butchers, meat inspectors and farmers.[lxxii] Brucellosis is not usually transmitted from person to person.[1]

Brucella infection seems more associated with humans engaged in livestock and livestock product activities than those engaged in other productive ventures.[lxxiii]

***Brucella* spp. are considered as the most common laboratory-acquired pathogens, and they are estimated to account for up to 2% of all laboratory-associated infections. [lxxiv,lxxv,]**

Greatest risk to human brucellosis was found to be associated with indirect contact with animals (the consumption of unpasteurized dairy products), as opposed to direct contact with animals in Saudi Arabia.[22]

Direct person-to-person spread of brucellosis is extremely rare. Mothers who are breast-feeding may transmit the infection to their infants and sexual transmission has also been reported. [lxxvi, lxxvii]

Pathogenesis

Brucellae can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, the respiratory tract, and the gastrointestinal tract.[67]

***Brucella* species have a unique ability of invading both phagocytic and nonphagocytic cells and surviving in the intracellular environment by avoiding the immune system in different ways, explaining why brucellosis is a systemic disease and can involve almost every organ system.[24] The principal virulence factors of *Brucella* species appear to be the cell-wall lipolysaccharide. Strains containing smooth lipolysaccharide have greater virulence and are more resistant to intracellular killing by the polymorphonuclear leukocytes (PMNLs).[55] *B. abortus*, *B. melitensis* and *B. suis* have been classically subdivided into biovars according to H2S production, CO2-dependence, sensitivity and distribution of the A and M epitopes.[56] However, because these tests are difficult to standardize, molecular markers have been investigated.[lxxviii]**

Risk factors for Human Brucellosis

Several studies have found greatest risk for human brucellosis to be associated with indirect animal contacts [thus] consumption of unpasteurized diary products[22]. Direct contact with domestic animals and consumption of raw products of animal origin are also found to be major risk factors. [22,lxxix] Assisting an aborting animal, proximity to neighborhoods, and christianity [have been] found to be risk factors for contracting human brucellosis.[79] Significant risk factors for Brucellosis were related to occupation as a farmer, shepherd or microbiologist and drinking fresh milk and or laban.[lxxx]

Being female and living in urban area have been found to be risk factors for human brucellosis in some studies.[lxxxi]

Epidemiology of Human Brucellosis

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.[lxxxii] Because of the worldwide distribution of brucellosis, international travel and military deployments increase the risk of exposure.[lxxxiii]

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.[82,lxxxiv] *Brucella* species vary in their

geographic distribution.[1] The global burden of human brucellosis remains enormous; it causes more than 500,000 infections per year worldwide. The heaviest disease burden lies in countries of the Mediterranean basin and Arabian Peninsula, and the disease is also common in India, Mexico, South and Central America.[24]

Brucellosis has been recognized to be a serious zoonotic disease in countries of the Eastern region for four decades.

The countries with the highest incidence of human brucellosis are Saudi Arabia, Iran, Palestinian Authority, Syria, Jordan and Oman. Brucellosis is a serious public health problem in many developing countries and in regions of high endemicity such as Africa, the Mediterranean, the Middle East, parts of Asia and Latin America.[24]

New foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening.[83]

It is an important human disease in many parts of the world especially in the Mediterranean countries of Europe, north and east Africa, the Middle East, south and central Asia and Central and South America and yet it is often unrecognized and frequently goes unreported.[53]

India has the largest livestock population in the world. It accounts for 57 percent of the world's buffalo population and 14 percent of the cattle population.[lxxxv] Considering the damage done by Brucella infection in animals in terms of decreased milk production, abortions, weak offspring, weight loss, infertility and lameness, brucellosis is one of the most serious diseases of livestock and also a major impediment for trade with India.

The first report of Brucellosis in Nigeria was in 1927and was reported as 10 cases of contagious abortions in cattle. [31]

Although brucellosis is a notifiable disease in Nigeria, the incidence, prevalence and distribution of the disease are difficult to determine as the system of disease surveillance and reporting is fragmentary and inefficient.[lxxxvi]

Brucellosis in humans is hardly diagnosed in hospitals in Nigeria despite suggestions that the magnitude of infections may be greater than appreciated.[lxxxvii,lxxxviii]

Clinical Disease

Clinical signs and symptoms of Brucellosis are non- specific and therefore require laboratory confirmation for definitive diagnosis. Fever is the most common sign and symptom of Human Brucellosis and occurring in 80- 100% of the cases.[24]

The Centers for Disease Control and Prevention's clinical description of brucellosis is "an illness characterized by acute or insidious onset of fever, night sweats, undue fatigue, anorexia, weight loss, headache and arthralgia.[lxxxix]

Brucellosis presents as a nonspecific febrile illness with a long and variable incubation period, usually 2 to 4 weeks in about 50% of patients, and as an acute systemic febrile illness in the remainder.[54]

In humans, brucellosis can be acute, sub-acute and/or chronic disease, but its geographical distribution follows the pattern found in animals.[xc] [This] is often overlooked and can mimic other conditions; and may involve various body sites.[5] *B. melitensis* is associated with acute infection whereas the infections with other species are usually sub-acute and prolonged.[10]

Typically, brucellosis begins as an acute febrile illness with nonspecific flu-like signs such as fever, headache, malaise, back pain, myalgia and generalized aches. Drenching sweats can occur particularly at night.[1] Some studies have found fever, arthralgia and sweating to be most frequent symptoms of human brucellosis.[xci]

Brucellosis induces inconstant fevers, sweating, weakness, anaemia, headaches, depression and muscular and bodily pain.[47]

In humans, brucellosis can cause a range of symptoms that are similar to the flu and may include fever, sweats, headaches, back pains, and physical weakness.[xcii]

A prolonged course of illness, often associated with suppurative destructive lesions, is associated with *B suis* infections. *B abortus* is associated with mild-to-moderate sporadic disease that rarely causes complications. *B canis* infection has a disease course that is indistinguishable from *B abortus* infection. *B canis* infection has an insidious onset, causes frequent relapses, and does not commonly cause chronic brucellosis.

***B pinnipediae* and *B cetaceae* are distinctive species that typically affect marine animals; however, these strains were recently described to cause disease in humans, mainly neurobrucellosis.[24]**

Human brucellosis is also known for complications and involvement of internal organs and its symptoms can be very diverse depending on the site of infection and include encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis.[xciii]

Symptoms can be nonspecific and influenza-like: intermittent fever, chills, malaise, diaphoresis, arthralgia, myalgia, headache, anorexia, and fatigue. Signs and symptoms of Brucellosis include malaise, arthralgia, body aches, sweats, hepatomegaly and splenomegaly.[xciv]

In humans, brucellosis can be a serious, debilitating and sometimes chronic disease that may affect a variety of organs.[1]

Other diseases from which Brucellosis is difficult to distinguish clinically are typhoid, rheumatic fever and other conditions causing pyrexia, frequently diagnosed as pyrexia of unknown origin (PUO).[3,13,16,19,xcv,xcvi,xcvii,]

Symptoms and signs of brucellosis usually referred as fever of unknown origin can be confused with other diseases including enteric fever, malaria, rheumatic fever, tuberculosis, cholecystitis, thrombophlebitis, fungal infection, autoimmune disease and tumors.[10]

Diagnosis

The only conclusive evidence of *Brucella* infection is the recovery of the bacteria from the patient. The recommended bacteriologic culture is the biphasic method of Castaneda which uses both solid and liquid medium in the same container. [53] The diagnosis of brucellosis is primarily dependent on clinical suspicion, which can be challenging since the presentation can be highly atypical.[72]

