PREVALENCE OF BOVINE TUBERCULOSIS AND KNOWLEDGE, ATTITUDE AND PRACTICE OF WORKERS IN TWO ABUJA ABATTOIRS, FEDERAL CAPITAL TERRITORY, NIGERIA

BY

DR KACHALLA MAIRO GUJBA

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BY

KACHALLA MAIRO GUJBA (A.B.U. 2003)

MPH (N-FELTP/MED/00025/2010-2011)

DEPARTMENT OF COMMUNITY MEDICINE, FACULTY OF MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA, KADUNA STATE.

FEBRUARY, 2014

ATTESTATION

I attest that the work in the thesis entitled PREVALENCE OF BOVINE TUBERCULOSIS AND KNOWLEDGE, ATTITUDE AND PRACTICE OF WORKERS IN TWO ABUJA ABATTOIRS has been performed by me in the Department of Community Medicine under the supervision of Dr M. Bello and Prof I. Ajogi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

KACHALLA, MAIRO GUJBA		**February, 2014**
Name of Student	**Signature**	**Date**

CERTIFICATION

This thesis entitled "PREVALENCE OF BOVINE TUBERCULOSIS AND KNOWLEDGE, ATTITUDE AND PRACTICE OF WORKERS IN TWO ABUJA ABATTOIRS by KACHALLA Mairo Gujba meets the regulations governing the award of the degree of Masters of Public Health in Field Epidemiology of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Prof I. Ajogi _____ _____
Chairman, Supervisory Committee **Signature** **Date**
Department of Vet. Public Health and
Preventive Medicine

Dr. M Bello _____ _____
Member **Signature** **Date**
Department of Vet. Public Health and
Preventive Medicine

Dr. M N Sambo _____ _____
Head of Department **Signature** **Date**
Department of Community Medicine

Prof. A A Joshua _____ _____
Dean **Signature** **Date**
School of Postgraduate Studies

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LIST OF ACRONYMS

BCG: Bacillus Calmette-Guérin

BTB: bovine tuberculosis

ELISA: Enzyme-Linked Immuno Sorbent Assay

HIV/AIDS: Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome

IFN-γ: Gamma Interferon

MDR: Multi Drug Resistant

OIE: Organization des Internationale Epizootic

PCR: Polymarese chain reaction

PPD-A or PPD-B: Purified Protein Derivative M. bovis or M. avium

PPD: Purified Protein Derivative

RDs: (Regions of Difference)

SICCT: Single Intra- dermal Comparative Cervical Tuberculin

TB: Tuberculosis

WHO: World Health Organization

SUMMARY

Mycobacterium bovis is present in animals in most developing countries where surveillance and control activities are often inadequate or unavailable and pasteurization is rarely practiced.

In Nigeria, the disease is sporadic. The current status on the actual prevalence rate of bovine tuberculosis at a national level is unknown but from the limited survey research which has been reported over 30 years, the prevalence of bovine tuberculosis due to *M. bovis* ranges from 2.5% in 1976 to 14% in 2007. The study was conducted to determine the prevalence of bovine tuberculosis in slaughtered cattle and assess the knowledge attitude and preventive practices of butchers and meat handlers.

A cross-sectional study was conducted at two abattoirs in Abuja, Nigeria from March 2013 - April 2013. Structured questionnaires were used to collect data on social demographics, medical information, work related exposure factors and preventive practices. The butchers and meat handler were selected by simple random sampling. Cattle slaughtered at the abattoirs were also screened for bovine tuberculosis using lateral flow technique and Ziehl Neelsen test. Bivariate and multivariate data analyses were done using Epi-info software.

Out of a total of 185 serum and 5 tissue samples screened at both abattoirs, 32 (17.3%) and all 5 were positive for the rapid test lateral flow technique and Acid Fast stain respectively. The only risk factor associated with bovine tuberculosis infection in these cattle was the sex (female) ($p = 0.01$). Of the 156 butchers and meat handlers on whom the questionnaire was administered, 115 (73.8%) were men and 41 (27.2%) were women. The following were significant by the level of knowledge; the length of time worked at the abattoir (Odds Ratio OR) = 3.4, 95% CI = 1.6-7.4, knowledge of mode of transmission (OR =2.4, 95% CI = 1.1-5.1), Potential risk associated with

your job (OR = 2.4, 95% CI = 1.1-5.0), eating or drinking at place of work (OR = 0.6, 95% CI = 0.2-1.4), Additionally, the following factor were significantly associated with poor knowledge of BTB ; drinking fresh milk (OR 0.4, 95% CI 0.2- 0.8), and not boiling their milk before drinking (OR 0.4, 95% CI 0.2- 0.9), Seen a bovine tuberculosis lesion in your slaughtered (OR = 0.2, 95% CI = 0.2-0.5), What's done to lesion (OR = 2.2, 95% CI = 1.0-4.8) and Do you consume meat with these lesions (OR = 2.2, 95% CI = 1.1-4.7). Multvariate analysis showed that length of work (Adjusted Odds Ratio [AOR] = 3.45, 95% CI= 1.3-9.0), and wether seen a bovine tuberculosis lesion on your slaughtered cattle (AOR = 0.29, 95% CI= 0.1-0.7) were significant by the level of knowledge.

The prevalence of bovine tuberculosis is under reported based on abattoir surveillance of tuberculous tissue only. Butchers and meat handlers have a an increase risk of exposure due to consumption of meat with lesion of tuberculosis and what is done when they come across lesion of bovine tuberculosis due to the inadequate knowledge of the disease and its mode of transmission. Butchers and meat handlers should be encouraged to seek for medical attention early when a cough persists.

Keywords: Bovine Tuberculosis, TB prevalence, Butchers, Abattoirs, Nigeria.

CHAPTER 1

1.0 INTRODUCTION

Tuberculosis (TB) is a chronic infectious and contagious Zoonotic disease of domestic, wild animals and humans [(1)]. Human and bovine tuberculosis (BTB) are two forms of tuberculosis that cause significant disease in mammals [(2)]. It is characterized by the formation of granulomas (tubercles) in tissues and organs more significantly in lungs, lymph nodes, intestine, liver and kidneys [(3)]. Though primarily a bovine problem but infect animals causing tuberculosis in camels, pigs, sheep, goats, horses, dogs, cats, badgers, lions, elephants, deers, primates and man [(4)].The disease is zoonotic and therefore of public health significance [(5)]. Human tuberculosis is mainly caused by Mycobacterium tuberculosis but in regions where bovine tuberculosis is prevalent in animals, human tuberculosis cases due to *Mycobacterium bovis* may occur [(6)]. Zoonotic bovine tuberculosis is present in most developing countries where surveillance and control activities are often inadequate or unavailable [(7)]. The disease is widespread and affecting livestock and human health in Africa [(8)]. It is among the fastest killer disease in Nigeria today [(9)] and developing countries as a whole [(10)]. In Africa, the occurrence of tuberculosis due to *Mycobacterium bovis* in human is difficult to determine accurately because of technical problems in isolating the microorganism [(11)]. Currently bovine tuberculosis in human is becoming increasingly important in developing countries like Nigeria as human and animals are sharing the same micro-environment and dwelling premises especially in rural areas [(12)]. Historically, tuberculosis caused by *Mycobacterium bovis* in human was associated with consumption of unpasteurized milk and this is still the most important route of exposure in developing countries [(13)]. Rural inhabitants and some urban dwellers in Nigeria still consume unpasteurized and soured milk potentially infected with *Mycobacterium bovis* [(12)]. The human cases of tuberculosis

associated with *Mycobacterium bovis* infection, both pulmonary and extra pulmonary has been documented in Nigeria [12, 14, 15]. From the limited survey which has been reported over the last 30 years in the country, prevalence of bovine tuberculosis due to *Mycobacterium bovis* ranges from 2.5% in 1970 to 14% in 2007. The disease has been on the increase as demonstrated by tuberculin test reports [12,16, 17, 18].

1.2 Research Problem

From the limited survey which has been reported over the last 30 years in the country, prevalence of bovine tuberculosis due to *Mycobacterium bovis* ranges from 2.5% in 1970 to 14% in 2007. The disease has been on the increase as demonstrated by tuberculin test reports [12,16, 17, 18].

Detection of bovine tuberculosis in Nigeria is carried out most commonly on the basis of tuberculin skin testing, abattoir meat inspection and rarely bacteriological techniques; however the current status on the actual rate of bovine tuberculosis at national level is unknown because in Nigeria, screening of cattle by the tuberculin skin test is sporadic [12].

The proportion of human tuberculosis due to *Mycobacterium bovis* is on the rise, isolation of *M. bovis* from sputum samples of patients with pulmonary TB has been reported in Nigeria. Of 102 *M. tuberculosis* complex isolates, 4 (3.9%) were *M. bovis* [14]

In Nigeria, Idigbe et al. found *M. bovis* in 4% ofpatients with lower respiratory tract symptoms [14].

In Nigeria diagnosis of Tuberculosis stops at the smear level, hence the specie involve in causing the disease are not known thereby making it difficult to study outbreaks, trace the route of transmission and also know the strain of Mycobacterium involved [12].

1.3 Justification

There is a substantial lack of knowledge of the distribution, epidemiological pattern, and zoonotic implication of bovine tuberculosis in developing countries especially in Africa [19].

Farmers, Abattoir workers, Butchers and Veterinarians are especially at high risk of acquiring the disease from animals [20].

The high prevalence of tuberculosis in human may be due to high prevalence of tuberculosis in slaughtered cattle and subsequent consumption of their products by humans [21].

The incidence of human tuberculosis due to species other than *Mycobacterium tuberculosis* has increased worldwide [22].

Human Immunodeficiency Virus/Acquired Immuno Deficiency Syndrome (HIV/AIDS) associated human tuberculosis due to *Mycobacterium bovis* and other Mycobacterium are major opportunistic infections in HIV/AIDS infected individuals, while HIV/AIDS is a major predisposing factor for tuberculosis including reactivation of the disease [23].

The current spreading of pandemic of HIV/AIDS infection in developing countries like Nigeria, especially where bovine tuberculosis is prevalent in domestic animals and wild animals, posses an additional serious public health problem [24].

International market requirement in trading of animals and their products require high standard and hygiene food animal's production [11]

Therefore, there is the need to conduct a systematic research to determine the association between *Mycobacterium bovis* in cattle slaughtered at the abattoir and abattoir workers to

provide information which can be used to formulate policies for control and eventual eradication of the disease in the country.

