PREVALENCE OF *MYCOBACTERIUM BOVIS* IN CATTLE AND GOATS SLAUGHTERED AT THREE MAJOR ABATTOIRS IN KADUNA STATE, USING IMMUNOCHROMATOGRAPHY TEST AND ACID FAST TECHNIQUE

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DEPARTMENT OF VETERINARY SURGERY AND MEDICINE
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AHMADU BELLO UNIVERSITY, ZARIA

FEBRRUARY, 2013

DECLARATION

Ideclare that the work inthis thesis, entitled, "Prevalence of*M.bovis* infection in slaughtered Cattle and Goats in three selected abattoirs in Kaduna state",using immunochromatography assay test and acid-fast techniquehas been carried out by me in the Department of Veterinary Surgery and Medicine,Ahmadu Bello University Zaria under the supervision of Professor,C.A.Kudi, andDrM. Bello.The information derived from the literaturehas been duly acknowledged in the text and in the lists of references provided.Nopartof this thesis was previously presented for another degree or diploma in any University.

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Signature Date

CERTIFICATION

This thesis entitled "Prevalence of *M.bovis* infection in slaughtered Cattle and Goats in 3selected abattoirs in Kaduna state", usingimmunochromatography assay test and acid-fast technique byNdenarimamShamakiYerimameets the regulations governing the award of the degree of Masters of Science of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to God Almighty for giving me the strength and to my loving son, Marvelous.

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ABSTRACT

This study was carried out in 3 selected abattoirs in Kaduna state,Nigeria,to determine the prevalence of *Mycobacterium bovis* infection in slaughtered cattle and goats. A total of 700 animals consisting of 500 cattle and 200goats were sampled. Sera and tissues suspected to be tuberculosis were collected from 3 selected abattoirs andanalyzed using the lateral flow (Sera) and ZhielNelseen test (tissue) methods. The results showed that 91(18.2%) of the cattle sampled were positive using immunochromatography assay test and that all these cattle were White Fulani(Bunaji).None of the SokotoGudali cattle examined was positive for tuberculosisusing immunochromatography assay test. By sex 71(16%) bulls were positive while 20(32%) of the females were positive.Majority of the cattle positive for the immunochromatography assay test were within the age range of 5-6 years old. Similarly, 25(12.5%) of the goats were positive for *M.bovis* and were all Red Sokoto while none of the Kano brown goats was positive for*M.bovis*using the immunochromatography assay test.Furthermore,seropositivity for the male and female goats was 13% and 9.6% respectively.Of the 500 cattle sampled, 2(0.4%) had lesions suggestive of tuberculosis and were acid fast positive.In conclusion this work reconfirms the existence of *M.bovis*infectionin the study area and further showed that it is on the increase.It is therefore pertinent that there is a need forurgent intervention, more so as it is of great public health significance.

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LISTOFABBREVIATIONS

1. AFB	Acid fast bacilli
2. BCG	Bacilli Calmett –Guerin
3. BtB	Bovine tuberculosis
4. DNA	Deoxyribonucleic acid
5. ELISA	Enzymelinkedimmosorbent essay
6. HIV	Human Immunodeficiency syndrome
7. ICA	Immune chromatographic assay
8. MOTT	Mycobacteria other than tuberculosis
9. MTBC	Mycobacterium tuberculosis complex
10. OIE	Office International des Epizooties
11. PCR	Polymerase chain reaction
12. PFGE	Pulse field gel electrophoresis
13. PPD	Purified protein derivative
14. REA	Restricted fragment analysis technique
15. RFLP	Restriction Fragment length polymorphism
16. SG	SokotoGudali breed of cattle
17. TB	Tuberculosis
18. Tb	tuberculosis
19. VNTR	Variable number of tandem repeat
20. WF	White Fulani breed of cattle
21. WHO	World Health Organization
22. ZN	ZiehlNeelsen

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Mycobacterium species are acid fast organisms and have relatively slow growth rate. They do not grow outside of the host except in cultured media. They cannot tolerate harsh environments such as prolonged exposure to heat, direct sunlight, and or dry conditions but the organisms can survive longer under cold, dark, and moist condition (Goodchild and Clifton-Hadley, 2001).

*Mycobacterium bovis*causes tuberculosis mainly in cattle but other warm blooded animals including humans are affected. *Mycobacterium bovis*is one of the members of the Mycobacterium tuberculosis complex. Members of the *Mycobacterium tuberculosis* complex (MTBC) include *Mycobacterium tuberculosis, M. bovis, M. africanum, M. microti, M. canetti*(Van Soolingen*et al.,* 1997) and are responsible for the severe disease in humans and animals (Falkinham, 1996; Cosivi*et al.*, 1998; Biet*et al*., 2005). *Mycobacterium bovis* has the widest range of pathogenicity of all *Mycobacteria species.*(Gibbons, 1963).

Other *Mycobacterium* species include the environmental non tuberculous or atypical mycobacteria which are not members of *Mycobacterium tuberculosis* complex but ordinary inhabitants on a wide variety of environmental reservoirs. Many of these atypical mycobacteria, particularly those of the *Mycobacterium avium-intracellulare*complex (MAC), have also been reported to be pathogenic to humans and animals though not as members of the MTBC (Falkinham, 1996; Biet*et al*., 2005).