A definitive diagnosis of Brucellosis is based on culture of *Brucella* strains from different samples, mainly blood.[xcviii]

Microscopic examination of stained smears can be useful for a presumptive diagnosis, particularly if the direct examination is supported by other tests.[1]

The diagnosis of human brucellosis cannot be made solely on clinical grounds due to the wide variety of clinical manifestations of this disease, and it is essential to perform bacteriological and serological tests.[53]

Due to many obstacles with culture, diagnosis relies mainly on serology, particularly the standard tube agglutination test (TAT).[108] Although several serologic techniques have been developed and tested, the tube agglutination test remains the standard method.[xcix]

Serology is useful in laboratory diagnosis, and a serum agglutination test (SAT) is available to detect both IgM and IgG antibodies. ELISA and PCR on blood samples are rapid tests at any stage of disease. Nasal swabs, sputum, and respiratory secretions may also be positive by PCR in the first 24 hours after exposure.[72]

Most human infections are diagnosed by serology.[1] Diagnosis of brucellosis is based on laboratory testing by serology and, ultimately by culture, in the context of clinical presentation and history of recent or past exposure.[5]

Definitive diagnosis of brucellosis is based on culture, serologic techniques, or both.

Clinically, identification to the genus level is adequate to initiate therapy, and the type of *Brucella* species involved does not alter the therapeutic agents used.[24]

Accurate and fast diagnosis of human brucellosis is very important as delay or misdiagnosis usually results in treatment failures, relapses, chronic courses, focal complications, and a high case-fatality rate.[c]

The following diagnostic methods are applied for diagnosis of human Brucellosis:

Brucella culture confirmed by light microscopy of Gram stained samples and Urease test.

Immunoassay tests which include Rose Bengal Test (RBT), Standard Tube Agglutination Test (STAT), Coombs test, Brucellacapt, Radioimmunoassay, ELISA.

Nucleic acid detection test which include mainly PCR.

The Rose Bengal test (RBT) is a rapid slide-type agglutination assay performed with a stained *B. abortus* suspension at pH 3.6–3.7 and plain serum. Because of its simplicity, it is often used as a screening test in human brucellosis and would be optimal for small laboratories with limited means. However, there is confusion about the value of this test so that present WHO guidelines recommend that RBT results be confirmed by other tests.[52,ci] Some authors have expressed concern of [RBT's] low sensitivity particularly in long evolution (chronic) cases.[cii]

Treatment

Although a safe and effective vaccine for humans is not available[49], human brucellosis can be treated with a combination of antibiotics.[85] The optimum antibiotic therapy for brucellosis is controversial but the treatment includes a six week course of a combination of antibiotics.[71] For humans with acute brucellosis, a minimum of 6 weeks of treatment is required.[82,ciii] The standard recommended oral treatment regimen includes doxycycline (100 mg, PO, q 12 h) in combination with rifampicin (600 to 900 mg, PO, q 24 h) for a 6-week period. An alternate recommended regimen includes administration of doxycycline (100 mg, PO, q 12 h) for 6 weeks in combination with streptomycin (15 mg/kg [6.8 mg/lb] or 1 g, IM, q 24 h) for 2 to 3 weeks. Although the latter regimen is identified as the gold-standard treatment and is more effective at preventing relapses, it is less practical because the streptomycin must be administered parenterally.[civ] Combination of doxycycline treatment (6 weeks' duration) with parenterally administered gentamicin (5 mg/ kg [2.3 mg/lb], IM, q 24 h) for 7 days is considered an acceptable alternate regimen; few other regimens have higher efficacy than the recommended regimens.[101,] For persons with contraindications for doxycycline (e.g. pregnant women), Trimethoprim- sulfamethoxazole may be considered as an alternative antimicrobial in consultation with their health-care providers. Brucellosis patients were successfully treated with a combination of Rifampicin 600 mg once daily and Septrin 800 mg twice daily for 6 weeks.[52]

Prevention and Control

Human brucellosis is usually prevented by controlling the infection in animals.[1]

Prevention of human brucellosis depends on the control of the disease in animals.[54] High percentage of relapses and therapeutic failures in spite of the use of currently recommended therapeutic regimens indicates the seriousness of this zoonosis and the need to control it.[cv]

Protecting humans against contact with fluids and tissues during assisted parturition of livestock may be an important means of reducing the risk of transferring brucellosis from livestock to humans.

Prevention of human brucellosis should be focused mainly on the elimination of infection in animals and humans along with hygiene, vaccination and effective heating and pasteurization of dairy products. [91]

Attempts at vaccinating people at risk have resulted in effective protection, but also provoked severe reaction in sensitized individuals or when administered incorrectly.[cvi] Vaccination alone will not eradicate *Brucella* as the immunity produced by *Brucella* vaccines are not absolute and can be circumvented by increasing the level of infection. It is obvious, therefore, that a policy of vaccination is more likely to succeed if combined with good measures of husbandry.[cvii]

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Kano is located in Northwestern Nigeria at 11˚30′N and 8˚30′E of the equator. It covers an area of 20,131 km^2 (7,772.6 sq mi). According to the population commission, the projected population in 2011 is 11,216,000 million people. Kano has diverse ethnic populations but the Hausa-fulanis predominate. A large proportion of the population is engaged in agriculture including livestock production. Kano metropolis is made up of six local government areas (LGAs) Dala, Fagge, Gwale, Municipal, Nassarawa and Tarauni LGAs.

One major hospital was recruited from each from each LGA. However, the main hospital in Gwale declined participation. Therefore five General hospitals within five local government areas in Kano metropolis were used:

S/No	LGA	Name of Hospital
1	**Dala**	**Sabo Bakin Zuwo General Hospital(SBZGH)**
2	**Fagge**	**Infectious Diseases Hospital(IDH)**
3	**Kano Municipal**	**Murtala Muhammed Specialist hospital(MMSH)**
4	**Nasarawa**	**Sir Muhammadu Sunusi General Hospital (SMSSH)**
5	**Tarauni**	**Aminu Kano Teaching Hospital(AKTH)**

3.2 Study Design

The research is a cross-sectional descriptive study.

3.3 Study Population

Study participants were drawn from the selected hospitals. Patients that attended the general out-patient department (GOPD) were recruited to participate in the research based on the selection criteria.

3.3.1 Inclusion Criteria:

Patients 5years and above that attended one of the selected hospitals with acute or insidious fever(>37.5^{o}C), plus any two or more of the following symptoms; night sweats, anorexia,

fatigue, weight loss, headache, joint pains, backaches ,flu like illness, spinal collapse and osteomyelitis.

3.3.2 Exclusion criteria:

Patients less than 5 years that were not having fever and those that did not consent to participate in the study.

3.4 Sampling and Sample size Determination

The sample size of 250 was calculated using the prevalence gotten from the findings of 8-28% in many parts of Nigeria.

Assumptions:

Desired Power - 80% with β= 0.2

Confidence interval- 95% with α=0.05

$$n = \frac{(1.96)^2 \, Pexp \, (1 - P)}{\sigma^2}$$

n = required sample size

Pexp = 18% (expected prevalence)

σ = 5% (desired absolute precision)

1 – P = 0.82

n = 223+ (10% expected attrition)

n = 250

3.5 Sampling Technique

Purposive sampling was employed based on the signs of the disease and the peri-urban nature of the state. There is more likelihood of people with recurrent fevers to attend general or tertiary institutions rather than the primary health centres'. One general/tertiary hospital was selected randomly per LGA within Kano metropolis.