1.4 Research Question

I. What is the prevalence of bovine tuberculosis at study site

II. What is the level of knowledge of bovine tuberculosis and attitude of butchers and meat handlers towards bovine tuberculosis at the study site

III. What are the preventive practices of bovine tuberculosis by butchers and meat handlers

1.5 General and Specific Objectives

1.5.1 General Objectives

Is to determine the prevalence of bovine tuberculosis in slaughtered cattle using two different methods (the rapid test lateral flow technique and Acid Fast stain)

1.5.2 Specific Objectives

I. Determine the prevalence of bovine tuberculosis in animals slaughtered at the abattoir in two Abuja abattoirs using blood sample and tissue samples

II. To examine the level of knowledge and attitude of butchers and meat handlers towards bovine tuberculosis

III. To assess the preventive practices of bovine tuberculosis by the butchers and meat handlers.

SCOPE OF STUDY

This study focuses on the cattle slaughtered and abattoir workers at the abattoirs under study at the Federal Capital Territory (FCT), Abuja, North Central, Nigeria.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1. Epidemiology of Bovine Tuberculosis

Bovine tuberculosis is a chronic bacterial disease of cattle that occasionally affects other species of mammals. This disease is a significant zoonosis that can spread to humans, typically by the inhalation of aerosols or the ingestion of unpasteurized milk [25]. Bovine tuberculosis is an infectious disease caused by *Mycobacterium bovis* that affects cattle, other domesticated animals and certain free or captive wildlife species. It is usually characterized by formation of nodular granulomas known as tubercles. Although commonly defined as a chronic debilitating disease, bovine tuberculosis can occasionally assume a more progressive course. Any body tissue can be affected, but lesions are most frequently observed in the lymph nodes (particularly of the head and thorax), lungs, intestines, liver, spleen, pleura, and peritoneum [26]. Cattle are considered to be the true hosts of M. bovis, although the disease has been reported in many other animals. Isolations have been made from buffaloes, bison, sheep, goats, equines, camels, pigs, wild boars, deer, antelopes, dogs, cats, foxes, mink, badgers, ferrets, rats, primates, South American camelids, kudus, elands, tapirs, elks, elephants, sitatungas, oryxes, addaxes, rhinoceroses, possums, ground squirrels, otters, seals, hares, moles, raccoons, coyotes and several predatory felines including lions, tigers, leopards and lynx [5,26, 27]

Mycobacterium bovis has been identified in humans in most countries where isolates of mycobacteria from human patients have been fully characterized. The incidence of pulmonary tuberculosis caused by M. bovis is higher in farm and slaughterhouse workers than in urban inhabitants. The transmission of M. bovis to humans via milk and its products is eliminated by the pasteurization of milk. One of the results of bovine

tuberculosis eradication programmes has been a reduction in disease and death caused by bovine tuberculosis in the human population [27].

2.1.1 Mycobacterium

M. bovis is a slow growing, facultative intracellular, aerobic and gram-positive bacterium with a dysgenic colony shape when cultured on Löwenstein-Jensen medium [28]. As all Mycobacterium specie., M. bovis has an unusual cell wall surface structure characterized by the dominant presence of mycolic acids and a wide array of lipids [29]. This waxy lipid envelope confers an extreme hydrophobicity, which renders the bacteria acid- and alcohol-fast, a feature that can be exploited to identify mycobacteria via the Ziehl-Neelsen staining technique [30]. The mycobacterial surface lipids also have a potent biologic activity and are thought to play a crucial role in pathogenesis [29]. M. bovis can be identified on the basis of specific biochemical and metabolic properties. For instance M. bovis requires pyruvate as a growth supplement, is negative for niacin accumulation and nitrate reduction, shows microaerophilic growth on Lebek medium and is generally resistant to pyrazinamide [28, 31]. Unlike *M. tuberculosis*, *M. bovis* lacks pyruvate kinase activity, due to pykA containing a point mutation that affects binding of Mg^{2+} cofactor [32]. Pyruvate kinase catalyses the final step of glycolysis, the dephosphorylation of phosphorenolpyruvate to pyruvate[32]. Therefore in *M. bovis* glycolytic intermediates are unable to enter into oxidative metabolism [32]. Although no specific studies have been performed, it seems that *M. bovis* must rely on amino acids or fatty acids as an alternative carbon source for energy metabolism [32].

2.1.2 Genome Structure

The *M. bovis* genome sequence is 4,345,492 base pairs in length, arranged in a single circular chromosome [32]. The genome contains 3,952 genes encoding proteins [32]. The genome is >99.52% identical at the nucleotide level to *Mycobacterium tuberculosis* [32]. Comparative sequencing with *M. tuberculosis* revealed 11 deletions from the genome of *M. bovis*, ranging in size from 1-12.7 kb, and has been confirmed by the genome sequence data [32]. *M. bovis* contains one unique locus termed TbD1, which is absent from the *M. tuberculosis* strain; therefore, it appears that deletion has been the mechanism in shaping the *M. bovis* genome [32].

2.2 Application to Biotechnology

M. bovis is the ancestor of the most widely used vaccine against tuberculosis, *M. bovis* bacillus Calmette-Guérin [32]. BCG is a strain that was created by growing *M. bovis* on potato slices soaked in ox-bile and glycerol over a period of 13 years [32].

2.3 Transmission and Susceptibility

Cattle are susceptible to M. bovis infection and are the preferred host for M. bovis. Cattle mount a demonstrable immune response to M. bovis infection. There is relatively limited knowledge about the factors which influence susceptibility/resistance to bovine TB and this is an obvious blind-spot in the knowledge and evidence base, especially locally. The risk factors which influence susceptibility can be divided broadly into the classical ‘nature’ (genetics) and ‘nurture’ (non- genetic or environmental) factors [33].

If these can be identified and quantified, several are likely to be amenable to intervention. There are different levels at which susceptibility and resistance act; resistance to infection, resistance to

disease, resistance to disease progression and resistance to onward transmission/excretion etc. Bovine TB, caused by the bacterium Mycobacterium bovis (M. bovis), is a chronic, primarily respiratory disease that can take a variable amount of time (from a few weeks to a lifetime) to develop from infection to clinical disease and to become infectious to other animals. The transmission rate between animals is low and the epidemic only progresses slowly. On average any infected animal is thought to transmit disease to just over one other animal. Animals infected with TB tend to shed M. bovis intermittently in relatively low numbers of organisms [(34)]. With some exceptions, it is agreed that cattle become infected with M. bovis by either the oral or respiratory routes [(35)]. The oral route is likely most important in calves nursing tuberculous cows, while the respiratory route is most common in cattle in general [(35)].

In the late 1990's, surveys of tuberculous cattle in Great Britain [(36)] revealed that 67% of tuberculous lesions were within the lungs and pulmonary lymph nodes (tracheobronchial and mediastinal). Cranial lymph node lesions were observed in 39%, while only 8% of lesions involved mesenteric lymph nodes. Although many studies demonstrate a tendency toward lesion development in pulmonary lymph nodes rather than in lungs, meticulous examination often reveals lesions in the lungs; most <1 cm in diameter [(37

Transmission can be either direct, through close contact, or indirect from exposure to viable bacteria in a contaminated environment (for example pasture and feed). The relative contribution of each of these routes has not been quantified. However, we know that M. bovis only lives and multiplies in mammalian hosts (or in a laboratory incubator on specific media which mimic the host) and only small numbers of viable bacteria are likely to persist in the environment for any length of time. Outside of a host, survival of M. bovis is thought to be usually short (measured in

weeks) as bacteria deteriorate quickly, especially if conditions are dry and sunny as they are killed by desiccation and UV light.

However, there is published evidence of survival for up to 11 months in ideal conditions i.e. moist and dark areas [(34)]. The main methods of cattle-cattle transmission of bovine TB are summarized below. Direct and indirect cattle-cattle transmission includes; within-herd transmission at housing, within-herd transmission at pasture, vertical (congenital) transmission, pseudo-vertical transmission (via milk), spreading bovine excreta on pasture , between-herd transmission through cattle movements, between-herd transmission across farm boundaries , From soil and silage, via drinking water, via arthropod vectors [(38)]. M. bovis can be transmitted by the inhalation of aerosols, by ingestion, or through breaks in the skin. The importance of these routes varies between species. Bovine tuberculosis is usually maintained in cattle populations, but a few other species can become reservoir hosts. Most species are considered to be spillover hosts. Populations of spillover hosts do not maintain M. bovis indefinitely in the absence of maintenance hosts, but may transmit the infection between their members (or to other species) for a time. Some spillover hosts can become maintenance hosts if their population density is high [(27)]

Transmission of M. bovis between hosts is thought to be mainly through the airborne route via aerosol droplets. Past studies have shown that low numbers of bacilli are needed to experimentally infect animals via the lung, whereas large doses are required to infect animals via ingestion. This is supported by the distribution of lesions found in cattle. Oral infection of man and calves was important when tuberculous milk was relatively common (when there was still clinical disease in the national dairy herd), although this is now much more rare with ongoing skin testing and removal of reactor cattle [(35)].

2.4. Distribution

Bovine TB is distributed throughout the world and has been reported on every continent (except Antarctica). Basically, where there are cattle there is, or has been, bovine TB [39] , with the notable exception of those countries that have eradicated bovine TB using a test-and-slaughter policy to remove infected cattle, such as Australia, some Caribbean islands (including Cuba) and parts of South America.

In other countries, notably the USA, Canada, South Africa and most of the European Union (EU), with the exception of the UK and Republic of Ireland (ROI), bovine TB has been reduced to negligible levels. Although sporadic, and sometimes persistent and damaging outbreaks associated with the import of infected cattle or the existence of wildlife reservoirs have been reported [39,40] . In New Zealand (NZ), the control of bovine TB in cattle and farmed deer has proven difficult, and a wildlife reservoir in brush-tailed possums has been implicated. Their control programme targets bovine TB in cattle and in local wildlife. Recent evidence indicates that NZ is now making significant progress towards eradication, although the epidemiology is different to that in the UK and ROI. The combination of cattle surveillance and controlling bovine TB in local wildlife has also limited persistent outbreak associated with white-tailed deer in Michigan (USA). The suspected wildlife reservoir in Michigan, Canada and the Kruger National Park in South Africa is itself of economic/social value.

Social issues and public approval could also be important factors in eliminating the disease, and has strong resonance for the UK, where the badger, a protected species, has been implicated as a reservoir host [39]. Although bovine tuberculosis was once found worldwide, control programs have eliminated or nearly eliminated this disease from domesticated animals in many countries. Nations currently classified as tuberculosis-free include Australia, Iceland, Denmark, Sweden,

Norway, Finland, Austria, Switzerland, Luxembourg, Latvia, Slovakia, Lithuania, Estonia, the Czech Republic, Canada, Singapore, Jamaica, Barbados and Israel. Eradication programs are in progress in other European countries, Japan, New Zealand, the United States, Mexico, and some countries of Central and South America.

Although bovine tuberculosis has been eradicated from the majority of U.S. states, a few infected herds continue to be reported, and a few states may periodically lose their disease-free status. In particular, a focus of infection in wild white tailed deer has complicated eradication efforts in Michigan. Similar problems exist with infected badgers in the U.K. and Ireland, and infected brush-tailed opossums in New Zealand. Bovine tuberculosis is still widespread in Africa, parts of Asia and some Middle Eastern countries [(26)].

2.5 Pathogenesis

The pathogenesis of bovine tuberculosis is not as well understood as the pathogenesis of tuberculosis in humans. Advances in the field of human tuberculosis have been made using various small animal models of M. tuberculosis infection. Extrapolations of findings from small animal models have been made to bovine tuberculosis. Such extrapolations may not always be appropriate as there are profound differences in host susceptibility, host immune response, environment, host ecology, and behavior[(41)]. Based on the predominance of tuberculous lesions in the respiratory tract and associated lymph nodes of diseased cattle, it was early believed, that BTB is transmitted from animals to animals through inhalation of infectious aerosols [(42)].

Further evidence for this route of transmission has come from a number of other studies [(42)]. Bacteria entering the respiratory tract, passing the mucociliary layer and gaining access to the alveolar space are thought to be phagocytized by macrophages, which may constitute the main cellular host for mycobacteria in vivo [(43)]. Following uptake into the phagosome, macrophages

attempt to kill the bacteria by phago-lysosome fusion and acidification. However, mycobacteria are able to prevent lysosomal delivery by manipulating the host cell signal transduction pathways using an array of bacterial effector lipids and proteins [(44)]. The organism replicates intracellularly after it has been taken up by the macrophages. A granuloma or tubercle forms as the body tries to wall off the infected macrophages with fibrous tissue. The granuloma is usually 1-3 cm in diameter, yellow or gray, round and firm. On cut section, the core of the granuloma consists of dry yellow, caseous, or necrotic cellular debris.