The classical MTBC agents, *M. tuberculosis*, *M. bovis,M. africanum,M. microti* and *M. canetti*cause pulmonary and extra pulmonary lesions in humans (Collins and Grange, 1983; Van Soolingen*et al*., 1997). Of these species, *M. tuberculosis* and *M. bovis* are highly pathogenic and clinically similar to each other, with humans and cattle being their natural hosts respectively. Cattle, goats and pigs are most susceptible to *M. bovis,* with sheep and horses showing a higher natural resistance (Radostits*et al*., 2000; Thoen*et al*., 2006). There has also been recognition of the fact that self-maintaining reservoirs of infection is present in wild life host in some countries (Morris *et al*., 1996).

Transmission is mainly through inhalation or ingestion of contaminated milk and meat from infected animal. Other uncommon routes of infection include intrauterine, intramammary and transcutaneous routes (Cosivi *et al.,* 1998). Reports have indicated that airborne infection of bovine tuberculosis continues to occur among meat industry and slaughter house workers, especially in regions where the infection is still prevalent in cattle (Thoen, *et al*., 2006). However, transmission to humans can be significantly reduced by pasteurization of milk; although only complete eradication of the disease can protect the farmer and his family. Transmission from cattle to humans in developed countries is an unlikely event nowadays but still occurs (Thoen, *et al*., 2006).

Bovine tuberculosis is a threat to animal and human health in several countries (Ameniand Tibbbo, 2003), as nearly 85% of cattle and 82% of the human population live in areas where the disease is prevalent or only partially controlled (Ayele*et al*., 2004). Some other studies in humans have shown the involvement of *M. bovis* in causing tuberculosis. For instance, in a study of 102 patients with pulmonary TB Nigeria, 3.9%

were found to be due to *M.bovis*(Idigbe *et. al.*, 1986). Cadmus *et. al.,* (2006) isolated 3 *M.bovis* strains in a study of 55 human sputum samples in Ibadan, Nigeria.

The global prevalence of human tuberculosis due to *M.bovis*has been estimated at 3.1% of all human tuberculosis cases (Ayele*et al.,* 2004). In addition to mammals, some avian species are susceptible to the infection including parrot-like birds, rock doves and North American crows (Geoghion*et al.,* 1989; Fhanninand Edwards, 1991; Dalovisio*et al.,* 1992; Grange and Yates, 1994.*Mycobacteriumbovis*in Nigeria may to some extent, mimic the pre-eradication period in Europe before the 1960s where the prevalence of bovine TB in the human population was relatively high.The free movement within country and trans-border smuggling of animals contributes significantly to inter and intra-species transmission and spread of *M.bovis* infection and thus contributes to the global upsurge in the prevalence of bovine tuberculosis in animals and humans especially in developing countries (Shitaye*et al.*, 2006).

1.2 Statement of Research Problem

Bovine TB has been widely distributed throughout the world with a serious effect on animal production. The disease is zoonotic and therefore of public health importance (O'Reilly and Daborn, 1995). The actual impact of animal Btb on human health is generally considered low or unknown in developing countries like Nigeria. The reason for that could be due to technical problems in the identification and isolation of *M. bovis* from human patients (Collins and Grange, 1983).

The public health significance of bovine TB in Nigeria remains largely unknown because few laboratories in the country are capable of differentiating *M. bovis* from *M. tuberculosis* and other members of the *M. tuberculosis Complex* (MTBC) (Cadmus *et al*.,

2006). The primary sources of infection for humans are consumption of unpasteurized milk and prolong close association between humans and animals. Rural inhabitants and some urban dwellers in Nigeria still consume unpasteurized and soured milk potentially contaminated with *M. bovis*(Abubakar, 2007).

Detection of Btb in animals in Nigeria is carried out most commonly on the basis of tuberculin skin testing, abattoir meat inspection and rarely on bacteriological examination.The current prevalence rate of Btb among the general public and at a national level is unknown because screening of cattle by the tuberculin skin test is sporadic and often times, diagnosis of tuberculosis in humans stops at the smear level and the particular species involved in causing thedisease are not known. This makes it difficult to study outbreaks and trace the routes of transmission of mycobacterium involved (Abubakar, 2007).

From the limited survey research which has been reported over the last 30 years in the country, the prevalence of bovine tuberculosis due to *M. bovis*ranges from 2.5% in 1976 to 14% in 2007(Abubakar,2007). The disease has been on the increase as demonstrated by the tuberculin test reports, and with isolation and identification of *M. bovis* in fresh and sour milk sold in local markets (Alhaji, 1976; Abubakar, 2007), as well as sputum and tissue samples from humans (especially among the Fulani herdsmen) with or without clinical signs of tuberculosis (Idigbe*et al*., 1986; Cadmus *et al*., 2006; Abubakar, 2007). Based on these limited studies, it was therefore established that *M. bovis* infection occurs in cattle and humans, with the highest prevalence of the human cases in the cattle herdsmen, abattoir workers and other handlers of livestock and livestock products (Abubakar, 2007).

Due to politico-economic reasons such as high cost of sustainable testing and slaughter of infected animals and the subsequent compensation to farmers. Poor implementation of control measures such as rigorous meat inspection at abattoirs, poor communication networks, insufficient collaboration with neighboring countries, lack of quarantine and smuggling of live animals across state boundaries have also been identified (Abubakar, 2007).

1.3 Justification

Bovine tuberculosis causes great economic losses and constitutes a continuous danger of transmission to man. There is the possibility of transmission by droplet inhalation as well as by contaminated food products. The economic loss to livestock industry due to tuberculosis is difficult to estimate in Nigeria but it is no doubt great. The overall reduction in efficiency of infected livestock may be reduced by 10-25% (WHO, 1994). It is therefore expected that eradication of bovine tuberculosis has been, and still is a major objective of many countries.