3.6 Study Instruments

The study instruments used were syringes, sample bottles, micropipettes, ice packs, plastic test tubes, hand gloves, ceramic tiles, toothpicks, Elisa and Rose Bengal test kits.

3.7 Data Collection Methods

3.7.1 Questionnaire Administration

Study participants were interviewed using a standardized questionnaire after pre testing. (Appendix I) .

Questions including socio-demographic data, potential risk factors, and clinical features of Brucellosis were explored.

Associated factors of human brucellosis were assessed based on contact with live animals, milk consumption, meat processing and consumption.

3.7.2 Blood collection

Blood was collected from each study participant to obtain sera for analysis.

Five millilitres of blood samples was collected aseptically by qualified medical personnel using sterile syringes and needles from cephalic veins of the patients into properly labeled sterile bottles. Blood samples were centrifuged at 704 x g for 10 minutes to separate sera from whole blood and serum samples were stored in freezer at -20^{o}C until required for serological analysis.

Screening of the Serum Samples using the Rose Bengal Test (RBPT)

The sera collected were first subjected to modified Rose Bengal test. This test is 100% sensitive. Antigens for the tests were obtained from University of Navarra, Microbiology and Parasitology Department, Irunlarrea, 1 31008 Pamplona Spain. Positive, negative and cut off reference sera were sourced from the same laboratory.

Four 25µL drops of saline (0.85% NaCl) were dispensed on the tile using a pipette. 25µL of positive plain serum was added to the first saline drop and mixed thoroughly by aspirating and expulsing the mixture several times with the pipette. The pipette tip was rinsed with saline and 25µL of the first dilution was transferred to the second saline drop. The second dilution was mixed thoroughly as in above and 25µL of it was transferred to the third saline drop. The third dilution was mixed thoroughly as in above and 25µL of it was transferred to the fourth saline drop. After mixing the fourth dilution thoroughly as in above, 25µL was taken and the rest discarded.

The RBT antigen was removed from refrigeration and equilibrated at room temperature and shaken to homogenize it using a toothpick. 25 µL of antigen was dispensed beside each drop of serum obtained from the dilution above using the automatic pipette. Immediately the antigen and serum were mixed with a tooth pick and the plate gently rocked clockwise and counterclockwise for exactly 4 minutes. Four sera were tested on a plate and results were read

at the end of the 4 minutes rocking period under a well illuminated plate. Account of the delay between mixing of each serum was taken.

Interpretation

Negative: uniform pink mixture, no clumps and no rim.

Positive: any perceptible agglutination (from fine clumps and some rim formation to coarse clumping and definite clearing).

The sera that tested positive to MRBPT were subjected to indirect Enzyme-linked immunosorbent assay (ELISA) to test IgG and IgM antibodies against *Brucella* to further confirm and show the evolution of the infection.

Enzyme-Linked Immunosorbent Assay (ELISA) IgG and IgM Tests

Antigens for the ELISA tests were obtained from VIRCELL S. L. Pza. Dominguz

Ortiz 1. Poligono Industrial Dos de Octubre. 18320 Santa Fe, Granada, Spain. Positive, negative and cut off reference sera were sourced from the same laboratory.

The Elisa method is based on the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme labeled antihuman globulin binds the antigen-antibody complex in a second step.

After a new washing step, bound conjugate is developed with the aid of a substrate solution (TMB) to render a blue colored soluble product which turns into yellow after the acid stopping solution.

Preliminary preparation of the reagent:

Washing solution was prepared in advance, just before the test was started. 50mls of 20x washing solution up to 1 liter with distilled water was prepared and stored at 2-8^{o}C. An incubator was set at 37^{o}C; all reagents were brought to room temperature 1 hour before use without removing the plate from the bag.

The number of wells to be used was determined. Four control wells were counted: Two for the cut off control and one each for the positive and negative sera. Wells not required for the test were returned to the pouch and sealed. 100µl of serum dilution was added to all wells, 5ul of each sample was added, 5µl of positive control, 5µl of cut off control and 5µl of negative control was added into the corresponding wells, and this was covered with a sealing sheet and incubated at 37^{o}C for 45minutes. The seal was removed and liquid aspirated from all wells, washed 5 times with 0.3ml washing solution per well. 100µl of conjugated solution was added to each well then covered with a sealing sheet and was incubated at 37^{o}C for 30 minutes. The seal was removed and liquid aspirated from the wells, washed 5 times with 0.3ml of washing

solution per well. 100µl of substrate solution was added into each well, covered with a sealing sheet and incubated at room temperature for 20 minutes protected from light.

Seal was removed and 50µl of stopping solution was added immediately into all wells. Reading was done with spectrophotometer at 450/620nm within 1 hour of stopping.

Interpretation of results:

The mean optical density for cut off serum was calculated, (Antibody index = sample optical density/mean cut off serum mean optical density x 10). Samples with an index of less than 9 was considered as not having IgG specific antibodies against *Brucella* samples (= negative result). Those with an index above 11 were considered as having IgG specific antibodies against *Brucella* (=positive result). Samples with index of 9-11 were considered as equivocal and were considered negative due to lack of reagents to retest.

Brucella IgM:

Principle of the test:

Detection of IgM against *Brucella* lipopolysaccharides (LPS) is suitable for the diagnosis of the acute form of the disease. The ELISA technique is sensitive and specific for detection of IgM antibodies against *Brucella*. The principle of the test is similar to that of IgG.

Interpretation of Results:

Antibody index = (sample optical density / cut off serum mean optical density) X 10

Samples with indexes below 9 were considered as negative

Samples with indexes above 11 were considered as positive

3.8 Data Management

3.8.1 Measurement of Variables

Socio-demographic characteristics of the patients including age, sex, occupation, educational status. Signs of the disease were measured using the medical information obtained.

3.8.2 Determination of the proportion of Human brucellosis in patients with Recurrent Fever

The proportion of the patients with recurrent fever that tested positive was calculated and compared to those with recurrent fever that were negative.

3.8.3 Determining Sensitivity of Clinical Diagnosis to Human Brucellosis

Sensitivity and specificity of the clinical diagnosis of Human brucellosis were calculated to see those that were captured clinically and tested positive in the laboratory analysis ie true positives.

3.8.4 Assessment of Risk Factors of Human Brucellosis

Risk factors of Human brucellosis were measured by doing bivariate and multivariate analyses to establish significant association of those factors with the disease or otherwise from this research.

3.9 Ethical Considerations

Introductory letter was obtained from the Department of Community Medicine and was presented to the Kano State ministry of health and the state Medical Ethical Committee. (Appendix II).

Ethical clearance was sought from the scientific committee of Kano State Hospitals Management Board (Appendix III).

Consent was sought from study participants and their accent documented (Appendix IV).

3.10 Limitations

The reluctance of the physicians to consider as one of their differentials human brucellosis in patients with recurrent fever and other signs suggestive of the disease.

Inability to get access to some patients in some of the hospitals so interpretation was done with caution.

Possibility of missing a positive case from those negative of RBPT.