The infection can spread hematogenously to lymph nodes and other areas of the body and cause smaller, 2-3 mm in diameter, tubercles. The formation of these smaller tubercles is known as "miliary tuberculosis". The histological lesions consist of necrotic cells in the center of the tubercle surrounded by epitheloid cells and multinucleated giant cells all encapsulated by collagenous connective tissue. The necrotic core of cells can often become calcified as the tubercle matures [(45)]. It has been stated that the route of transmission of M. bovis can be deduced by the pattern of lesions observed in the infected animal [(46)]. Moreover, when infection occurs across mucous membranes such as the pharynx or intestine, the initial lesion in the mucous membrane may not be visible while lymph node lesions are obvious [(47)].

2.6 Pathology

In many cases, the course of the infection is chronic and signs may be lacking, even in advanced cases when many organs may be involved. When present, clinical signs vary; lung involvement may be manifested by a cough, which can be induced by changes in temperature or manual pressure on the trachea. Dyspnoea and other signs of low-grade pneumonia are also evidence of

lung involvement [27]. In advanced cases, lymph nodes are often greatly enlarged and may obstruct air passages, the alimentary tract, or blood vessels. Lymph nodes of the head and neck may become visibly affected and sometimes rupture and drain. Involvement of the digestive tract is manifested by intermittent diarrhoea and constipation in some instances. Extreme emaciation and acute respiratory distress may occur during the terminal stages of tuberculosis. Lesions involving the female genitalia may occur. Male genitalia are seldom involved [27].

At necropsy, tubercles are most frequently seen in bronchial, mediastinal, retropharyngeal and portal lymph nodes and may be the only tissue affected. In addition, the lung, liver, spleen and the surfaces of body cavities are commonly affected. Early nodular pulmonary lesions can often be detected by palpation. The lesions are usually non-odoriferous. Other anatomical sites can be infected and should be examined [27]. Macroscopically, a tuberculous granuloma usually has a yellowish appearance and is caseous, caseo-calcareous, or calcified in consistency.

Occasionally, its appearance may be purulent. The appearance may be more purulent in cervids and camelids. Some nontuberculous granulomas may be indistinguishable macroscopically from tuberculous granulomas [27]. The caseous centre is usually dry, firm, and covered with a fibrous connective capsule of varying thickness. Lesion size ranges from small enough to be missed by the unaided eye, to involvement of the greater part of an organ. Serial sectioning of organs and tissues may be required to detect the small lesions contained within the tissue [27]. Indeed, most studies of naturally infected cattle describe the majority of lesions within the lung, pulmonary lymph nodes and cranial lymph nodes [48].

The conclusion is that this distribution of lesions suggests a respiratory route of infection. Investigations in naturally infected cattle suggest that lesions of the alimentary tract, although not

common generally, are more common in temperate climates where conditions favor dissemination and survival of M. bovis on forage, while respiratory tract lesions are more common in arid climates where conditions favor aerosolization of M. bovis [48,49]. The principal route of transmission of tubercle bacilli among herds of cattle is the aerogenous one although a small minority of infections may be congenital. milk-borne or sexually transmitted [50]. The primary pulmonary lesion in cattle is very similar to that occurring in man: namely a small lesion at the site of infection and considerable enlargement of the lymph nodes draining the affected segment of lung [50]. While in man such primary complexes often heal spontaneously, this seems to happen only rarely in cattle [50]. In the latter the disease may appear to be latent or only slowly progressive but such lesions are often open. Therefore a tuberculin-positive COW with only minimal lung lesions may spread the infection through- out the entire herd [50].

The primary focus of infection in the lung may extend locally or more widespread dissemination by way of the lymphatic or circulatory systems may occur. Local spread manifests as a localized or generalized bronchopneumonia: cavity formation is not so obvious in cattle as in man [50]. More wide- spread disease is relatively uncommon : post-mortem studies revealed that the disease was restricted to the thorax in 80-90%of animals [51]. In the animals with extrathoracic disease the affected organs were. in descending frequency, the liver, kidneys, spleen, uterus and udder [51].

2.7 Diagnosis

Detection of bovine tuberculosis in cattle and other susceptible animal species is often made on history, clinical and necropsy findings, tuberculin skin tests and abattoir meat inspections. Definitive diagnosis is made on culture with morphological appearance and biochemical tests [52] and molecular biological techniques like PCR [53]. Therefore, in countries with endemic bovine

TB, postmortem diagnosis by detection of gross lesions has been proposed [54]. In diagnosis of mycobacterial infections, culture is still considered the international gold standard [55] .However, due to dysgonic and slow growth characteristics, the identification of *M. bovis* by culture and biochemical methods is cumbersome and time consuming [56] For convenience the methods for the diagnosis of tuberculosis in cattle are examined in two broad categories namely direct and indirect tests [57].

The direct tests are those designed to directly identify the organism in the host animal. Primarily this relates to the post mortem examination of animals and the associated tests used to confirm infection [57].

Indirect tests are those that identify infection in the live animal using indirect indicators of infection.

This category of test is dominated by those based upon immunological markers, i.e. those that identify and measure immune responses in the animal to the M. bovis organism [57]. They fall into 2 catego

ries, those that are based upon the cellular immune response, primarily the intradermal skin test and the interferon-gamma test, and those based upon antibody responses. Of course there is considerable overlap in how the various tests are or could be implemented. For example most post mortem histopathology and culture is carried out on animals found infected using an immunological test [57]. Passive abattoir surveillance can be a cost effective method for surveying TB in animals [58]. In reality most of these animals will be infected but the post mortem surveillance, typically in conjunction with bacteriology and histopathology, will have failed to identify infection [38].

Today, the two most widely used tests for the diagnosis of BTB are the single intra- dermal comparative cervical tuberculin (SICCT) test and the Bovigam® test (Prionics) [59]. Both are based on the detection of the early cell mediated immune (CMI) response in tuberculosis infection. The SICCT test measures the delayed-type hypersensitivity response to intra-dermally injected bovine and avian tuberculin. Tuberculin or the so-called purified protein derivative (PPD) is a crude extract of water soluble proteins from a heat-treated culture of M. bovis (PPD-B) or M. avium (PPD-A) [59]. PPD-B and PPD-A are injected side by side into the skin of an animal's neck and skin swelling is measured 72 hours later. According to the OIE standard procedure, the test result is considered positive, if the difference in the increase of skin thickness at the site of PPD-B injection is more than 4 mm greater (> 4 mm) than the increase in skin thickness at the site of PPD-A injection [60].

The Bovigam® test measures the in vitro gamma interferon (IFN-γ) production of whole blood cells after exposure to PPD-B and PPD-A for 16-24 hours [59, 61]. IFN-γ production is quantified by a sandwich enzyme-linked immunosorbent assay (ELISA). A large disadvantage of the Bovigam® test is the requirement to quickly process the blood samples soon after collection; however, the method appeared to be somewhat more sensitive than SICCT [59, 61]. The polymerase chain reaction (PCR) is a technique for identifying the presence of bacteria-specific sequences of DNA. The genome of M. bovis has been sequenced [62]. For example in one study an optimised PCR test applied to bovine post mortem tissues resulted in a test sensitivity of 61-65% compared to conventional pathology and culture [63].

2.7 Identification of the Agent

In cattle, clinical evidence of tuberculosis is usually lacking until very extensive lesions have developed. For this reason, its diagnosis in individual animals and an eradication programme

were not possible prior to the development of tuberculin by Koch in 1890. Tuberculin, a concentrated sterile culture filtrate of tubercle bacilli grown on glycerinated beef broth and, more recently, on synthetic media, provides a means of detecting the disease [26].

2.7.1 Microscopic examination

Mycobacterium bovis can be demonstrated microscopically on direct smears from clinical samples and on prepared tissue materials. The acid fastness of M. bovis is normally demonstrated with the classic Ziehl– Neelsen stain, but a fluorescent acid-fast stain may also be used. Immunoperoxidase techniques may also give satisfactory results [26].

2.7.2 Culture

To process specimens for culture, the tissue is first homogenised using a mortar and pestle, stomacher or blender, followed by decontamination with either detergent (such as 0.375–0.75% hexadecylpyridinium- chloride [HPC]), an alkali (2–4% sodium hydroxide) or an acid (5% oxalic acid). The alkali or acid mixture is shaken for 10–15 minutes at room temperature and then neutralised. Neutralisation is not required when using HPC. The suspension is centrifuged, the supernatant is discarded, and the sediment is used for culture and microscopic examination.

It is recommended that, as a minimum, pooled lymph node samples from the head and thorax be cultured when no visible lesions are detected in tuberculin or interferon test positive animals at post-mortem examination [26]. For primary isolation, the sediment is usually inoculated on to a set of solid egg-based media, such as Lowenstein–Jensen, Coletsos base or Stonebrinks; these media should contain either pyruvate or pyruvate and glycerol. An agar-based medium such as Middlebrook 7H10 or 7H11 or blood based agar medium [64] may also be used. Cultures are incubated for a minimum of 8 weeks (and preferably for 10–12 weeks) at 37°C with or without

CO2. The media should be in tightly closed tubes to avoid desiccation. Slopes are examined for macroscopic growth at intervals during the incubation period. When growth is visible, smears are prepared and stained by the Ziehl–Neelsen technique. Growth of M. bovis generally occurs within 3–6 weeks of incubation depending on the media used [26].

2.8 Nucleic Acid Recognition Methods

Specific identification of an isolate as M. bovis can be made using PCR targeting a mutation at nucleotide positions 285 in the oxyR gene,169 in the pncA gene, 675/756/1311/1410 and 1450 of the gyrB gene and presence/absence of RDs (Regions of Difference) [65, 66, 67, 68]. Alternatively molecular typing techniques, such as spoligotyping will identify M. bovis isolates and provide some molecular-typing information on the isolate that is of epidemiological value [69].

2.8. 1 Delayed hypersensitivity test

2.8.1.1 The tuberculin test (the prescribed test for international trade)

A correct injection technique is important. The injection sites must be clipped and cleaned. A fold of skin within each clipped area is measured with calipers and the site marked prior to injection. A short needle, bevel edge outwards and graduated syringe charged with tuberculin attached, is inserted obliquely into the deeper layers of the skin. The dose of tuberculin is then injected. A multi-dose syringe or multiple injection guns may be used provided that delivery of the volume and safety are assured. The dose of tuberculin injected must be no lower than 2000 International Units (IU) of bovine or avian tuberculin.

A correct injection is confirmed by palpating a small pea-like swelling at each site of injection. The distance between the two injections should be approximately 12–15 cm. In young animals in

which there is no room to separate the sites sufficiently on one side of the neck, one injection must be made on each side of the neck at identical sites in the centre of the middle third of the neck. The skin-fold thickness of each injection site is re-measured 72 hours after injection. The same person should measure the skin before the injection and when the test is read [26].