The rising incidence of tuberculosis in humans, particularly in immune compromised persons, has given a renewed interest in the zoonotic importance of *M. bovis*, especially in developing countries more so with the ease and frequency of the spread of disease from animals to humans in an uncontrolled environment (Thoen, *et al*., 2006). At present, there is also an association between mycobacteria infection, HIV/Aids pandemic and conditions such as diabetes in the developing world (Federal ministry of Health 2006).

Nigeria has one of the largest livestock populations and Kaduna state is the Centre of trade in livestock feed, attracting investors from neighboring states and countries. The state currently has 11 large livestock markets that make up 2.9% of Gross state

production (Anon, 2010). Since the real incidence of *M.bovis* on human health is unknown, it is essential to advance the eradication of Bovine tuberculosis worldwide by means of adequate programme, especially in developing countries (Grange, 2001). There is the need therefore, for regular surveillance and determination of the prevalence of Bovine tuberculosis in animals slaughtered in our abattoirs, thus necessitating this study with the following basic aim and objectives:

1.4 Aim of Study

To determine the prevalence of *Mycobacterium bovis* infection in slaughtered cattle and goats in three selected abattoirs in Kaduna State,Nigeria.

1.5 Objectives

1. To determine the prevalence of*M.bovis* infection using:
    a. Immunochromatography assay test
    b. Gross lesion and acid fast staining
2. To determine the level of association among sex, breed and age of cattle and goats to *M.bovis* infection.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bovine Tuberculosis

Bovine Tuberculosis (TB) is a contagious disease, which can affect most warm-blooded animals, including man. Cattle, goats and pigs are the domestic livestock most susceptible to infection, while horses are relatively resistant. The disease has been prevented and controlled in most developed countries by regulations, due to its ability to infect humans and cause significant livestock production losses (Cosivi*et al*., 1998).

These countries are conscious of local and international implications of the disease for trading in animals and animal products. In Africa, however, bovine TB represents a potential health hazard to both animals and humans, as nearly 85% of cattle and 82% of the human population live in areas where the disease is prevalent or only partially controlled. (Theon and Steele, 1995).

The WHO estimated that for the years 1990–1999, humantuberculosis (TB) incidence and mortality was 88 million and 30 million respectively, with mostcases occurring in developing countries.(Anon,1994) The annualglobal incidence of TB was predicted to increase to10.2 million by 2000, a 36% increase from 1990. In1995, 3.3 million cases were reported to the WHO Global Tuberculosis Programme. Of these, 62% occurred in South-East Asian and Western Pacificregions, 16% in sub-Saharan Africa, and 7–8% in eachof the regions of the Americas, Eastern Mediterraneanand Europe. Given the rapidly spreading globalhuman immunodeficiency virus (HIV) epidemic in developingcountries,

the WHO estimated that 70%(6 million) of humans co-infected with TB and HIVlive in sub-Saharan Africa (Cosvi*et al*, 1998)

2.2 Biology of Mycobacteria

The *Mycobacteria* are rod-shaped, aerobic bacteria that do not form spores. They do not readily take up stains, but once stained will resist decolourisation by acid-alcohol and are therefore referred to as acid-fast bacilli. Mycobacteria are strict aerobes, although growth is enhanced by an increased carbon dioxide (CO_2) tension. The organism which grows slowly on special media at 37^0C is a non-motile, straight rod, measuring about 0.2-0.6 by 1.0-10um. There are more than fifty *Mycobacterium* species, many of which are saprophytic (Chadwick, 1982; Rose and Barron, 1983; Brooks *et al*., 2007).

2.2.1 Constituents of tubercle bacilli

The Mycobacterial cell walls can induce delayed hypersensitivity and some resistance to infections and can replace whole Mycobacterial cells in Freund's adjuvant. Mycobacterial cell contents only elicit delayed hypersensitivity reactions in previously sensitized animals. The constituents listed below are found mainly in cell walls (Brooks *et al.,* 2007).

2.2.1.1Lipids;-

Mycobacteria are rich in lipids such as mycolic acids (long-chain fatty acids C78-C90), waxes, and phospholipids which are largely bound to proteins and polysaccharides (Quinn *et al.* 1994; Brooks *et al.* 2007).

Muramyl dipeptide (from peptidoglycan) complexes with mycolic acids can cause granuloma formation; phospholipids induce caseous necrosis. Lipids are to some extent

responsible for acid fastness of the organism. Their removal with hot acid destroys acid-fastness, which depends on both the integrity of the cell wall and the presence of certain lipids. Acid-fastness was lost after sonication of Mycobacterial cells. Analysis of lipids by gas chromatography reveals patterns that aid in the classification of different species (Quinn et al. 1994; Brooks *et al.* 2007)

"Serpentine cords" in which acid-fast bacilli are arranged in parallel chains are found in virulent strains of tubercle bacilli. Therefore, cord formation is correlated with virulence. A "cord factor" (trehalose-6, 6'-dimycolate) has been extracted from virulent bacilli with petroleum ether. It inhibits migration of leucocytes, causes chronic granulomas, and serves as an immunologic "adjuvant" (Quinn *et al.*1994; Brooks *et al.* 2007).

2.2.1.2 Polysaccharides

Polysaccharides found in Mycobacteria can induce the immediate type of hypersensitivity and can serve as antigens in reactions with infected sera but their role in the pathogenesis of disease is uncertain (Brooks *et al*. 2007).