CHAPTER FOUR

RESULTS

4.1 Socio-Demographic Features of the patients

There were 250 respondents obtained from the five hospitals. From Aminu Kano Teaching Hospital (AKTH), there were 59 respondents out of which 9 (15%) were positive. Fifty three respondents were obtained from Infectious Diseases Hospital (IDH) out of which 9 (27%) were positive. Fifty eight respondents were obtained from Sabo Bakin Zuwo General Hospital (SBGZH) out of which 11(19%) were positive. From Sir Muhammadu Sunusi Specialist Hospital (SMSSH), there were 56 respondents out of which 10(18%) were positive and from Murtala Muhammad General Hospital (MMGH) there were 44 respondents of which 12(25%) were positive (Table 4.1).

More male respondents were obtained from all the hospitals except one SBGZH (Table 4.2).

There were 131 males and 119 female respondents. Of the 131 male respondents 31(24%) were positive and of the 119 female respondents 19(16%) were positive. More males were positive of the disease than females but there was no statistical association between gender and the disease.

All the 250 respondents were 5years and above. The mean age of the respondents was 24years with a standard deviation of 16 (Table 4.5).

Out of the 250 respondents, 66(27%) were in the age group 21-30years and 13(26%) of the 50 found to be positive of *Brucella sp*, were also in the same age group. Out of the 31 males found to be positive ten were within the age group 31-40years representing twenty percent of the all positive cases (Figure 4.1). Age had no statistical association with the disease (Table 4.15).

Out of the 250 respondents 125(50%) were single, 120(48%) were married, 3(1.2%) were divorced and 1(0.8%) was a widow. Out of the 50 positive cases, 25(50%) were single and the other 25(50%) were married (Figure 4.2).

Marital status had no association with human brucellosis (Table 4.16).

Out of the 250 respondents the highest number 90(36%) had secondary school education, 84(34%) had primary school education, 47 (18%) had informal education, 18 (12%) had tertiary education and 11(10%) had no form of education at all. Out of the 50 positive cases the highest 18(36%) had secondary school education while the lowest 2(4%) had tertiary education (Table 4.3). Educational status had no significant association with human brucellosis.

Of the 249 respondents that stated their occupational status, most were unemployed 92(37%), followed by students 57(23%) and the rest were employed informally and formally (27%) while others were 57(23%). (Table 4.4)

Sixteen (32%) of the 50 positive cases were employed, 15(30%) were unemployed, 13(26%) were students and others were 6(12%). Being employed had significant association with human brucellosis and was found to be protective against the disease (Table 4.16).

4.2 Clinical Information of Respondents

Out of the 250 respondents, 181(72%) had fever on presentation, spinal collapse 12 (5%), osteomyelitis 19(8%), cough/sneeze 145(58%), headache 186(74%), joint pains 196 (78%), muscular pains 125(50%), fatigue 174(70%) and night sweats 94(38%).

Orchitis (swollen testes) was only applicable to males and out of 131 male respondents only 5(4%) had it.

Out of the 50 positive cases 44(88%) had fever on presentation, headache 38(76%), cough/sneeze 36(72%), fatigue 36(72%), joint pains 38(76%), muscular pains 31(62%), night sweats 20(40%), osteomyelitis 13(26%), spinal collapse 8(16%) and recurrent fever 32(64%). Out of the 31 male cases only 5(16%) had orchitis (Table 4.7).

Clinical signs that had significant association with human brucellosis were fever on presentation (p 0.00), recurrent fever (p 0.01), cough/Sneeze (p) osteomyelitis (p 0.00) and 0.0270) (Table 4.16).

4.3 Results of Laboratory Analysis

4.3.1 Characteristics of Respondents from Rose Bengal Plate Test

Out the 250 respondents screened and tested with RBPT, 50(20%) tested positive. Out of the 50 positive cases 31(62%) were males and 19(38%) were females (Figure 4.3).

4.3.2 Characteristics of ELISA Positive Cases

Out of the 50 cases tested with ELISA, 14(28%) were positive using ELISA IgM while 36 (72%) were positive of ELISA IgG alone.

Out of the 14 that were IgM positive 9 (64%) were males while 5 (36%) were females; and of the 36 that were IgG positive 22(61%) were males while 14(39%) were females (Table 4.7).

4.4 Proportion of Recurrent Fever with Human Brucellosis

Out of the 250 respondents 121(48%) had recurrent fever while 36 (64%) out of the 50 positive cases had recurrent fever (Table 4.9).

There was significant association between recurrent fever and human brucellosis (p 0.01) (Table 4.16).

4.5 Sensitivity of Laboratory Analysis to Clinical Diagnosis of Human Brucellosis

The sensitivity of laboratory analysis to clinical diagnosis was 90% while the specificity was 85%.

4.6 Risk Factors for Human Brucellosis

4.6.1 Milk

There was no significant association between milk consumption and human brucellosis (p 0.19) and source of milk consumed (p 0.08) but there was significant association between type of milk consumed and human brucellosis.

Consumption of fresh milk (p 0.00) and kindirmo (p 0.00) were significantly associated with the disease (Table 4.8).

There was no significant association between milk pasteurization and human brucellosis (p 0.42) (Table 4.8).

4.6.2 Raw Meat

There was no association between cutting raw meat and human brucellosis (p 0.04) but there was significant association between processing raw meat (p 0.00) and eating raw meat (0.00) with the disease. (Table 4.9).

4.6.3 Live Animals

There was significant association between keeping live animals (p 0.00); (Table 4.10), types of animals kept (goats p 0.00); (Table 4.11) and breeding animals (p 0.00) (Table 4.12) with human brucellosis.

There was significant association between assisting animals' parturition and human brucellosis (p 0.00) (Table 4.13).

4.7 Independent Risk Factors for Human Brucellosis

Independent risk factors for human brucellosis from multivariate analyses are consumption of fresh milk (p 0.02) and kindirmo (p 0.00); keeping goats (p 0.00), assisting animal parturition (p 0.01), processing raw meat (p 0.03) and eating raw meat (p 0.00).

Clinical signs that had strong association with human brucellosis were recurrent fever (p 0.04), cough/sneeze (p 0.02) and osteomyelitis (p 0.00).

Being employed (p 0.04) was found to be protective of human brucellosis.

Table 4.1: Distribution of Respondents by Hospitals

Name of Hospital	Positive (%)	Negative	Total (100%)
AKTH	9(15.3)	50(84.7)	59
IDH	9(27.3)	24(72.7)	33
SBZGH	11(19.0)	47 (81.0)	58
SMSSH	10(17.9)	46(82.1)	56
MMGH	11(25.0)	33 (75.0)	44
Total	50	200	250

Highest number of respondents 59(24%) were obtained from AKTH while the lowest number 33(13%) were obtained from IDH.

Table 4.2: Distribution of Respondents by Gender and Hospitals

Hospital	Positive		Total	Negative		Total
	Females (%)	Males		Females (%)	Males	
AKTH	4	5	9	22	28	50
IDH	1	8	9	8	16	24
MMGH	4	7	11	16	17	33
SBZGH	10	1	11	47	0	47
SMSGH	0	10	10	7	39	46
Total	19	31	50	100	100	200

More males participated in the research than females and of all the hospitals studied SBZGH was one that most respondents were females; those that tested positive were 10(98%) and no male amongst them.