2.9 Blood-Based Laboratory Tests

Besides the classical intradermal tuberculin test, a number of blood tests have been used [70] .Due to the cost and the more complex nature of laboratory-based assays, they are usually used as ancillary tests to maximise the detection of infected animals (parallel testing), or to confirm or negate the results of an intra-dermal skin test (serial testing). There is also evidence that when an infected animal is skin tested, an enhanced blood test can occur during the following week. This allows for better separation of in-vitro blood test responses leading to greater test accuracy. The gamma-interferon assay and the lymphocyte proliferation assay measure cellular immunity, while the ELISA measures humoral immunity.

a) **Gamma-interferon assay** (the alternative test for international trade)

In this test, the release of a lymphokine gamma interferon (IFN-γ) is measured in a whole-blood culture system. The assay is based on the release of IFN-γ from sensitised lymphocytes during a 16–24-hour incubation period with specific antigen (PPD-tuberculin) [71]

b) Lymphocyte proliferation assay

This type of in-vitro assay compares the reactivity of peripheral blood lymphocytes to tuberculin PPD (PPD- B) and a PPD from Mycobacterium avium (PPD-A). The assay can be performed on whole blood [(72)] or purified lymphocytes from peripheral blood samples [(73)].

c) Enzyme-linked immunosorbent assay

An advantage of the ELISA is its simplicity, but sensitivity is limited mostly because of the late and irregular development of the humoral immune response in cattle during the course of the disease. Specificity is also poor in cattle when complex antigens such as tuberculin or M. bovis culture filtrates are used. However, a comparison of antibody levels to PPD-B and PPD-A has been shown to be useful in increasing specificity in the ELISA [(74)].

For example, a lateral flow-based rapid test (TB StatPak) has been shown to be useful for detecting tuberculous animals, particularly in some domestic animals, wildlife [(75)] and zoo animals such as South American camelids , badgers [(76)] nonhuman primates or elephants [(77)] where no cellular immunity tests like the gamma- interferon tests are available and where skin testing has been proven unreliable [(26)].

Summary

In countries with endemic bovine TB, postmortem diagnosis by detection of gross lesions has been proposed [(54)]. In diagnosis of mycobacterial infections, culture is still considered the international gold standard [(55)] .However, due to dysgonic and slow growth characteristics, the identification of *M. bovis* by culture and biochemical methods is cumbersome and time consuming [(56)]

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Passive abattoir surveillance can be a cost effective method for surveying TB in animals [58]. In reality most of these animals will be infected but the post mortem surveillance, typically in conjunction with bacteriology and histopathology, will have failed to identify infection [38].

Tuberculin, a concentrated sterile culture filtrate of tubercle bacilli grown on glycerinated beef broth and, more recently, on synthetic media, provides a means of detecting the disease [26].

Mycobacterium bovis can be demonstrated microscopically on direct smears from clinical samples and on prepared tissue materials[26].

2.10 Treatment

Antimicrobial treatment has been attempted in some species, but the treatment must be long term, and clinical improvement can occur without bacteriological cure. The risk of shedding organisms, hazards to humans and potential for drug resistance make treatment controversial. In some countries, it may be illegal [25]. M. bovis is resistant to pyrazinamide, which is widely used in the treatment of infections caused by M. tuberculosis Complex in humans [78]. Cattle should not be treated at all and as such farm animals with tuberculosis must be slaughtered (culled) [25, 78]

2.11 Prevention and Control

In developed countries BTB has nearly been eradicated or drastically reduced in farm animals to low levels by control and eradication programmes [4, 79, 80]. Animal TB is endemic in many African countries because the economic constraints preclude the use of skin test and slaughter

control strategies, which have proved effective in the developed world [81]. The basic strategies required for control and elimination of bovine tuberculosis are well known and well defined. However, because of financial constraints, scarcity of trained professionals, lack of political will, as well as the underestimation of the importance of zoonotic tuberculosis in both the animal and public health sectors by national governments and donor agencies, control measures are not applied or are applied inadequately in most developing countries [79] .The level of testing and control of bovine tuberculosis in Africa is also considerably constrained by the lack of infrastructure, both veterinary and transport related [82]. Bovine tuberculosis can be controlled by test-and-slaughter or test-and-segregation methods. Affected herds are re-tested periodically to eliminate cattle that may shed the organism; the tuberculin test is generally used. Infected herds are usually quarantined, and animals that have been in contact with reactors are traced.

Only test-and-slaughter techniques are guaranteed to eradicate tuberculosis from domesticated animals [25]. Animals in traditional African farming systems are seldom culled and there is a greater chance for chronic tuberculosis in old cows, particularly those subjected to stress [83]. Even when cows are infected, it is difficult to force culling, because the cattle value is deeply interwoven with the social system and they are the savings of the rural poor [83]. Whole herd test and slaughter programmes have been used world-wide for the control of bovine tuberculosis as well as other diseases such as brucellosis and contagious bovine pleuropneumonia. A number of versions of the tuberculin test have been applied, but most programmes now use a purified protein derivative from culture of M. bovis (PPD-B) [84].

2.12 Public Health Significance

The link between animal and human tuberculosis has long always been known to be strong, as shown by the works of Villemin in 1865 [85,86] which demonstrated the cross- adaptability of the

tubercle bacilli from one species to another to cause disease; pointing out the danger that tuberculosis could be transmitted from animals to humans [85]. This was corroborated in 1902 by Ravenel [87], who demonstrated Mycobacterium bovis in a child with tuberculous meningitis. Human TB is mainly caused by M. tuberculosis but in regions where bovine TB is prevalent in animals, human TB cases due to M. bovis may occur [5,88] resulting from ingesting contaminated unpasteurised milk and raw meat and also by inhaling cough spray from infected livestock [4, 79, 88, 89]. Bovine TB is endemic and zoonotic TB as M. bovis is posing serious public health threats in most of Africa [4, 79,88]. Poverty therefore is not only a predisposing factor for the emergence of TB but also a consequence of it. Other risk factors include food hygiene practices like consumption of unprocessed dairy products, consumption of raw meat and HIV infection [79]. In addition human to human transmission of M. bovis and the emergence of multidrug resistant (MDR) M. bovis have also been documented, raising infection control concerns in health care settings [90, 91].

2.13 Risk Factors in Humans

Indirectly, man acquires the disease from animal sources by ingestion of meat and meat products from slaughtered infected cattle and consumption of unpasteurised infected milk [79,88,92]. Ingestion of unpasteurized contaminated milk products poses a greater risk than ingestion of infected meat products because badly infected carcasses are condemned; parts of carcasses that are processed as meat products are inspected and thoroughly cooked [93]. Thorough cooking [170°F (76.7°C) for 30 min] removes virtually all risk of infection [94, 95].

People suffering from M. bovis tuberculosis can retransmit the infection to cattle; however, this is not common [96] .The existing eating culture (eating of raw meat and drinking of raw milk), the very common close contact of animals with humans (most common in rural areas), inadequate meat inspection and the prevailing low standard of hygienic practices are potential risk factors that favours the spreading of zoonotic tuberculosis [3]. Development and spread of multidrug resistant strains and increased immigrant population from infected area to free zone or communities have been reported to increase the risk of infection [97]. People with HIV/AIDS, other forms of immmuno-suppression and debilitating disorders such as chronic renal disease, cancer, diabetes, etc are reported to be at risk of being infected on exposure to tuberculosis [98, 99].

If the apparent difference in virulence of M. bovis and M. tuberculosis in humans is the result of differences in responsiveness of the host defense mechanisms, HIV- induced immunosuppression could well lower host defences leading to overt disease after infection [79] there have been reported cases of HIV related human tuberculosis due to M. bovis. Other risk factors include food hygiene practices like consumption of unprocessed dairy products, consumption of raw meat and HIV infection /AIDS [79].

2.14 Individual at Risk

Research findings revealed that at risk, individuals are persons in contact with potentially infected animals such as veterinarians, abattoir workers, meat inspectors, autopsy personnel, farmers, milkers, animal keepers (as well as those in the zoo), animal dealers, laboratory personnel and owners of potential tuberculous pets (e.g. monkeys) [100,10

Summary

Retrospective study of bovine and human tuberculosisin abattoirs and hospitals in Enugu State, Southeast Nigeria reported underestimation of the real situation.

Mycobacterium bovis has been identified in humans in most countries where isolates of mycobacteria from human patients have been fully characterized. The incidence of pulmonary tuberculosis caused by M. bovis is higher in farm and slaughterhouse workers than in urban inhabitants [(27)]

There is relatively limited knowledge about the factors which influence susceptibility/resistance to bovine TB and this is an obvious blind-spot in the knowledge and evidence base, especially locally [(33)]

The basic strategies required for control and elimination of bovine tuberculosis are well known and well defined. However, because of financial constraints, scarcity of trained professionals, lack of political will, as well as the underestimation of the importance of zoonotic tuberculosis in both the animal and public health sectors by national governments and donor agencies, control measures are not applied or are applied inadequately in most developing countries [(79)]

Poverty therefore is not only a predisposing factor for the emergence of TB but also a consequence of it. Other risk factors include food hygiene practices like consumption of unprocessed dairy products, consumption of raw meat and HIV infection [(79)]

Indirectly, man acquires the disease from animal sources by ingestion of meat and meat products from slaughtered infected cattle and consumption of unpasteurised infected milk [(79,88,92)]

The existing eating culture (eating of raw meat and drinking of raw milk), the very common close contact of animals with humans (most common in rural areas), inadequate meat inspection

and the prevailing low standard of hygienic practices are potential risk factors that favours the spreading of zoonotic tuberculosis [3]

CHAPTER 3
3.0 MATERIAL AND METHODS

3.1 Area of Study

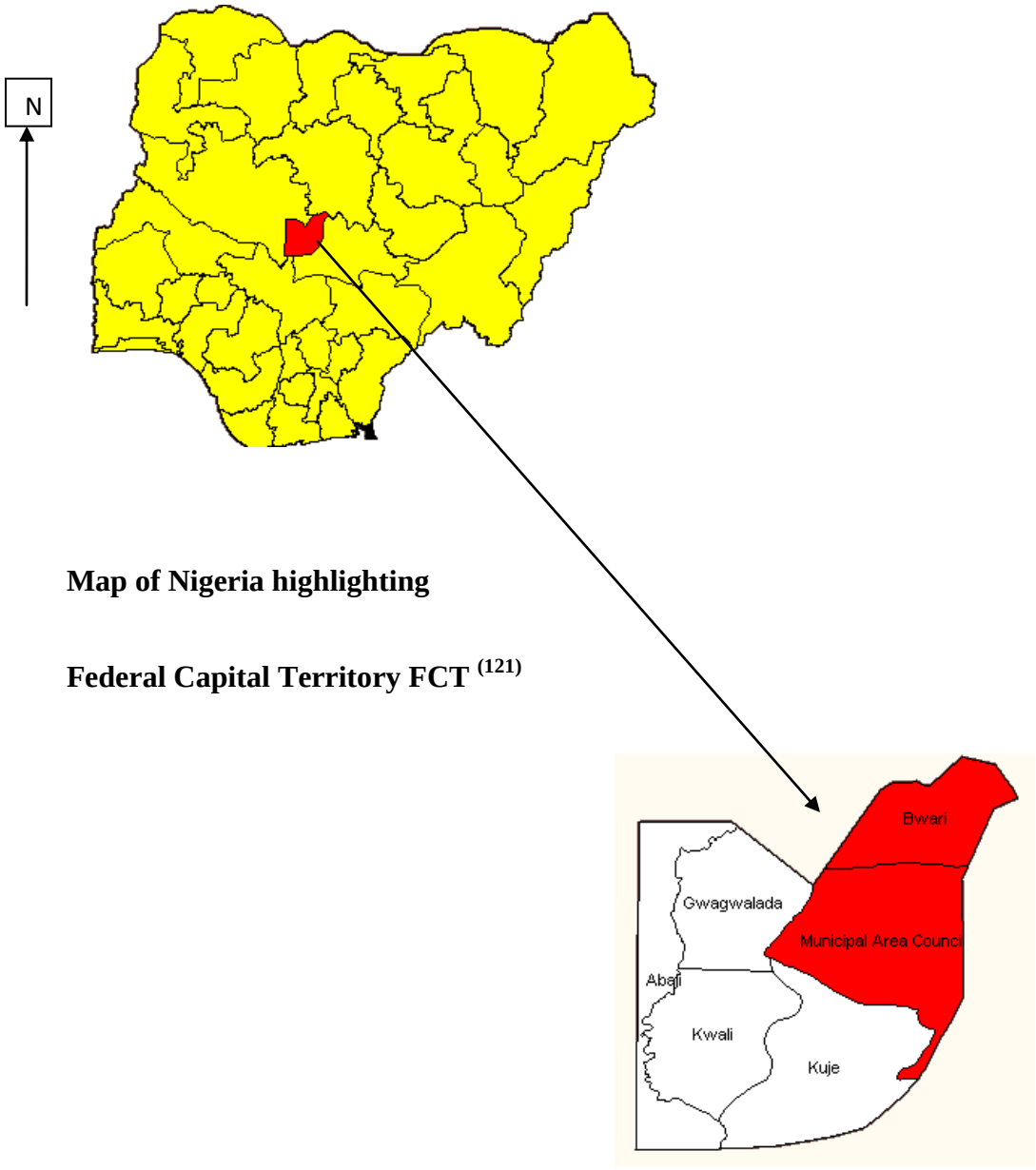
Abuja is the home of Federal Capital territory , the capital of Nigeria. The territory was formed in 1976 from parts of former Nassarawa, Niger and Kogi states. It is the central region of the country located just North of the confluence of Rivers Niger and Benue. It is bordered by the state of Niger to the West and North, Kaduna to the Northeast, Nassarawa to the east and south, and Kogi to the southwest lying between the latitude 8^0 50' N and 8.833^0 N, and longitude $7^0$10' E and 7.167^0 E. It covers total area of7,315 km^2 , of which the actual city occupies 275.3 km^2 and a total population of 1,405,201 according to 2006 census. It is situated within the Savannah region with moderate climatic conditions.