2.2.2 Classification of Mycobacteria

Conventional methods for classification and identification of Mycobacteria include observation of rate of growth, colony morphology, pigmentation, and biochemical profiles. The conventional methods require 6-8 weeks for identification. Growth rate separates the rapid growers (growth in less than 7 days) from other Mycobacteria (Table 2.1). Photochromogens produce pigment in light but not in darkness; scotochromogens develop pigment when growing in the dark; nonchromogens (nonphotochromogens) are non-pigment or have light tan or buff-coloured colonies. Individual species or complexes are defined by additional biochemical characteristics (e.g., positive niacin test as shown

in M. tuberculosis, reduction of nitrated, production of ureases or catalase arylsulfatase test, and many others (Brooks *et al.* 2007).

Table 2.1: Runyon Classification of Mycobacteria.

Classification	Organism
TB complex	*M. tuberculosis*
	M. africanum
	M. bovis
Photochromogens	*M. asiaticum*
	M. kansasii
	M. marinum
	M. simiae
Scotochromogens	*M. flavescens*
	M. gordonae
	M. scrofulaceum
	M. szulgai
Nonchromogens	*M. avium complex*
	M. celatum
	M. hemophilum
	M. gastri
	M. genavense
	M. malmoense
	M. nonchromogenicum
	M. shimoidei
	M. terrae
	M. trivale
	M. ulcerans
	M. xenopi
Rapid growers	*M. abscessus*
	M. fortuitum group
	M. chelonae group
	M. phlei
	M. smegmatis
	M. vaccae

Source :(Brooks *et al.*, 2007)

2.3 World Distribution of Tuberculosis

All species are not equally susceptible, and are oftengrouped into spill-over (end) hosts and maintenancehosts. Cattle and other bovine species are consideredthe primary and most well-known reservoirs or maintenancehosts (Dabornet *al*.1993). In countries where maintenance hostsare present endemically in the wild, infection fromthese populations to domestic cattle or other farmanimals is difficult to avoid.(Dabornand Grange, 1993).

Bovine species are natural hosts to thedisease, but a wide spectrum of domestic and wildanimals, as well as man, can be infected (Theon and Steele, 1995). Animalsreported as having being infected with *M. bovis*includeNorth American bison (*Bison bison*), buffaloes (*Synceruscaffer*), elk (*Cervuselaphus*), domestic and wildpigs (*Susscrofa*), goats (*Capra hircus*), camels (*Camelusbactrianus*), dogs (*Canisfamiliaris*), cats (*Feliscatus*), sheep (*Ovisaries*), possums (*Trichosurusvulpecula*),badgers (*Melesmeles*), mink (*Lutreolavison*),ferrets (*Putoriusfuro*) and non-human primates (Dabornand Grange,1993)

The global prevalence of human TB due to *M.bovis*has been estimated at 3.1%. Of all human TBcases, 2.1% and 9.4% are pulmonary and extra-pulmonary TB cases respectively(Cosivi,*et al.,* 1998). In industrialized countries, human TB due to *M. bovis*is relativelyrare as a result of TB control in cattle. Nevertheless,an estimated 1% of all TB cases are reported tobe caused by *M. bovis*, probably due to reactivationof dormant lesions among the elderly (Grange, 1996).

Many factors account for the failure of developingcountries to control and eradicate bovine TB. Added to the high costs ofa sustainable testing programme are problems of socialunrest due to political instability and ethnic wars, resultingin the displacement of large numbers of human and animal populations. Furthermore, lack of veterinary expertise and communication networks, insufficient collaborationwith bordering countries and hence a lack of quarantine and smuggling of live animals across stateboundaries constitute stumbling block in the eradication and control of the disease. Scarce human and financial resources areoften absorbed by action against the high incidence ofother acute and fatal diseases, such as contagious bovinepleuropneumonia, foot and mouth disease, Africanand classical swine fever and parasitic diseases (Blood and Radostis, 1989).

2.4 Tuberculosis in Africa

In Africa, as in most developing countries, *Mycobacterium bovis*infection remains an uninvestigated problem. For this reason, the WHO, with the participation of the Food and Agriculture Organization (FAO), convened a meeting on zoonotic bovine TB in November, 1993 in Geneva, Switzerland, where the public health significance of *M. bovis*in humans and animals worldwide was discussed. Data collected from most developing countries, mainly from sub-Saharan Africa, were insufficient to represent the true epidemiological picture of the disease. It was therefore recommended that collection of scientific data on human TB due to *M. bovis*should be prioritized (Anon, 1994a).The epidemiology of TB has been affected in recent decades by the upsurge in HIV infection. As many HIV-infected individuals are co-infected with TB, the incidence of the disease may rise in the coming years, (Zumla*et al*, 1998). The correlation between the prevalence

of *M. bovis*infection in humans and that in local cattle populations highlights the potential threat of this disease for humans. (Dabor*et al*, 1996). Bovine TB is present in almost all African countries affecting both domestic and wild animals (Anon, 1994). Daborn and Grange (1993) reported that the disease was prevalent the livestock of 33 (80%) of 43 African member countries of the Regional Commission of the Office International des Epizooties (World Organization for Animal Health, OIE).

Twenty –five of fifty five African countries reported sporadic/low occurrence of bovine TB; six reported enzootic disease; two, Malawi and Mali, were described as having a high occurrences. (Cosivi*et al*., 1998; Amanfu, 2006).