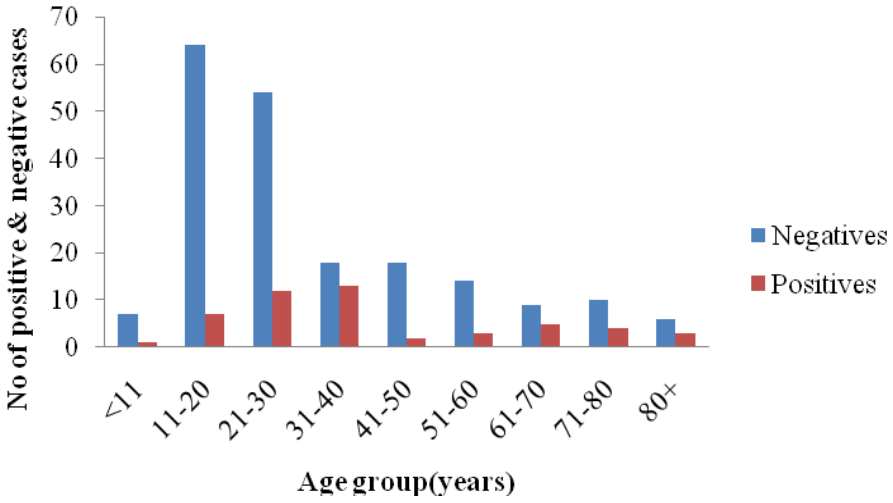


Figure 4.1: Distribution of Respondents by Age groups

The highest number of positive cases (27%) was in the age group 31-40 years and the lowest (13%) was in the age group < 11years.

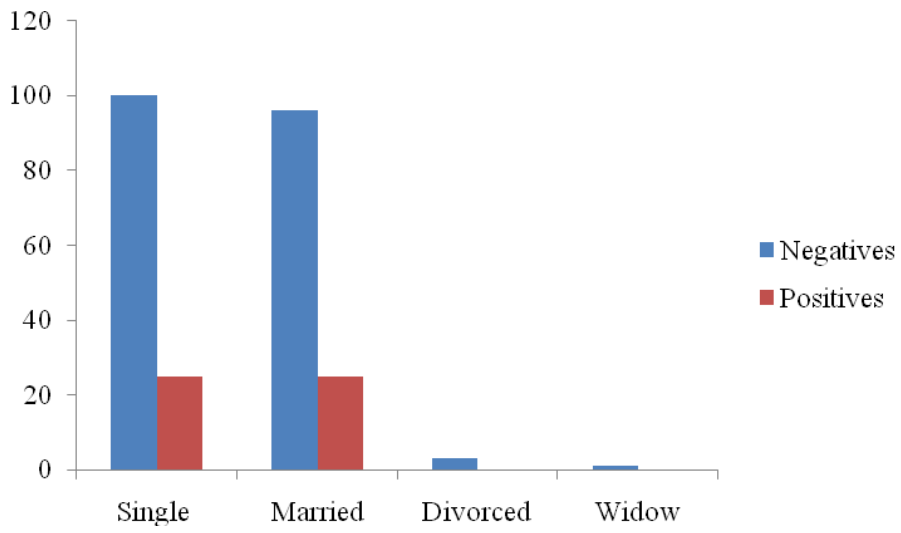


Figure 4.2: Distribution of Respondents by Marital Status

Amongst the 250 respondents half (125) were single and the other half were married. Out of the 50 positive cases, 25(50%) were single and the other 25(50%) were married.

Table 4.3: Distribution of Respondents by Educational status

Educational Status	Positive (%)	Negative (%)
None	3(6.0)	8(4.0)
Islamic	10(20.0)	37(18.5)
Primary	17(34.0)	67(33.5)
Secondary	18(36.0)	72(36.0)
Tertiary	2(4.0)	16(8.0)
Total	50	200

Highest number of respondents had secondary school education 90(36%) of which 18 tested positive while those with no form education were lowest 11(4%) of which 3 were positive.

Table 4.4: Distribution of Respondents by Occupation

N=249

Occupation	Positives (%)	Negatives (%)
Unemployed	15(31.3)	87(46.7)
Informal employment	8(16.7)	20(9.2)
Formal employment	8(12.5)	31(7.2)
Others	6(12.5)	17(15.9
Students	13(22.9)	44(21.0)
Total	50	199

Highest numbers of respondents were unemployed 102(48%) of which 15 tested positive and that represent thirty percent of the positive cases.

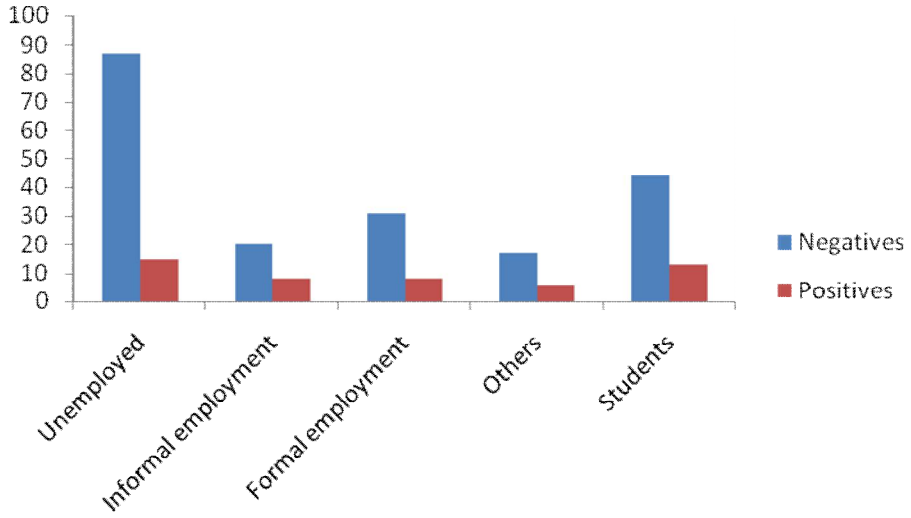


Figure 4.3: Distribution of Cases by Occupation

Highest number of respondents was unemployed 102 (48%) and fifteen of them tested positive.

Table 4.5: Association between Medical Information of Respondents and Human Brucellosis

Medical Information	Positives (%)	Negatives (%)	OR(95%CI)	p-value
Fever on presentation	44(88.0)	137(68.5)	3.33(1.37-8.33)	0.00
Headache	38(76.0)	148(74.0)	1.11(0.54-2.29)	0.39
Cough/Sneeze	36(72.0)	109(54.5)	2.15(1.09-2.23)	0.01
Fatigue	36(72.0)	138(69.0)	1.16(0.58-2.29)	0.35
Joint pains	45(90.0)	151(75.5)	2.92(1.10-7.77)	0.01
Muscular pains	31(62.0)	94(47.2)	1.82(0.97-3.44)	0.032
Night sweats	20(40.0)	74(37.0)	1.14(0.60-2.14)	0.35
Osteomyelitis	13(26.0)	6(3.0)	11.36(4.06-31.08)	0.00 (F.exact)
Spinal collapse	8(16.0)	4(2.0)	9.33(2.69-32.44)	0.00 (F.exact)
Recurrent Fever	32(64.0)	89(44.0)	2.22 (1.17-4.21)	0.01

Fever on presentation (p 0.00), recurrent fever (p 0.04), cough/sneeze (p 0.02), joint pains (p 0.01), osteomyelitis (p 0.00) and spinal collapse (p 0.00) were the medical symptoms associated with brucellosis.

Table 4.6: Distribution of ELISA positivity by Gender

Elisa positivity	Elisa IgM	Elisa IgG	Total
Male	9	22	31
Female	5	14	19
Total	14	36	50

Twenty eight percent of those that tested positive with RBPT were positive of Elisa IgM while 72% were positive of Elisa IgG. More males tested positive to both Elisa IgM and IgG.