The territory is currently made up of six local councils, comprising the city of Abuja and five Local Government Areas, namely: Abaji, Gwagwalada, Kuje, Bwari, Kwali.

The study is to be conducted in 2 major abattoirs in randomly selected two area councils namely Bwari and Abuja municipal area councils managed by the Federal Capital Territory Administration.

Cattle are brought and slaughtered from livestock markets, Fulani herds and private farms mostly from Northern part of the country and neighboring African countries of Cameroun, Chad, Mali and Niger.

Abattoir operations are not standard and unhygienic giving room for potential contamination of the premises.



Map of Nigeria highlighting

Federal Capital Territory FCT [121]

Figure 1: Map of FCT Highlighting the Area Councils of Study State

3.2 Study Design

Cross-sectional study was carried out to determine the prevalence of bovine tuberculosis in serum of cattle slaughtered at study site. An analytical cross-sectional study was also conducted to determine factors responsible for the positivity of the sera against bovine tuberculosis. Cross-sectional study was carried out on the butchers and meat handlers at study site to determine the

3.3 Study Population

Human population

3.3.1 Inclusion criteria

All butchers and meat handlers who are 18 years and above present at the abattoir at the time of the study were included

3.3.2 Exclusion criteria

All individuals at the abattoirs at the time of the study who are not butchers or meat handlers and below 18 years of age were excluded.

Animal population

Inclusion criteria: All cattle at the abattoir at the time of sample collection were included.

Exclusion criteria: All animals at the abattoir other than cattle and cattle whose owner did not consent at the time of the study were excluded.

3.4 Sample Size Determination

Animal sample size for determining antibodies

$$N = \frac{Z^2 pq}{d^2}$$

where q = 1-p

N = Minimum sample size + an additional 10% of randomly selected animals in cases some samples may not be used

P = prevalence rate 12.5% [103]

d = desired absolute precision 5% (0.05)

Z = appropriate value for standard normal deviation at 95% C.I (1.96)

Therefore $\frac{1.96^2 \times 12.5\% \times (1 - 12.5)}{0.05^2} = 168.1$

Approximately 185 blood samples and only tissue samples suggestive of tuberculosis will be collected

Human sample size for assessment

$$N = \frac{Z^2 pq}{d^2}$$

where q = 1-p

N = sample size

P = proportion rate 28.1%[104] of individuals with knowledge of BTb

d = desired absolute precision 8% (0.08)

Z = appropriate value for standard normal deviation at 95% C.I (1.96)

Therefore $\frac{1.96^2 \times 28.1\% \times (1 - 28.1\%)}{0.08^2} = 121.3$

10% attrition rate of 121.3 = 12.1

121.3+12.1= 133.4

157 Questionnaires was administered to butchers and meat handlers

3.5 Sampling Technique

Animal sampling

Selection of animals was done systematically. Whole Blood was collected during bleeding of animal while only tissues suggestive of tuberculosis was collected for Acid Fast Test. In veterinary practice lymphnode from respiratory tract, lung and liver tissue, lymphnodes from gastrointestinal tract and pus or caseous material from open tuberculous cavities are, in that order, the specimen most frequently submitted for laboratory examination. They are usually selected at veterinary inspection in slaughterhouses when macroscopic lesions suggestive of tuberculosis are detected [105]. Animals were examined just before slaughter to record body

condition; as good, fair, or poor, the age; as adult or young and sex; male or female. About 5mls of whole blood was collected. Samples was labeled and transported on ice in a cooler to the laboratory to be stored in refrigerator for not more than three days until processed. Individual information on animal such as age, sex, breed, body condition scores was taken during blood collection.

Slaughtered animals were examined, at post mortem for TB lesions. Visceral organs and lymph node were inspected through careful visual palpation and incision procedures for nodules' and granulamatous lesions as described by [55, 106] .Tissue samples were collected aseptically from animal with suspected lesions of TB in sterile screw – capped containers and transported on ice box to the laboratory where they were kept frozen until processed

Human Sampling

Stratified sampling was used for selection of study subjects. Two groups of butchers and meat handlers were used. Sampling frame for each group was prepared from abattoir register and from each group individuals were selected by simple random sampling by ballot.

Informed consent was obtained from study participant and confidentiality was assured.

Study was conducted from February, 2013 to April, 2013. Data was collected through interviewer administered structured questionnaire by veterinary personnel trained for the interview at the time of sample collection from butchers, meat handlers.

3.7 Data Collection Method

Technique for Laboratory

Acid fast stain (Ziehl Neelsen)

A small amount of the sample (tissue) is placed on a glass slide stained and heated. The cells in the sample hold on to the dye. The slide is the washed with an acid solution, after which a different stain is applied. The ability of the sample holding on to the first dye even after the acid wash gives a positive result [107].

Steps of Acid Fast Staining Procedure

1. Drop suspension of sample onto slide
2. Air dry slide 10 minutes at 60 °C, heat-fix slide 10 minutes at 90 °C
3. Flood slide with Carbol Fuchsin (Primary stain), the cell will hold on to the dye
4. Hold a flame beneath the slide until steam appears but do not allow it to boil
5. Allow hot slide to sit for 3 to 5 minutes, rinse with tap water
6. Flood slide with 3% hydrochloric acid in isopropyl alcohol(Decolourizer)
7. Allow to sit for 1 minute, rinse with tap water
8. Flood slide with Methylene Blue (Counter stain)
9. Allow to sit 1 minute, rinse with tap water
10. Blot dry
11. View under oil immersion lens [108, 109]

LATERAL FLOW TECHNIQUE (One step Anigen Bovine TB Antiboby Rapid Test)

Is a solid phase chromatographic immunoassay for the qualitative detection of *Mycobacterium bovis* antigen in serum or plasma.

Recently, the MPB 70 protein was revealed to be a highly species specific protein secreted by *Mycobacterium bovis*, resulting in the development of the Anigen Rapid Bovine TB Ab test kit.

Principle: Immunochromatographic assay using Direct Sandwich Method

- (Rec. MPB70 Ag capture)-(Antibody in blood)-(Rec.MPB70 Ag detector)
- Capture and detector material: Recombinant MPB70 Antigen

Purpose: Detection of antibodies (IgM, IgG) against *Mycobacterium bovis*

Sensitivity: 90% vs BTB confirmed by Bacterial isolation

85.1% vs Purified Protein Derivative (PPD) test

Specificity: 98.6% vs Purified Protein Derivative (PPD) test

No cross reaction against *Mycobacterium avium, Mycobacterium tuberculosis*

Questionnaire Administration

The structured questionnaire contained the information on the following variables will be administered to butchers and meat handlers to assess for their level of awareness and risk factors of Bovine tuberculosis infection.

- Demographic variable - Name, Age, Sex, Marital status, Educational qualification, Duration of work at abattoir and No of people living in residence
- Knowledge of the Disease - Mode of transmission, Keeping animals and other animal hosts
- Work related exposure factors - eating and drinking at work place,frequency of drinking unboiled fresh and sour milk, consumption of meat with lesion, practice upon seeing organ with tuberculous lesion
- Medical History – history of persistent cough and duration, family history of tuberculosis treatment and BCG
- Preventive practices – history of receiving training and willingness to receive more training

The questionnaire was pre – tested in Lafia abattoir. Interviews were conducted on eligible participants during sample collection

3.8 Data Management and Analysis

All completed questionnaires were reviewed on the field to ensure it was administered correctly by the interviewers. Data collected was entered, cleaned and analysed using Microsoft excel and Epi info 7 version. A total of 156 questionnaires were administered and all the study participants responded appropriately giving a response rate of 100%. The analysis of the data was done in a step wise manner; univariate, bivariate and multivariate analyses. The study results are presented in different sections in line with the specific objectives of the study. Descriptive and analytical statistics were used to summarize the data obtained (percentage, 95% confidence interval, means, standard deviation). The level of knowledge of disease was compared across the investigated variables using odds ratio. Test results were considered as significant if p-value was ≤ 0.05. Factors that were found to be significant in the Bivariate analyses were subjected to Multivariate analyses to control for confounding and effect modification. Tables and figures were used for an all-inclusive presentation of the results.

3.9 Ethical Considerations

Approval for this study was obtained from the Federal Capital Territory Health Research Ethics Committee as well as the Scientific and Ethical Committee of the Ahmadu Bello University Teaching Hospital, Zaria. Permission was also obtained from the Management of each abattoir where the study was carried out. Informed consent was obtained from each eligible abattoir worker before questionnaire administration and sample collection. Confidentiality of information obtained was assured and maintained.

3.10 Limitations

The study did not include cattle whose owner did not consent and other food animals such as sheep, goat and pigs, although operations for this animals are carried out at a different section of the abattoir.

The administration of the questionnaires through face to face interviews may increase likelihood of respondents' inclination to give socially acceptable answers giving a false increase in the result.

CHAPTER 4

4.0 RESULTS

4.1 The number of serum samples analysed in two abattoirs

The number of serum samples analysed as shown in table 1; The average daily slaughter figure in the two abattoirs was 87 cattle, out of which 73 were in Karu and 14 were in Kubwa abattoirs. A total of 185 samples were analysed, out of which 155 were from Karu and 30 from Kubwa.

4.2 Prevalence of *Mycobaterium bovis* in Sera of Cattle at Slaughter

The prevalence of bovine tuberculosis in cattle screened at the two abattoirs; Karu and Kubwa are as shown in table 2. Out of a total of 185 serum samples screened at both abattoirs, 32 (17.3%) was positive for the lateral flow technique.