2.5 Bovine Tuberculosis (*M.bovis*) in Nigeria

The earliest report of Bovine tb in Nigeria was among pure bred and half bred stock that were kept indoors. These animals were tested by the use of tuberculin test, post mortem and laboratory examination (Manly, 1929; Alhaji, 1976).The suspected origin of infectionwas from a herd that wasimportedfrom Germany. A study conducted in Sokoto abattoirs in Nigeria had aprevalence rate for tuberculosis as 0.69%(Ajogi*et al*. 1995).Another survey of tuberculosis in Maiduguri and Ibadan abattoirs indicated 1.21% prevalence in slaughtered cattle in Nigeria(Atsanda and Agbede, 1990).Bovine tuberculosis primarily appeared in the pulmonary form in the abattoir study conducted by Igbokwe*et al.,*(2001),who recorded TB prevalence in Damaturu(1.9%),Gashua(2.6%),Maiduguri(2.5%),and Damboa abattoirs(20%).Ibrahim (2007) in his epidemiological studies of Bovine tuberculosisin Jigawa Statehad a prevalence of 10.8%.Okaiyeto*et al*.(2008) carried out a study in 120 lactating Friesian

cows andestablished an overall prevalence of 5% based on tuberculin test and a prevalence of 1.7% based on acid fast staining of milk samples.

2.6 Tuberculosis in Livestock

Mycobacterium bovis and certain serovars of *Mycobacterium avium* complex are the most important mycobacterial species for livestock. Cattle,sheep,goats,pigs,and horses are susceptible to infection with *Mycobacterium bovis*in varying degrees (Amanfu, 2006).The insidious nature of the disease and the fact that it does not cause fulminating outbreaks with high mortality as other trans boundary animal diseases may be the cause of the decreased reporting and absence of measures for its control(Amanfu,2006).In industrialized countries, animal TB control and elimination programmes, together with milk pasteurization, have drastically reduced the incidence of the disease caused by*M.bovis* in both cattle and humans(Cosivi*et al*.,1998).

2.7 Epidemiology

All species, including humans, and age groups are susceptible to *Mycobacterium bovis*with cattle, goats and pigs most susceptible and sheep and horses showing a high natural resistance (Radostits*et al*. 2000). In developed countries, tuberculosis in animals is a rarity with occasional severe outbreaks occurring in a small group of herds. The presence of the disease is usually signaled by detection in carcasses at abattoirs (Radostits*et al*,2000).Although the prevalence of the disease within a country varies from area to area, the highest incidence of bovine tuberculosis is generally observed where intensive dairy production is most common, notably in the milk sheds (Cosvi*et al*, 1998). In addition, in some industrialized countries such as United States, where bovine TB is

close to elimination as a result of proper meat hygiene, bio security and disease surveillance and control measures, large dairy herds that are crowded together under intensive system of management represent the main source of infection (Cosvi *et al*, 1998). The primary sources of infection for humans are consumption of unpasteurized milk and close association between humans and animals (Daborn*et al,* 1996). Rural inhabitants and some urban dwellers in Africa still consume unpasteurized and soured milk potentially contaminated with *M. bovis.* Milk-borne infection is the main cause of non-pulmonary TB in areas where bovine TB is common and uncontrolled (Daborn*et al*., 1996).

The current problem of *M. bovis*in developing countries may, to some extent mimic the pre-eradication period in Europe before the 1960s, where the prevalence of bovine TB in the human population was relatively high (Pavlick,2002).

2.7.1 Aetiology

Bovine TB is caused by *M. bovis.* Although cattle are considered to be the primary hosts of *M. bovis*, the disease has an exceptionally wide mammalian host range, which includes humans(Blood, et al,1989, O'Reily and Daborn,1995).*M. bovis*is a member of a closely related group of mycobacteria referred to as *Mycobacterium tuberculosis* complex *(*MTC),which comprises *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium microti*and *Mycobacterium bovis*bacilli Calmette-Guérin (BCG) .New members of the group include *M. canetti* and *M. caprae.Mycobacterium tuberculosis* complex (MTC) bacteria are usually regarded as subspecies and are characteristically 99.9% similar at the nucleotide level, with identical

16S rRNA sequences. However, there are distinct phenotypic differences between the subspecies and not least their host range and pathogenicity *(*Van Sooligen*et al.,* 1997).

*Mycobacterium bovis*closely resembles *M. tuberculosis*, and precise identification of and distinction between the two can be established by biochemical and molecular biology techniques. In the public health centres of most developing countries, Löwenstein-Jensen (LJ) medium, with pyruvate or Glyceral a medium on which *M. bovis*may grow poorly or not at all, is commonly used for the isolation of *M. tuberculosis.* Inoculated media are often incubated insufficiently for *M. bovis*cultures to appear (Grange, 1996). This may help to explain the low number of bovine type human TB cases reported in developing countries *Mycobacterium bovis,is* a robust pathogen and may survive in the environment, in buildings, on transport vehicles, on pasture and in slurry (Grange, 1996). The organism has been reported to survive in cow faeces for more than 5 months in winter, 4 months in autumn, 2 months in summer, and in soil for up to 2 years (Wray, 1975). Manure fertilization of arable land is common practice in developing countries. Survival of *M. bovis*in soil and slurry therefore implies pasture and vegetable contamination, representing a potential source of infection to animals and humans, alike (Wray, 1975).

2.7.2 Transmission

Inhalation of *M. bovis*is the most probable and principal route to bovine infection and is facilitated by close, prolonged contact between infected and healthy animals. Ingestion of *M. bovis*directly from infected animals or from contaminated pasture, water or utensils may also be very common in some regions(Neils, 1994). Congenital infections and vertical transmission have been recorded. These routes, like genital transmission, which occurs when reproductive organs are, infected (Neils, 1994).