Table 4.7: Association between Source of Milk Consumed and Human Brucellosis

Source of milk	Positives (%)	Negatives (%)	Odds Ratio(95% CI)	p-value
Market	33 (66)	152(76)	0.61(0.31-1.60)	0.0799
Farm	2(4)	5(3)	1.63(0.31-8.63) 0.4244(F.exact)	
Homes	2(4)	2(1)	1.05(0.57-30.03) 0.1796(F.exact)	

No association between source of milk consumed and human brucellosis.

Table 4.8: Association between Types of Milk consumed by Respondents and Human Brucellosis

Type of Milk consumed	Positives (%)	Negatives (%)	Odds Ratio(95% CI)	p-value
Fresh milk	17(34)	23(12)	3.96(1.97-8.22)	0.0001
Evaporated milk	11(22)	34(17)	1.38(0.64-2.96)	0.2076
Powdered milk	21(42)	99(50)	0.74(0.40-1.36)	0.1743
Yoghurt	18(36)	70(35)	1.05(0.55-1.99)	0.4444
Kindirmo	35(70)	73(37)	4.06(2.03-7.93)	0.0000
Butter/Ghee	1(2)	13(7)	0.30(0.04-2.29)	

0.1897(f.exact)

The unprocessed types of milk have significant association with human brucellosis and they are fresh milk (p. 0.00) and kindirmo (p.0.00).

Table 4.9: Association between Milk Pasteurization and Human Brucellosis

Pasteurization	Positives	Negatives	Total
Pasteurize milk	28	111	139
Do not pasteurize milk	22	89	111
Total	50	200	250

Odds Ratio (95%CI): 1.03(0.46-2.01); p-value 0.4203

There is no association between milk pasteurization and human brucellosis.

Table 4.10: Association between Raw Meat Consumption and Processing with Human Brucellosis

Meat	Positives (%)	Negatives (%)	Total	Odds Ratio(95%CI)	p-value
Cut raw meat	20(40)	54(27)	74	1.80(0.94-3.44)	0.0398

Process raw meat	**25(50)**	**29(14)**	**54**	**5.76(2.96-11.37)**	**0.0000**
Eat raw meat	**25(50)**	**20(10)**	**45**	**9.00(4.37-18.52)**	**0.0000**

Processing raw meat (p 0.00) and eating raw meat (p 0.00) were found to be associated with the disease.

Table 4.11: Association between Keeping Live Animals and Human Brucellosis

Keep Animals	Positives	Negatives	Total
Yes	**28**	**64**	**92**
No	**22**	**136**	**158**
Total	**50**	**200**	**250**

Odds Ratio (95%CI): 2.70(1.44-5.10); p-value 0.0010

There was significant association between keeping live animals and human brucellosis

Table 4.12: Association between Types of Animals kept and Human Brucellosis

Type animals	Positives Total=50(%)	Negatives Total=200(%)	OR(95%CI)	p-value
Cattle	**5(10.0)**	**11(5.5)**	**1.91(0.63-5.77)**	**0.1954**
Sheep	**6(12.0)**	**25(12.5)**	**0.95(0.37-2.37)**	**0.4763**
Goats	**19(38.0)**	**20(10.0)**	**5.52(2.65-11.50)**	**0.0000**

Table 4.13: Association between Breeding Animals and Human Brucellosis

Breed Animals	Positives	Negatives	Total
Yes	28	63	91
No	22	132	154
Total	50	195	245

Odds Ratio (95%CI):2.10(1.10-4.11); p-value 0.0172

Table 4.14: Association between Assisting Animal Parturition and Human Brucellosis

Assist Parturition	Positives	Negatives	Total
Yes	28	63	91
No	22	132	154
Total	50	195	245

Odds Ratio (95%CI):2.83(1.23-6.51); p-value 0.00

Table 4.15: Unconditional logistic Regression; Risk factors of Human Brucellosis; December, 2011 (Initial Model)

S/No	Factor	OR (95% CI)	P -value
1	Age	0.77 (0.08 – 7.16)	0.8149
2	Sex	1.63(0.86 – 3.08)	0.1306
3	Educational status	1.25 (0.66 – 2.37)	0.4910
4	Occupational status	0.53 (0.26 – 1.08)	0.0790
5	Marital status	1.01 (0.54 – 1.88)	0.9757
6	Drink milk	0.00 (0.00 – 1.00)	0.9672
7	Consume fresh milk	3.96(1.91-8.21)	0.0002
8	Consume kindirmo	3.69(1.91-7.95)	0.0001
9	Pasteurize milk	1.02(0.55-1.90)	0.9493
10	Breeding animals	2.10 (1.08 – 4.11)	0.0298
11	Keeping animals	2.70(1.44 – 5.09)	0.0020
12	Keeping goats	5.52(2.65-11.49)	0.0000
13	Assist animals' parturition	5.59(0.92- 33.92)	0.0617
14	Have contact with animals' placenta	0.44(0.06- 3.32)	0.4275
15	Having fever on presentation	1.00(0.54- 5.86)	1.0000
16	Recurrent fever	2.19(1.01- 4.78)	0.0484
17	Joint pain (Arthritis)	2.92(1.09-7.77)	0.0318
18	Osteomyelitis	11.36(4.06-31.79)	0.0000
19	Spinal collapse	9.33(2.69-32.69)	0.0004

Table 4.16: Unconditional Logistic Regression; Risk Factors of Human Brucellosis; December, 2011 (Final Model)

S/No	Factors	OR (95% CI)	P-value
	Occupational status	0.47 (0.23 – 0.97)	0.0406
	Consumption of fresh milk	2.61 (1.14 – 5.98)	0.0227
	Consumption of kindirmo (local yoghurt)	2.77 (1.32 – 5.82)	0.0072
	Keeping goats	4.59(5.73-12.16)	0.0021
	Assist animals' parturition	2.83(1.23-6.51)	0.0141
	Processing raw meat	2.47 (1.07 – 5.68)	0.0340
	Eating raw meat	5.69 (2.42-13.35)	0.0001
	Recurrent fever	2.19(1.01-4.79)	0.0482
	Osteomyelitis	7.82(2.37-25.84)	0.0007
	Cough/Sneeze	2.15(1.09-4.23)	0.0270

CHAPTER FIVE

DISCUSSION

There were 250 respondents obtained in the study; 131 males and 119 females. Mean age of the respondents was 24years with a standard deviation of 16. Of the 250 respondents, 50 were found to be positive for Brucella species. The sensitivity of laboratory analysis to clinical diagnosis was 90% while the specificity was 85%. Thirty six (72%) of the positive cases were IgG positive.

Amongst the socio-demographic features of the respondents, employment status had significant association with the disease while age, gender, marital and educational status had no association. Clinical features significant for brucellosis were recurrent fever, cough/sneeze and osteomyelitis.

Significant risk factors for human brucellosis in this study were consumption of fresh milk and kindirmo, processing and consumption of raw meat, keeping goats and assisting animals' parturition.

Brucellosis is one of the seven neglected zoonoses as described by WHO. Within sub-Saharan Africa which Nigeria is part of, many of these known infectious diseases both directly and indirectly transmitted; do occur commonly but are poorly controlled in both livestock and human populations and ultimately these diseases have a great impact on economic development.[cviii]

Human brucellosis affects all ages and sexes. In this study, human brucellosis was found to affect both sexes. The cases of Brucellosis was highest in 31-40years (30.2%) and lowest in <11 years (2.3%) age groups. This is in agreement with series of reports that found brucellosis to affect people in their third to fifth decades of life but is in contrast with findings of Abu-Shaqra[cix] in Jordan, where he found the cases of brucellosis in his study to be lowest in below 4 years and highest in the 5 – 14 years age group. The findings in this study and others in with it may be because active rearing of animals and working in livestock industry is common amongst adults as against those within the younger age groups.