4.3 Visual Identification of Lesion in Tissues of Cattle Slaughtered

Of the 185 cattle from which blood for serum was taken, 5 (2.7%) had visible lesions suspected to be bovine tuberculosis as shown in table 2.

4.4 Microbacterial detection of *Mycobacterium bovis* in suspected tuberculous tissues

Five (5) suspected tissue samples were screened at both abattoirs all 5 were positive for the Ziehl Neelsen test as shown in table 2.

4.5 Prevalence of bovine tuberculosis in male and female cattle slaughtered

Out 185 serum samples 48 were from female and 137 were from male as shown in table 3. Fourteen (14) of the 48 were positive for lateral flow technique giving a prevalence of 29.2% in female while 18 of the 137 were positive for lateral flow technique giving a prevalence of 13.1% in male.

All tissue samples analyzed were from female and all were positive for Ziehl Neelsen test.

4.6 Body condition data of sero-positive cattle

Table 4 shows body condition data of sero-positive cattle. Six (6) out of 28 cattle with fair body condition were positive for lateral flow technique giving a prevalence of 17.6%, 23 out of 119 cattle with good body condition were positive giving a prevalence of 16.2% and 3 out of 6 cattle with poor body condition were positive giving a prevalence of 50%.

4.7 Analysis of Questionnaire

Of the 156 Butchers and Meat handlers on whom the questionnaire was administered115 (73.8%) were men and 41 (27.2%) were women. The proportions of the various abattoir workers in the category of each variable investigated during the course of this study are shown in Table 5

Table 1: The number of samples analysed in two abattoirs in F.C.T, North central, Nigeria, March, 2013

Study site	Average slaughter figure	Proportion	No of samples
Karu	73	83.9	155
Kubwa	14	16.1	30
Total	87	100	185

Table 2: Prevalence of bovine tuberculosis in cattle slaughtered at two abattoirs in F.C.T, North central, Nigeria, March, 2013

Site	No of samples	Rapid test positive	Percentage %	Ziehl Neelsen positive	Percentage %
Karu	155	28	18.1	4	80
Kubwa	30	4	13.3	1	20
Total	185	32	17.3	5	100

Table 3: Prevalence of bovine tuberculosis in male and female cattle slaughtered at the two abattoirs

Sex	No of samples	Rapid test positive	Percentage %	Ziehl Neelsen positive	Percentage %
Female	48	14	29.2	5	10.4
Male	137	18	13.1	0	0

Table 4: Body condition data of sero-positive cattle slaughtered at the two abattoirs in F.C.T, North Central, Nigeria, March, 2013

Body Condition of Cattle	Sero-positive	Confidence Interval 95%	Sero-negative	Confidence Interval 95%	Percentage %
Fair	6	[7.2-36.4]	28	[12.5-25.4]	17.6
Good	23	[53.3-86.3]	119	[70.4-84.1]	16.2
Poor	3	[2.0-25.0]	6	[1.5-8.3]	50.0

Table 5: Bivariate analysis of sero-positive cattle slaughtered at the two abattoirs in F.C.T, North Central, Nigeria, March, 2013

Variable	Odds Ratio	Confidence Interval (CI) 95%	P-value
Body condition	1.3	[0.6 -3.2]	0.3
Sex	2.7	[1.2 – 6.0]	**0.01**

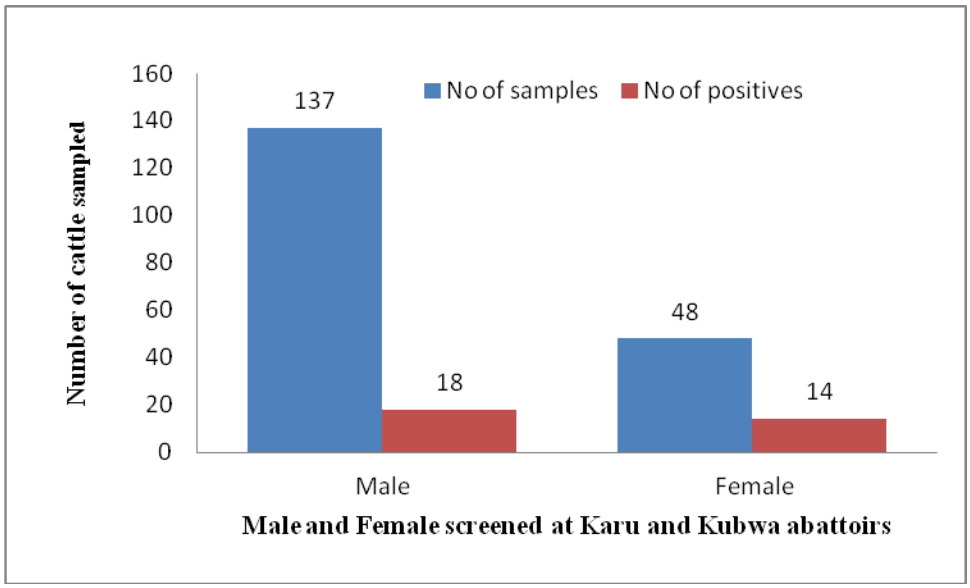


Figure 2: Sex distribution of cattle screened at Karu and Kubwa abattoirs,F.C.T, North Central Nigeria.

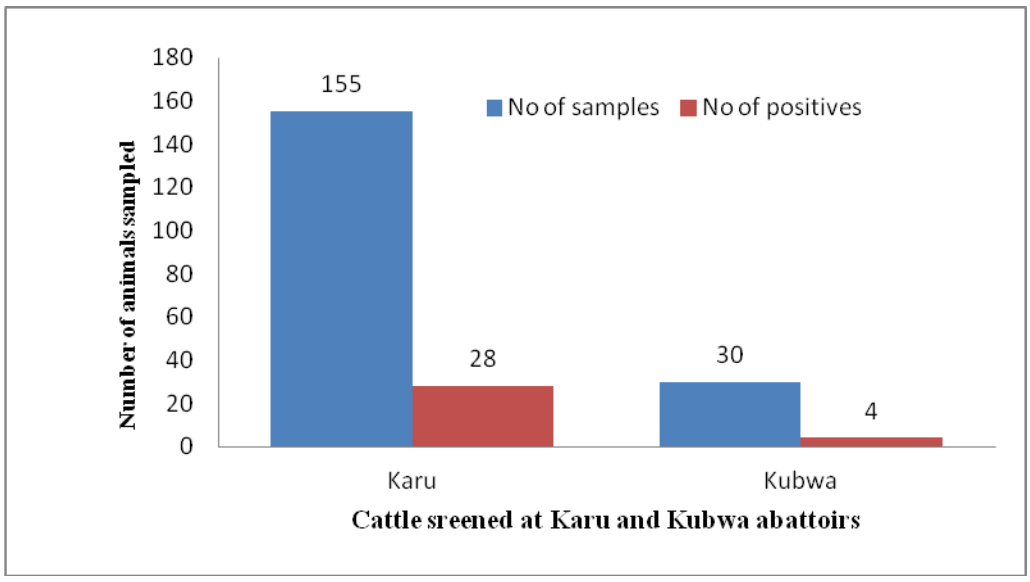


Figure 3: Distribution of seropositives among cattle slaughtered at Karu and Kubwa abattoirs, F.C.T, North Central Nigeria, March, 2013

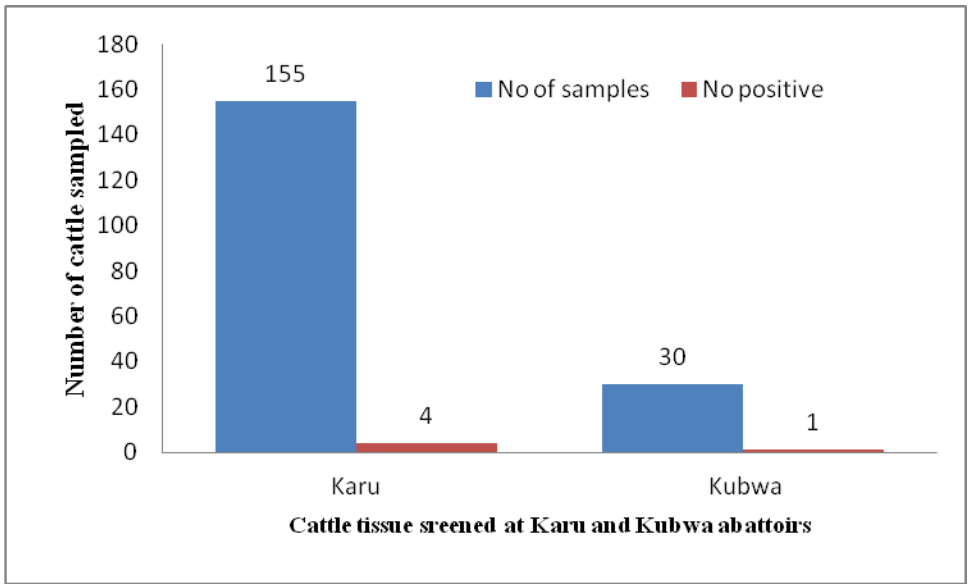


Figure 4: Distribution of tissues positive to Ziehl Neelsen test among cattle slaughtered at Karu and Kubwa abattoir abattoirs, F.C.T, North central Nigeria, March, 2013

Table 6: Demographic characteristics of butchers and meat handlers at two abattoirs in FCT, North Central Nigeria, March, 2013

Characteristics	Female (n=41)	Male (n=115)
Age (Mean and Standard Deviation)	25.5 ± 5.3	30.5 ± 9.4
Marital Status		
Single	18(43.9%)	35(30.2%)
Married	20(48.8%)	80(69.8%)
Divorced	0(0%)	0
Widowed	4(9.6%)	0
Educational Status		
Primary	22(53.6%)	45(39.1%)
Secondary	18(43.9%)	60(52.2%)
Tertiary	1(2.4%)	3(2.6%)
Others (Qur'an)	0	7(6.1%)
Length of work at abattoir		
1 -2 Years	27(65.8%)	20(17.4%)
3 – 5 Years	3(7.3%)	30(26.1%)
>6 Years	19(46.3%)	57(49.6%)

Mean age of the butchers and meat handlers was approximately 29.2years. Most (73.7%) of the participants were male and 63.7% of all the participants were married. Majority (75.6%) of the participants claim to know bovine tuberculosis. Of the 156 butchers and meat handlers, 67 (42.9%) had primary education while 78 (49.7%) had secondary education.