2.7.2.1 *Animal-to-animal transmission*

Infectious animals may shed *M. bovis*in a number of ways: in faeces, milk, discharging lesions, saliva and urine. (Cosvi*et al*.1995). Intensive livestock farming promotes close contact between animals, favouring the spread of *M. bovis*. Extensive livestock farming, however, especially transhumance with no housing system, raises the question as to how bovine TB transmission can take place. Close contact between animals occurs for example, at watering points such as ponds, wells and streams. In Africa, grazing animals usually gather at night for protection from predators. (Daborn *et al.* 1996). Vaccination and artificial insemination centres, dipping tanks, auction stations, market places and transportation are the commonest animal gathering places, and are sites where transmission of infection could easily occur. (Neils*et al.*1991). Due to the high ambient temperature in tropical zones, animals tend to concentrate under trees or other shaded areas for parts of the day, preferring to graze early in the morning and late in the afternoon. Possibly, the most dangerous spots for nose-to-nose or mouth-to-mouth contact among animals are salt supplementing points. (Neils*et al*.1991). Therefore, while extensive farming is safer than zero level grazing systems to prevent disease transmission, some of the above situations simulate the dangers of intensive farming in relation to disease transmission (Neils*et al*, 1991).

2.7.2.2 Animal-to-human transmission

In industrialized countries, the incidence of TB due to *M. bovis*in humans is almost at zero level as a result of pasteurization of milk and milk products and eradication of bovine TB in cattle populations (Collin and Grange, 1983).However, in developing

countries, bovine TB in animals can be widely distributed in regions where control measures are not applied or are conducted sporadically and pasteurization of milk is rarely practiced (Cosivi *et al*. 1995).In Industrialized countries, the direct correlation between *M. bovis*in cattle and TB due to *M. bovis*in humans has been well documented, whereas little information is available from developing countries (Collin *et al.* 1983). Pulmonary tuberculosis due to *M. bovis*is more common in rural dwellers, as a result of inhalation of dust particles or bacteria-containing aerosols shed by infected animals, while urban dwellers acquire the infection via the gastrointestinal route and develop extra-pulmonary TB (Daborn*et al.*, 1996). In countries with a relatively high prevalence of bovine TB in cattle, abattoir and farm workers are the groups most exposed to infection due to their constant contact with animals. Current economic and social globalization has created greater opportunities for the spread of zoonotic diseases such as TB. When considering the revival of TB in countries previously declared to be free of the disease. (Grange, 1996).

2.7.2.3 Human-to-animal transmission

The role of humans in infecting *cattle* with bovine TB stressed the potential danger that patients with smear-positive pulmonary TB due to pathogenic mycobacteriosis may pose to animals. However, reports of human infection of cattle are rare (O'Reilly *et al*, 1995). The genitor urinary tract in humans is a site of non-pulmonary TB due to *M. bovis*; genitourinary TB may appear to be of little importance to epidemiologists in studying human infection, but this route of infection from man to cattle is well-documented. Grange and Yates (1994) reported that farm workers urinating in cowsheds may represent a source of infection for animals. An analogous situation is thought to occur in rural

Africa, where patients with genitourinary TB may urinate on pasture: animals craving salt preferentially graze on this grass and may succumb to infection. (Grange *et al,* 1994)

2.7.2.4 Human-to-human transmission

Human TB caused by *M. bovis*as a result of human to-human transmission was reported in the Netherlands in 1994 (Van Sooligen*et al.*1994). Evidence of transmission of *M. bovis*between humans is considered rare and largely anecdotal, and the rate of transmission seems insignificant compared to animal-to-animal or animal-to-human infection (O'Reilly, *et al,* 1995). Human-to-human transmission of *M. bovis*is considered less efficient than that of *M. tuberculosis*. However, transmission among HIV-infected humans, where immune suppression increases the susceptibility of the host to the organism may be different. *Mycobacterium bovis*has been isolated from HIV-infected individuals in some industrialized countries, with an additional serious complication of high primary resistance to isoniazid, streptomycin and pyrazinamide (Van Sooligen, 2001).

2.7.3 Pathogenesis

There are numerous ways in which cattle can become infected with *M. bovis.* These can be affected by, environment and climate, and prevailing farming practices (Pollock, *et al*, 2002). Under natural conditions, the main route of *M. bovis*infection in cattle is by inhalation. This mode of transmission is dominating in industrialized countries, where intensive farming is practiced (Neils*et al*.1994). In field case studies of bovine TB in these countries, lesion distribution and pathology show predominant involvement of the upper and lower respiratory act and associated lymph nodes (Neils*et al*. 1994).

Confirmed tuberculin reactors frequently appear to have an absence of lung lesions. However, lesions when present within the lung parenchyma are usually too small to be easily detected during meat inspection. A generally accepted concept is that infection with *M. bovis*can become established in cattle by inhalation of the tubercle bacilli, possibly a single bacillus, in an aerosol droplet that lodges within the respiratory tract, probably the alveolar surface of the lung. The bacilli are phagocytized by macrophages, and subsequently interact with cells involved in innate and acquired immune responses in tissue or draining lymph nodes (Neils*et al*. 1991).This often results in nonvascular nodular granulomas known as 'tubercles'. Characteristic tuberculous lesions occur most frequently in lungs and retropharyngeal, bronchial and mediastinal lymph nodes. Lesions can also be found in the mesenteric lymph nodes, liver, spleen, serous membranes, pleura and other organs. (Blood *et al.*1989). The role of activated mononuclear macrophages is considered most important in protecting the host against *M. bovis*(Thoen and Hums, 1986). Macrophages are involved in processing mycobacterial antigens and presenting them to T-lymphocytes, which are considered a key recognition unit in the immune response to mycobacteria (Thoen and Hums, 1986).The characteristic lesion caused by *M. bovis*in cattle is described as having a centre of caseous necrosis, usually with some calcification, with a boundary of epithelioid cells, some of which form multi-nucleated giant cells and few to numerous lymphocytes and neutrophils (Theon*et al.,*1995, Neils *et al.*1994).Primary lesions in cattle, unlike man, are rarely contained by the immune response, and dissemination from a lesion may occur by natural ducts such as bronchi, by lymphatic spread or by haematogenous spread when massive miliary TB occurs(Francis,1947). It is worth noting that despite the many studies on bovine TB over the years, it was recently concluded that a better understanding of the dynamics of the

events following *M. bovis* exposure of cattle and subsequent infection would be of significant benefit to diagnosis and disease control (Pollock *et al*., 2002).