In this study there was no association between brucellosis and sex; this is in agreement with the findings of Baba *et al* [13] in Maiduguri where they found no association between brucellosis and sex but is in contrast with a study on childhood brucellosis in Jordan.[cx]

The sensitivity of clinical diagnosis to Human brucellosis was 90% which was quite high. This may be attributed to the fact that the awareness of the clinicians on the disease was raised during the study period. Specificity of the RBPT was 85% which is also high. Therefore RBPT being relatively cheap and easy to perform can be adopted in states where there are incidences of animal and human brucellosis. This is in agreement with the findings of Moriyon

et al [66] that tested sera of patients during clinical practice from 1975-2001; Maichom[3], Muriuki[cxi] in Kenya and Oomen.[9]

RBPT had a diagnostic performance comparable to ELISA in this study as all positive cases picked by RBPT were confirmed by ELISA even though there is possibility of missing a case because only cases picked by RBPT were subjected to ELISA.

Consumption fresh milk and kindirmo (locally prepared yoghurt) both unpasteurized dairy products are independent risk factors for Human brucellosis in this study. This is in agreement with the findings of Masomeh *et al* in Iran, Al- Shamahi *et al* in Yemen, [80] Benjamin and Annobil in Saudi Arabia[cxii] and El Amin et al in Oman.[cxiii] Dairy production and development is a priority of Kano government; therefore this and other related researches should raise awareness of the state government and other Northern states in general on the importance of dairy production using best practices and to sensitize its populace implication of consuming these unprocessed milk products which is routine in most households in the region.

Arthritis showed significant association with Brucellosis in a study in Turkey[cxiv] and Andalucia while this study shows osteomyelitis as the most significant symptom with p=0.00.

Keeping goats have been found to be a risk factor for human brucellosis in this study. This is in agreement with studies of Staszkiewiz *et al* in Michigan USA and another that found raw goat cheese to be the source of Brucellosis outbreak in Andalucía Spain[cxv] but reports from Refai,[25] Al Talafhah *et al,*[cxvi] in Jordan and El sherbinni *et al*[cxvii] in Egypt incriminated sheep as the first species for contracting human brucellosis.

Assisting animal parturition was found to be a significant risk factor of human brucellosis in this study with p=0.01.Owners of animals and livestock workers do assist parturition without gloves and other necessary protective clothing and consequently contract the infection if the animals being assisted are infected. This agrees with the findings of El sherbinni where contact with placenta of aborted animals is a risk factor.

Processing and eating raw meat are risk factors in this study. Processing raw meat has been identified as risk factor for Brucellosis in Central Sudan[cxviii] through contact with infected tissue and also through cuts or abrasions found by Young.[54] The social habit of eating raw meat such as offals has been found to be an important risk factor for human brucellosis in Uganda[cxix] and also in Nigeria where it is common amongst butchers and meat sellers which may predispose to infection if one ingests parts from infected tissues.

CHAPTER SIX

6.1 Conclusions

Out of the 250 respondents recruited from five general hospitals in Kano metropolis 50 tested positive to ELISA out of which 14 (28%) had acute infection (IgM) while 36 (72%) had chronic infections (IgG).

The sensitivity of clinical diagnosis based on serology in Kano state was high. Clinical features consistent in those diagnosed positive were recurrent fever, osteomyelitis and cough/sneeze.

Significant risk factors for Human brucellosis in this study were consumption of fresh milk and kindirmo, processing and consumption of raw meat, keeping goats and assisting animals' parturition.

6.2 Recommendations

Physicians should raise the index for diagnosis of human brucellosis by including it in their differential diagnosis in patients that present with some signs suggestive of the disease which include recurrent fever, arthralgia, osteomyelitis and cough/sneeze.

Health care administrators should consider including screening test in hospitals to enable evaluation of patients suspected of human brucellosis.

The state government should optimize surveillance of brucellosis in livestock so the extent of its occurrence in animals known, and institute deliberate control program, so also reduce the rate of infection in humans.

The government should sensitize the populace on importance of this disease, conduct public education and enlightenment on the risks and some clinical features suggestive of human brucellosis.

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APPENDICES

Appendix I

Department of Community Medicine,

Faculty of Medicine,

Ahmadu Bello University, Zaria

RESEARCH QUESTIONNAIRE

Introduction

Good day dear respondent, my name is Aisha Nasiru Isa a postgraduate student of the department of community medicine ABU Zaria and a resident of the Nigerian Field Epidemiology and Laboratory Training Program (N- FELTP), Federal Ministry of Health, Abuja.

I am working on a research; Case control study of human brucellosis in Kano State. I seek your permission to administer this questionnaire which will assist me in finding the risk factors associated with the disease in Kano State. Your positive response will certainly help the research to come up with recommendations that would lead to instituting interventions towards control and prevention.

I am hoping that your sincere response to these questions will go a long way in enabling me to achieve these objectives. I appreciate the few minutes you will spend in responding to these questions and sincerely assure you that utmost confidentiality will be maintained.

Would it be okay with you to follow-up at a later date?

Thank you for your cooperation.

A. Personal Identification:

Identification Number……..

Unique Number……. Name of Hospital…….……

Cases: (1) Case (2) Control For Cases only: Temp (^{o}C)……..

A1 Interviewer's Name…….……..

A2 Patient Name: ……

A4 Full Residential Address……..

…….

……

……

A5 Phone number ……

A6 Local Government Area……

B. Socio Demographic Information

B1 Age……..