Table 7: Assessment of Questionnaire

Variable	Mean score	Percentage %
Knowledge of the Disease	4.5	53.2
Work related exposure factors	8.8	75
Medical history	1.46	42.3
Preventive practices	1.2	21.8

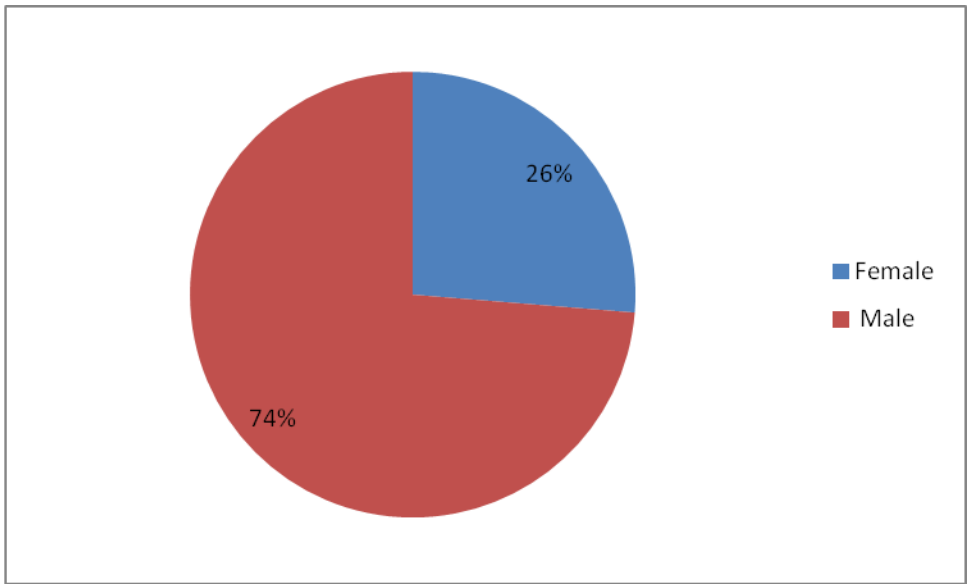


Figure 5: Sex distribution of butchers and meat handlers at the two abattoirs in F.C.T, North Central, Nigeria, March, 2013

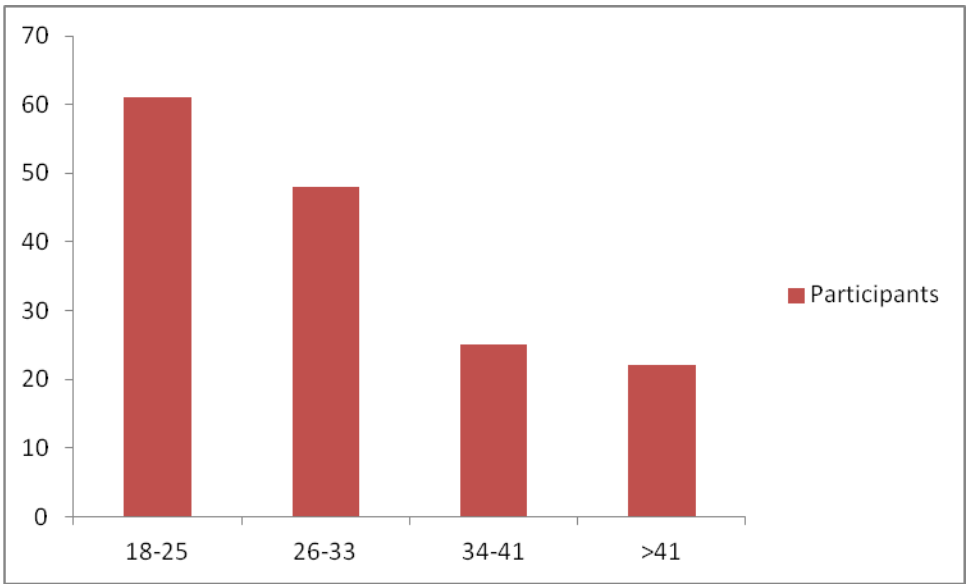


Figure 6: Age distribution of Butchers and meat handlers at the two abattoirs in F.C.T, North Central, Nigeria, March, 2013

Majority of the participants fell within the age group of 18-25 (39.1%)years

Table 8: Bivariate analysis of risk factor for bovine tuberculosis in butchers and meat handlers in two abattoirs in FCT, North Central, Nigeria, March, 2013

Variable Exposure	Odds Ratio	Confidence Interval [CI 95%]	P - value
Length worked at the abattoir >6 / <6 years	3.4	[1.6 -7.4]	0.001*
Do you know the mode of transmission- Yes/ No	2.4	[1.1-5.1]	0.02*
Do you keep animals at home- Yes/ No	1.8	[0.8-4.6]	0.1
Are you aware of BTB in wild animals- Yes /No	1.4	[0.7-3.1]	0.2
Potential risk associated With your job- Yes /No	2.4	[1.1-5.0]	0.02*
Do you eat or drink at place of work- Yes /No	0.6	[0.2-1.4]	0.2

Table 9: Bivariate analysis of risk factor for bovine tuberculosis in butchers and meat handlers in two abattoirs in FCT, North Central, Nigeria, March, 2013

Variable **Exposure**	**Odds Ratio**	**Confidence Interval** **[CI 95%]**	**P - value**
Do you drink fresh milk- Yes/ No	0.4	[0.2-0.8]	0.006*
Do you boil your milk before drinking- Yes/ No	0.4	[0.2-0.9]	0.02*
Do you drink sour milk- Yes/ No	1.2	[0.5-3.3]	0.5
Seen a BTB lesion in your slaughtered animal- Yes/ No	0.2	[0.1-0.5]	0.0001*
What's done to lesion-call meat inspector/ Ignore	2.2	[1.0-4.8]	0.04*

Table 10: Bivariate analysis of risk factor for bovine tuberculosis in butchers and meat handlers in two abattoirs in FCT, North Central, Nigeria, March, 2013

Variable Exposure	**Odds Ratio**	**Confidence Interval**	**P - value**
Do you consume meat with these lesions- Yes/ No	2.2	[1.1-4.7]	0.03*
Have/had persistent cough- Yes/ No	1.8	[0.7- 4.2]	0.2
Vaccinated with BCG- Yes/ No	0.7	[0.3-1.4]	0.2
Received any training on BTB- Yes/ No	0.8	[0.3-1.7]	0.3

- Values significant at ≤ 0.05

The odds ratio revealed the following:

Those that worked at the abattoir for greater than 6 years were associated with 3.4 times increased risk of exposure compared to those than worked for less than 6 years.

Those that did not know the mode of transmission were 2.4 times more at risk of exposure than those that knew.

Keeping animals at home was associated with increased risk compared to those that did not keep animals at home.

Lack of awareness of disease in wild animals is 1.4 times associated with increased risk compared to those that aware of disease in wild animals.

Those that did not know the potential risk associated with their job were 2.4 times more at risk than those that knew.

Those that did not eat or drink at place of work are 0.6 time associated with decrease risk of exposure compared to those that ate and drank at place of work.

Drinking fresh milk without boiling is 0.4 time more likely to be exposed than those that boil milk before drinking.

Drinking sour milk increases the risk of exposure 1.2 time than not drinking.

Those that saw lesion were 0.2 time more at risk than those that did not see lesion.

Those that ignore lesion were 2.2 times associated with increased risk of exposure than those that called the attention of the meat inspector.

Consuming meat with lesion inreases the risk of exposure 2.2 times than those who do not consume meat with lesion.

Having or had persistent cough increases risk of exposure 1.8 times compared to those that do not have or never had.

No BCG vaccination or not receiving training on BTB were 0.7 and 0.8 time respectively associated with increased risk of exposure than those that have been vaccinated or have received training on BTB.

Bivariate analysis showed the following to be significant at p-values of ≤ 0.05: length worked at the abattoir >6 years, do you know the mode of transmission, potential risk associated With your job, do you drink fresh milk, do you boil your milk before drinking, seen a BTB lesion in your slaughtered animal, what's done to lesion, do you consume meat with these lesions.

Table 11: Shows values of multivariate analysis of risk factors for bovine tuberculosis in butchers and meat handlers in two abattoirs in FCT, North Central, Nigeria, March, 2013

Term	Odds Ratio	95% C.I.			P-Value
Length Work	3.4705	[1.3340	-	9.0288]	0.0108*
Transmission	1.5010	[0.5949	-	3.7876]	0.3898
Risk to job	1.5534	[0.5793	-	4.1652]	0.3815
Drink fresh Milk	0.4643	[0.1895	-	1.1376]	0.0933
Boil Milk	0.8607	[0.3248	-	2.2813]	0.7630
Lesion in animal	0.2993	[0.1193	-	0.7512]	0.0102*
Action on lesion	1.2999	[0.5062	-	3.3385]	0.5857
Consume lesion	1.8299	[0.7445	-	4.4975]	0.1878

***Values that remained significant in the unconditional logistic regression model.**

Two factors – working at the abattoir for over six years (p = 0.01) and seeing lesion in slaughtered animal (p = 0.01) remained statistically significant among all factors that were significant at Bivariate analysis. These two factors remained as independent risk factors for exposure to bovine tuberculosis in abattoir workers.

4.8 Multivariate Analysis of Risk Factor For Bovine Tuberculosis in Butchers And Meat Handlers

Multivariate analysis

To perform multivariate analyses, an unconditional logistic regression model was developed using the "step-up" approach. The final model developed for this analysis is shown in Table 4.8. After controlling for the length of time working at the abattoir and consumption of meat with lesion were more likely to be exposed (Adjusted Odds Ratio (AOR) = 3.**5**; 95% Confidence Interval (CI) = 1.3 – 9.0, p = 0.01) and 0.3; 95% Confidence Interval (CI)=0.1-0.8, p=0.01 However there was no association found between Knowledge and other variables investigated (Table 4.8).

4.9 Risk Factors Associated With Sero-Positivity in Cattle Slaughtered at The Two Abattoirs In Abuja

Factors associated with seropositivity to BTB Infection in cattle screened at the two Abuja abattoirs are shown in Table 5. The only risk factor associated with BTB infection in these cattle was the sex (p = 0.01).

CHAPTER 5

DISCUSSION

Serum analysis with lateral flow technique revealed a prevalence of 17.3% as shown in table 2. This is higher than the prevalence value of 12.5% using the same lateral flow technique reported by Dan birnin et al [(103)] but lower than prevalence value of 60% reported by Awah Ndukum et al[(110)] . The difference may be due to number of animals and location. The prevalence of 2.7% was revealed based on the visual identification of lesions in tissues of cattle slaughtered as shown in table 2, is similar to prevalence of 2.8% reported by Igbokwe [(111)] but higher than values of 0.6% reported by Cadmus et al [(112)] . The difference may be due to the number of animals used for the study and for detection of bovine tuberculous lesions in particular depend on the work load, time and the diligence of the inspector conducting the examination Corner et al. [(113)]. Despite this, detection of tuberculous lesions in abattoirs can be affected due to early infection or parasites, non-specific reactions and due to infection other than *M. bovis* as reported by Corner, [(55)] and other irregularities of abattoir meat inspections as was documented by Edwards et al. [(114)]. The tissue stained with Ziehl Neelsen revealed a prevalence of 100% as all 5 tissue samples were positive as shown in table 2. This is higher than a prevalence value of 31% reported by Awah Ndukum et al[(110)] . The difference could be due to the proportion of the tissues with lesions detected.

The prevalence from this study suggests that meat being sold for public consumption is either diseased or exposed to several levels of contamination which could endanger the public health. The risk of disease transmission from abattoir to the public at large is very high.

The Seroprevalence was more in Female 29.2% than in Males 13.1% (OR = 2.7, 95% CI = 1.2 - 6.0) , prevalence due to visual identification lesion on tissues 2.5% in females and 0% in males as well as in the Ziehl Neelsen test 100% in female and 0% in males all shown in table 3. This is

consistent with the herd prevalence of 50% reported by Firdessa et al [115] where 94% of the positives were females. This demonstrates husbandary conditions as a major influence on the prevalence of BTB as reported by Ameni et al [116]. Intensification, stress and overcrowding are possible explanations for such relationship. The same report of higher prevalence in female than male was reveal in a study by Maxwell et al [117] . COW with only minimal lung lesions may spread the infection through- out the entire herd [50].

The higher prevalence in female animals suggest that they shed the infection for a long time, spreading the disease to other animals through feaces close contact and to humans through consumption of milk and close contact with animal handlers.