2.7.4 Diagnosis

Tuberculosis can be diagnosed clinically, but usually only in the later stages of the disease. The tuberculin skin test (TST) is universally recognized and is generally used for preliminary diagnosis in bovine TB control programmes. However, in countries with low disease prevalence or disease free status, meat inspection is used for diagnosis and surveillance. Other tests, such as an enzyme-linked immunoassay (ELISA) and the gamma-interferon assay, have been used as supplementary tests in eradication and control of the disease. Assays for bovine interferon-gamma have recently been applied to indicate infection. Confirmation of infection, however, often relies on isolation and identification of *M. bovis*from tissue(Neill *et al.,* 1994).

2.7.4.1 Clinical examination and necropsy

Many cattle with bovine TB are clinically normal. Some cows with extensive military tuberculous lesions also appear clinically normal, but progressive emaciation unassociated with other signs should arouse suspicion of TB. Capricious appetite and fluctuating temperature are also commonly associated with the disease. Pulmonary involvement is eventually characterized by chronic cough, together with dyspnoea and other signs of low-grade pneumonia. Affected animals are docile and sluggish, but the eyes remain bright and alert (Blood *et al.,* 1989). A report from Great Britain on the pathological examinations of cattle over 6 months old stated that the primary complex in TB is in the lungs and their associated lymph nodes. More recent studies reported by

Neill *et al*. (2001) and Pollock and Neill (2002) indicate that the precise processes by which cattle become infected by *M. bovis*may not be completely characterized or understood.

*Mycobacterium bovis*can be demonstrated microscopically on direct smears from clinical samples, and on prepared tissue materials. Tissue smears from affected organs stained by the Ziehl-Neelsen (ZN) method can be used to demonstrate the presence of acid-fast mycobacteria (red bacilli on a pale blue background after methylene blue staining), and appear on a green background if re-stained with malachite green. Examination of a haematoxylin-eosin-stained section of lesion for each case that is positive on smear is valuable. This technique is cheap, practical and a useful preliminary diagnostic step in developing countries where other laboratory facilities are not available. Furthermore, identification in clinical samples and pathological specimens can be performed rapidly (Pritchard, 1988).

2.7.4.2 Serological assay

It is well established that cattle infected with *M. bovis*develop early immune responses, dominated by cell-mediated immunity (Alito *et al*, 2003). Therefore, serology is less efficient to identify cattle in the early stages of infection, when antibody titres are low (Wood, 1994). There are several advantages to using serological methods, like, ELISA, for the diagnosis of Btb. These tests require one handling of the animals and only one visit of the veterinarian to the farm. Blood sampling can be repeated as often as necessary, without altering the immune status of the animal. That the interpretation is based on numerical values makes it more objective than the observation of swelling of the skin (Lilenbaum*et al.,* 2001). The indirect ELISA technique measures the binding of

specific antibodies to an antigen (Lilenbaum*et al.,* 1999). In order to diagnose cattle infected by *M. bovis,* antigens usually employed are the PPD and single or associated purified antigens from *M. bovis*(Fifis*et al.,* 1994). Regarding purified *M. bovis*antigens, Ag85 complex represents a major part of the secreted proteins (Wiker*et al* 1992). Nevertheless, antibodies directed against these antigens may also be present in non-infected individuals (Wiker*et al*., 1992), leading to false positives. In Brazil, Lilenbaum*et al* (2001) used an ELISA with the Ag85 as its capture antigen and achieved a sensitivity of 91.3% and specificity of 94.8%. Although other studies reported high specificity, the sensitivity of ELISAs systems employing this antigen were lower (Fifis*et al*.1994). The MPB70 protein, which represents approximately 10% of the PPD (Wiker*et al*.1992), has been identified as a B-cell target in tuberculous cattle and also as a reasonably specific protein. Medeiros, (Guerrero *et al,* 1997).New diagnostic procedure of bovine tuberculosis in Brazil has suggested that MPB70 and its highly homologous protein MPB83, secreted mycobacterial proteins with limited species distribution, are both involved in host protein cell binding. These proteins are highly expressed in *M. bovis*, but minimally expressed in *M. tuberculosis in vitro* and probably *in vivo*. Wiker*et al* (1998) demonstrated the diagnostic potential of these proteins, and reported that most monoclonal antibodies identify shared epitopes on these molecules, and that the linear epitopes of MPB70 were the major antibody targets, both upon immunization with protein preparations and during infection with *M. bovis.* Although MPB83 presents some minor differences in comparison with the MPB70, new studies demonstrated the usefulness of the MPB83 to diagnose tuberculosis earlier than MPB70 in experimentally infected animals. In that regard, MPB83- ELISAs detected specific antibodies four weeks post infection, whereas MPB70-ELISAs detect specific antibodies 18-22 months after