B2 Sex: 1. Female 2. Male

B3 Marital Status: 1. Single 2. Married 3.Widowed 4. Divorcee 5. Others

B4 Highest level of education attained: 1.None 2.Islamic 3. Primary

4. Secondary 5.Tertiary 6.Others

B5 Religion: 1. Islam 2. Christianity 3.Others

B6 Occupation: 1. Unemployed 2. Informal employment 3.Formal employment

4. Trade (specify)…….. 5. Others (specify)…….

Risk Factors

C. Milk

C1 Do you take milk and or milk products? 1. Yes 2. No

C2 If yes, which types do you take? 1. Whole milk 2.Powdered milk

3. Evaporated milk 4.Yoghurt 5. Kindirmo 6. Butter/Ghee 7. Others……..

C3 Where do you get milk / milk products? 1. At home from your animals

2. Farm 3. Market 4.Others (specify)……..

C4 Are milk products always pasteurized? 1. Always 2. Sometimes 3.Never

4. Don't know

C5 How often do you take milk or milk products? 1. Always 2. Sometimes

3. Never

D. Live Animals

D1 Do you keep or have contact with animals? 1. Yes 2. No

D2 If yes, how frequent? 1. Always 2. Sometimes 3. Never

D3 If yes, what type of animals? 1. Cattle 2.Sheep 3.Goats 4. Dogs 5.Cats

6. Others (specify)…….

D4 Do you breed animals? 1. Yes 2. No

D5 If yes, where do the animals live? 1. At home 2. On the farm 3. Roam around

D6 Do you slaughter animals? 1. Yes 2. No

D7 Do you process animals? 1. Yes 2. No

D8 Do you milk animals? 1. Yes 2. No

D9 Did/Do you help parturient animals? 1. Yes 2. No

D10 Did/Do you have contact with animals' placenta? 1. Yes 2. No

Meat

E1 Do you participate in cutting raw meat? 1. Yes 2. No

E2 Do you participate in processing raw meat? 1. Yes 2. No

E3 Do you eat raw meat? 1. Yes 2. No

Medical Information

F1 Did (Do) you have fever within the last 3 months? 1. Yes 2. No

3. Don't know 4. Can't remember

F2 Do you have fever now? 1. Yes 2. No 3. Don't know

F3 How often do you have fever? 1. Daily 2. Weekly 3. Every other week

4. Fortnightly 5. Monthly 6. Over 3 months 7. Biannually 8. Annually

F4 Do you have cough or sneeze? 1. Yes 2. No

F5 Did (Do) you have headaches? 1. Yes 2. No

F6 Did (Do) you have joint pains? 1. Yes 2. No

F7 Did (Do) you have muscular pains? 1. Yes 2. No

F8 Did (Do) you have fatigue? 1. Yes 2. No

F9 Do you have night sweats? 1. Yes 2. No

F10 Do you have swollen testes? 1. Yes 2.No

F11 Do you have spinal collapse? 1. Yes 2. No

F12 Do you have osteomyelitis? 1. Yes 2. No

Case Control Study of Human Brucellosis in Kano State

Information Leaflet for Research Assistants

Aisha Nasir Isa

9/27/2011

NAME OF HOSPITAL:_____

My Name is Aisha Nasiru Isa, an MPH student of the Department of Community Medicine, Faculty of Medicine, Ahmadu Bello University, Zaria.

The subject of our Research is Case Control study of Human Brucellosis in Kano state.

Below are the Inclusion and Exclusion criteria for selection of our Cases and Controls

Please take time to explain to patients or patients' wards as the case may be the objective of this Research as you've seen it done by the researcher at the practical sessions with patients

Please ensure that all patients that agreed to participate in this study sign or thumb print on the consent form as the case may be.

Please ensure that all questions are asked as stated and answers are clearly written or selected as given by the patients enrolled in the study.

1. Cases
   Inclusion criteria- Patients to be enrolled into the research

Any patient aged 5years and above that has temperature above 37.5^{o}c plus any two or more of the following symptoms:

- Night sweats
- Anorexia: doesn't have appetite to eat
- Fatigue: easily gets tired
- Weight loss
- Headache
- Flu like illness: cough, sneeze, sore throat, catarrh
- Joint pain
- Backaches
- Osteomyelitis
- Spinal collapse

Each of the patients enrolled from the above criteria should sign or thumbprint the consent form and be administered the questionnaire. Temperature of the above patients should be written in the space provided on the questionnaire

5mls of the blood should be withdrawn from the cephalic vein of each patient by the personnel designated to do so. Sera is separated from whole blood and stored in a -20^{o}c freezer.

Exclusion criteria- Patients that must never be enrolled into the research

Any patient that is below 5years and does not have fever

Patients that do not want to participate in the Research

2. Controls-

Inclusion criteria-Patients to be enrolled into the research

Any patient aged 5years and above that does not have fever and has temperature below 37.5^{o}c plus the patient does not have any two of the following symptoms:

- Night sweats
- Anorexia: doesn't have appetite to eat
- Fatigue: easily gets tired
- Weight loss
- Headache
- Flu like illness: cough, sneeze, sore throat, catarrh
- Joint pain
- Backaches

5mls of the blood should be withdrawn from the cephalic vein of each patient by the personnel designated to do so. Sera is separated from whole blood and stored in a -20^{o}c freezer.

Exclusion criteria- Patients that must never be enrolled into the research

Any patient less than 5 years of age

Patient with laboratory confirmed diagnosis of malaria or typhoid fever.

The patients that do not want to participate in the Research

Above is the detailed explanation for the Research assistants to use and refer to for proper recruitment of Cases and Controls for this Research after going through practical sessions of Data and sample collection with the Lead Researcher.

Please feel free to call the numbers below for any clarification

Aisha Nasiru Isa- 08032014200 and 08075300006

Thank you

Read and understood by Research Assistants

1. Name……

Signature& Date……

2. Name……

Signature& Date……

Appendix II

DEPARTMENT OF COMMUNITY MEDICINE

FACULTY OF MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA

VICE CHANCELLOR: Professor Abdullahi Mustapha,
B.Sc (Hons) Pharm, A.B.U. Ph.D, (London) FPSN
A.g. HEAD OF DEPARTMENT: Dr. S.H. Idris,
MBBS, DMLD, FWACP (COMM. HEALTH)

Telephone: 069-551718
E-mail: commmedabu@yahoo.com
Fax: 069-550022

Our Ref: MPH/FE-MED/0066/2009-10

Date: 4th May, 2011

Dear Sir,

INTRODUCTION OF MASTER IN PUBLIC HEALTH IN FIELD EPIDEMIOLOGY STUDENT AND RESEARCH PROJECT:- DR. AISHA NASIRU ISA

This is to certify that the above named MPH student is conducting her Research Project on the topic:- "Case control study of Human Brucellosis in Kano State".

I shall be grateful for your assistance in giving her all the necessary information/data relevant to the project topic to enable her handle the assignment with confidence and success.

Thank you.

Yours faithfully

Dr. S.H. Idris
HEAD OF DEPARTMENT

Appendix III

KANO STATE
HOSPITALS MANAGEMENT BOARD
BOARD HEADQUARTERS
P.M.B 3540, Post Office Road, Kano

HMB/GEN/488/II 06/10/2011 (9/10/1432AH)

Dr. Aisha Nasiru Isa
Department of Community Medicine,
Ahmadu Bello University, Zaria,
Kaduna State.

PROVISIONAL ETHICAL CLEARANCE

Sequel to your application to conduct research titled "CASE CONTROL STUDY OF HUMAN BRUCELLOSIS" at M.M.S.H., M.A.W.S.H., Sabo Bakin Zuwo Maternity I.D.H. and S.M.S.G.H.in the Light of the above, I am mandated to convey provisional clearance to proceed on your study based on the following conditions.

i. That the consent of all participants must be obtained by filing inform consent form.
ii. That you should apply all standard place ways of attending patients including procedures and all necessary consents where applicable.
iii. That there should be a good records keeping of all patients recruited with all procedures done them and results obtained.
iv. That you should liaise with the management of the facility of your focus for appropriate guidance.
v. All recruited participants should be traceable.
vi. That any publication related to the study should be brought to the knowledge of the ethical committee for approval.
vii. That a copy of your finding should be submitted for documentation, record and final approval, please.

Best wishes.

Nazir Ahmad Ya'u
Senior Assistant Secretary
FOR: CHAIRMAN ETHICAL COMMITTEE

Cc

The Chief Medical Director,
M. M. S. H., and M. A. W. S. H.
Kano.

Above is for your information and record, please.

Nazir Ahmad Ya'u
Senior Assistant Secretary
FOR: CHAIRMAN ETHICAL COMMITTEE

Appendix IV

INFORMED CONSENT FORM

I, …….

Whose address is …….

Hereby give consent to participate in the research project as has been explained to me by

……

All the terms of this consent have been explained to me in a language that I understand. I am also aware that strict confidentiality will be maintained in this study. That I am not required to answer a question(s) if I choose not and i can voluntarily withdraw from the study without any punitive measures taken against me.

Thumb print……. Names/Signature…….……

Participant Interviewer