The seroprevalence according to the body score of the cattle as shown in table 4 were 17.6%, 16.2% and 50% for Fair, Good and Poor body condition respectively. This is similar to a study reported by Firdessa et al where the body condition scoring of the nearly 3,000 animals suggested no significant differences between tuberculin reactors and non-reactors and a poor correlation was also seen among the 36 slaughtered animals [115] .

The knowledge of the butchers and meat handlers was found to be an average score of 4.5 and it was reveal that 53.2% of the participant had a good knowledge of the disease and it's means of transmission as shown in table 7.

The work related exposure factor had a mean score of 8.8 and 75% of the participants priotize the message pertaining to bovine tuberculosis as shown in table 7.

The medical investigation had mean score of 1.46 and 42.3% of the respondents had a good medical history as shown in table 7.

The assessment of preventive practices reveal a mean score of 1.2 and 21.8% claim to practices safely as shown in table 7.

This is consistent with findings from the few studies that assessed knowledge and behaviour amongst high risk groups in similar settings in other African countries. Swai et al assessed the knowledge and practice of animal health workers and livestock keepers in Tanzania. They concluded that, inadequate awareness (53.2%) and lack of knowledge of zoonoses (46.8%) combined with food consumption habits and poor animal husbandry are likely to expose respondents to an increased risk of contracting zoonoses [118] .

Amenu et al also documented the lack of accurate knowledge on transmission of zoonoses and the prevalence of risky behaviour, as consumption of raw animal products and unsafe slaughtering practices in a rural district in Ethiopia [119] .

Similarly, Mfinanga et al investigated the level of knowledge and prevailing practices in rural Tanzania and found that about 40% of respondents practice habits deemed to be 'high risk' for exposure to BTB, while 75% exhibited poor knowledge of TB[120] .

The unhygienic and unwholesome practices of hiding infected products as shown in table 9 increase the risk of selling to the public infected and contaminated meat product.

Over time and with repeated meat inspections butchers acquire ample knowledge about the nature of pathologies that can lead to condemnation of carcasses just from observing the activities of the veterinary staff. Unruly butchers could obstruct inspection of their animal carcasses or hide lesions from unassisted inspectors. Similar findings have been reported by Cadmus and Adesokan in Nigeria that pathological cases including zoonoses in slaughtered animals were missed due to uncooperative attitudes of butchers in ensuring thorough meat inspection [112]

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The study establishes the presence of the Infection in the animals slaughtered as shown in table 2, with higher prevalence in female as shown in table 3 at the two Abuja abattoirs. The study also confirmed that routine abattoir surveillance of bovine tuberculosis through identification of lesions in meat under reports the prevalence of the disease as shown in table 2.

The study has also shown that butchers and meat handlers have an inadequate knowledge of the disease, work related exposures are eminent and preventive practices are not in use as shown in table 7.

6.2 Recommendations

Based on the findings of this study, the following recommendations are hereby suggested;

1. The FCT Department of Agriculture and Rural Development which manages these abattoirs should;

a) Carry out further screening of cattle for bovine tuberculosis to establish the true prevalence of the disease in the Federal Capital Territory

b) Encourage livestock farmers to screen their animals regularly to control the disease at farm level.

c) Organize public enlightenment campaigns aimed at highlighting the zoonotic importance of bovine tuberculosis among abattoir workers who are at high risk of acquiring the disease because close contact

d) Have regular surveys organized by the Federal and State Ministries of Agriculture on Bovine Tuberculosis among abattoir workers and others at risk because of their occupation as a lot of data could be generated for the National Animal Disease Surveillance and Response (NADIS) of Federal Department of Livestock for its control.

2. Non Governmental Organizations or Animal Insurance Organizations should formulate a policy of compensating owners of positive animals or condemned organs to augument efforts of government.

3. Government at all levels should organize public enlightenment campaigns aimed at highlighting the zoonotic importance of Bovine Tuberculosis among Butchers and meat handlers who are at high risk of acquiring the disease.

   a. Butchers and meat handlers should be discouraged from eating raw meat and educated on adherence to safe animal-product handling practices

   b. Educate those involved in animal handling in any form on the need to adopt strict hygiene measures especially washing their hands properly with soap by using the electronic and print media.

   c. Encourage Butchers and meat handlers to seek medical care early to treat persistent cough.

   d. Health education and the use of personal protective equipment by abattoir workers can go a very long way in preventing infection in those at risk.

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Appendix 1: Questionnaire

PREVALENCE OF BOVINE TUBERCULOSIS IN TWO ABUJA ABATTOIRS

AWARENESS QUESTIONAIRE No ☐

I am Dr KACHALLA Mairo Gujba, a resident of the Nigeria Field Epidemiology and Laboratory Training program (NFELTP)

This questionnaire is to assess the knowledge attitude and practices among butchers and meat handlers on bovine tuberculosis (BTB) in the abattoirs.

The data gathered is intended to be used to improve on the current situation with (BTB) in the abattoirs in Abuja, FCT.

I hereby solicit your participation in this survey and assure you that all information will be treated in confidence.

Filling the questionnaire at will is considered as your consent.

Section A: Personal Information.

Name: _____

First Last

1. Age: _____
2. Sex: _____
3. Marital status: _____
4. Educational Qualification: list
   (a) (b) (c) (d)

5. How long have you worked at the abattoir? _____

6. Number of people living with you in your residence: _____

Bovine Tuberculosis awareness information:

7. Do you know what Bovine Tuberculosis (Tarin fuka) is?

Yes ☐ No ☐

If yes, for how long, _____

8. Do you know the mode of transmission (contacting) of disease?

Yes ☐ No ☐

If yes, how many modes of transmission (contacting) do you know? _____

10. Do you keep animals at home?

Yes ☐ No ☐

11. Are you aware of BTB in wild animals?

Yes ☐ No ☐

If yes, what animals do you know that have TB, _____

Work related exposure

12. Do you know the potential risk of contracting the disease associated with your job?

Yes ☐ No ☐

13. Do you drink fresh milk?

Yes ☐ No ☐

If yes, how often?

Daily ☐ Weekly ☐ Monthly ☐ > Monthly ☐ Never ☐

14. Do you boil your milk before drinking?

Yes ☐ No ☐

15. Do you drink sour milk?

Yes ☐ No ☐

If yes, how often?

Daily ☐ Weekly ☐ Monthly ☐ > Monthly ☐ Never ☐

16. Have you ever seen a BTB lesion?

Yes ☐ No ☐

If yes, how often do you see these lesions?

i. Always ☐

ii. Sometimes ☐

17. What do you do when you come across such lesions?

a. Call the attention of the meat inspector ☐

b. Discard immediately ☐

c. Ignore ☐

18. Do you consume meat with these lesions?

Yes ☐ No ☐

19. Do you always agree with the meat inspector's decision regarding the lesion?

Yes ☐ No ☐

Medical History

20. Do you or have ever had cough?

Yes ☐ No ☐

If yes, what was the duration?

1 month ☐ 3 months ☐ 6 months ☐ 1 year ☐ > 1 year ☐

21. Have any of your resident members had/have been treated for BTB in last 12 months?

Yes ☐ No ☐

If yes, at what age did they receive the treatment?

Specify ☐

22. Are all members of your household vaccinated with BCG?

Yes ☐ No ☐ Don't know ☐

Preventive practices

23. Have you ever received any training on BTB?

Yes ☐ No ☐

24. Are you willing to improve on your knowledge of BTB?

Yes ☐ No ☐

Thank you for providing the above information.

Appendix 2: Sample Collection Form

PREVALENCE OF BOVINE TUBERCULOSIS IN ABUJA ABATTOIRS

Name of Abattoir

Date

Animal ID No

Sex Male ☐ Female ☐

Age Adult ☐ Young ☐

Breed Local ☐ Exotic ☐

Body Condition of animal Good ☐ Fair ☐ Poor ☐

Specimen type:

Blood for serum ☐

Tissue with suspected TB lesion (specify) ☐

Appendix 3: Ethical Considerations

Ahmadu Bello University Teaching Hospital

P.M.B. 06, Shika - Zaria, Kaduna State, Nigeria. 069-876305
website: www.abuth.org abuthshika@yahoo.com Abuthshika@gmail.com

Chairman of Board:
Chief Medical Director: **DR. LAWAL KHALID,** *MBBS, FMCS, FWACS, FRCS(ED) mni*
Chairman, Medical Advisory Committee: **DR. ABDULLAHI MOHAMMED,** *MBBS, FWACP FICS*
Director of Administration: **BARR. ISHAK BELLO,** *LL.B, BL, LL.M, PGDM, AHAN, FCAI*

Our Ref: ABUTH/HREC/TRG/36 26th March, 2013

Dr. Aishatu Bintu Gubio
Public health,
Faculty of Medicine,
ABU, Zaria

ETHICAL CLEARANCE.

Your application for ethical clearance on the research proposal titled: - **"Seroprevalence of Rubella Virus among pregnant women in Kaduna State Nigeria."** refers.

This is to convey ethical approval for you to commence the study. The ABUTH Scientific and Health Research Committee require an annual update from the Principal Investigator.

Thank you.

PROF. J.U OKPAPI
CHAIRMAN, ABUTH HREC

Appendix 4: Test Kit and Illustration of procedure



Test Kit



Result Window

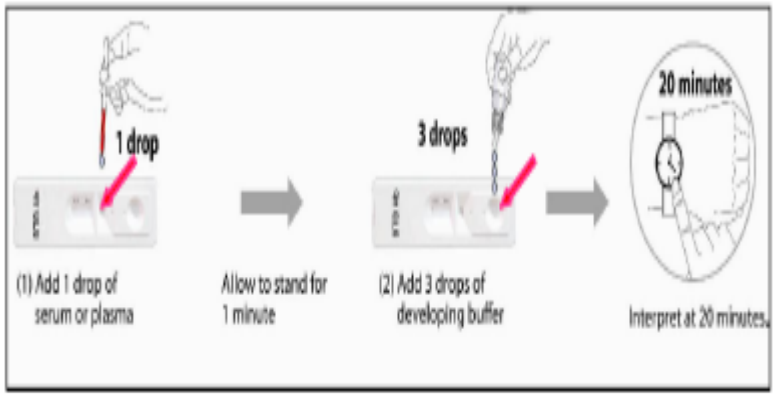


Illustration of the 20 minutes procedure

Test kit -The test kit is removed from the foil pouch, and placed on a flat dry surface. With a capillary tube One drop (10µl) of serum is added to the sample hole marked 'S' on the test device. Three drops of developing buffer is dispensed into developing hole.

Result window - A purple band in the result window of the kit will be seen in 20 minutes. A color band will appear in the left section of the result window to show that the test is working properly. This band is the Control line **(C)**. The right section of the result window indicates the test results. If another color band appears in the right section of the result window, this band is the Test line **(T)**. BioNote Incoporated

Appendix 5:

WORK PLAN

S/N	Project Activities	Physical Targets	Time Frame	Major expected outcome
1	Procurement of stationeries, questionnaire design and pilot testing of questionnaires	Standardization of questionnaire	1 month	Study Question
2	Traveling to Study areas, collection of samples and conduct of survey(Car Hire) Printing and Photocopying of Questionnaires	Collection of Samples from cattle slaughtered. Administration of questionnaire	2 months	Samples for Laboratory Analysis and Qualitative Data
3	One step Anigen Bovine TB Antiboby Rapid Test kit Procurement of Lab consumables and Laboratory Analysis	Laboratory analysis of serum samples collected from cattle slaughtered Laboratory analysis of tissue samples collected from cattle slaughtered	3 months	Results of Laboratory Analysis
4	Allowances for Research Assistants and Interviewers			
5	Others	Honarium and Incentives		