experimental infection (Wiker, 2009). Furthermore, ELISAs using MPB70 as capture antigens have good specificity (88 to 96%), whereas sensitivity was quite variable, ranging from 18 to 73% (Wiker, 2009). The MPB70 has some cross-reactivity with *Nocardia*was the impetus to test specific epitopes of the protein, in an attempt to increase the test specificity. Furthermore, rM70-83-E6, a recombination of antigens MPB70, MPB83 and ESAT-6, specifically reacts with bovine tuberculosis-positive sera, also improving ELISA sensitivity and specificity (Liu *et al,* 2007). Employing bovine PPD as capture antigen in ELISA, McNaire*et al*. (2001) reported 90% sensitivity and 89.8% specificity for diagnosis of Btb. Lilenbaum*et al*. (1999) reported 86.7% sensitivity and 90.6% specificity in dairy herds in Rio de Janeiro, Brazil. Perhaps recombinant antigens are not antigenically identical to their native counterpart. Therefore, they may not be recognized by the immune system of infected animals (Fifis *et al.,* 1994). There is sensitivity in proportion to the number and distribution of lesions found in the anatomopathological tests, suggesting that the sensitivity of ELISA depends on the duration and severity of herd infection. Although serological assays cannot be considered first choice diagnostic methods, many researchers describe strategies for their use (Goodchild*et al.,* 2006). Their recommendations are based on the existence of animals not showing signs of the disease (McNaire*et al*., 2001), as well as increased antibody titres in more advanced stages of the disease (Pollock *et al.,* 2002). Using ELISA, Lilenbaum& Fonseca (2006) identified tuberculous cows in 18 herds involved on a tuberculosis control programme, and subsequently confirmed infection by isolation of *M. bovis*from lung lesions. In those cases, ELISA was employed as a complementary diagnostic test and improved the control of tuberculosis by identifying anergic cows.

Immunochromatographic assay using direct sandwich method, MPB7O protein was shown to be a highly species specific test for protein secreted by *M. bovis*as used for the capture and detector material. MPB7O is present predominantly in *M. bovis* and to a very limited degree in *M. tuberculosis* and other mycobacterial species. The purpose of this test is detection of antibodies (1gM, lgG) against *M. bovis* from the serum or plasma. The aim is to enhance sensitivity and differential specificity, ease of use, adaptability to automation, computerized interpretation and reporting for individual, herds and populations and to reduce the cost compared to tuberculin test (Adams, 2001) in TB diagnosis. However, it is generally agreed that antibody-based tests are not stand-alone tests, as they fail to detect many *M. bovis*-infected cattle with subclinical disease in a population (Thoen*et al.* 1975). More over, non-specific responses may be observed on these tests because of responses elicited by other mycobacteria or organism that share antigens with *M. bovis* (Thoen*et al*., 2006).

2.7.4.3 Culture and artificial media and identification

The best way to detect *M. bovis* in tissue fragment is by culturing decontaminated samples on Lowenstein-Jensen and Stone brink Leslie solid culture medium (Wray, 1975) subsequently incubated at 37oC for up to 60 days. The sample decontamination aim is to inactivate other bacteria that might be present in the sample, to avoid their growth and media nutrients exhaustion, which renders *M. bovis* growth unfeasible. One of the most used decontamination method used decontamination method used in Veterinary Medicine is the Petroff method that uses 4% NaOH with posterior neutralization with1NHCL, which is applied, traditionally in tissue fragments samples refrigerated or frozen (Rosemberg*et al.,*1990). Alternatively, these fragments may be

immersed in saturared solution of sodium borate. The advantage of this procedure is to provide conservation for two months, without the need of refrigeration or freezing which makes it suitable to be used in any kind of slaughter house and also in field conditions. It is important to consider that the sample contamination rate may vary according to the kind of sample, the way it was collected and preserved, and also transporting time to the laboratory (Corner, 1994).

Following centrifugation, the sediment is often inoculated onto a set of solid media slopes consisting of egg-based media such as Lowestain Jensen, Coletsos base and Stone brink's media. Slopes of these media containing pyruvate are inoculated, usually in duplicate, and an agar-based medium, such as Middle brook 7H10 or 7H11, is also often used (Abe *et al,* 1999). Stone brink's medium containing sodium pyruvate without glycerol is possibly the best medium. Colonies of *M. bovis*are expected to appear after 3–5 weeks of incubation at 37^0C. A radiometric culture method such as the semi automated BACTEC 460 system employing a liquid supplemented Middle brook medium can also be used. Radio-labelled carbon dioxide obtained following metabolism of palmitic acid incorporated into the culture medium, is measured in an ion chamber system as an indicator of bacterial growth-stained smears from suspect culture growth are examined by direct light microscopy. Suspected mycobacterial cultures are inoculated into liquid or solid media and incubated for at least 8 weeks at 37^0C in air with or without increased carbon dioxide. Identification of presumptively identified mycobacterial isolates is commonly carried out by determining cultural and biochemical properties (Abe *et al,*1999). The radiometric method provides results significantly more rapidly than traditional culture, but is often sensitive to fungal contamination, particularly when

certain animal specimens are used, and will grow atypical mycobacteria or non mycobacterial organisms not restricted by the antibiotics incorporated (Kubica*et al.,* 1984). The disadvantages of this method are that the BACTEC method is more expensive, requires an instrument to read the culture vials, and involves handling of radioisotopes. It should be noted that acid-fastness is not restricted to the mycobacteria.Other acid-fast organisms include *Corynebacterium*spp., *Nocardia*spp., and *Rhodococus*spp. Colony of MTC organisms in primary cultures can be observed after 4–6 weeks on solid media, and after 13–15 days in radiometric and automated culture systems. The drawback of using radioisotopes has been overcome in a newer system of the BACTEC 'MGIT'960 culture system, which employs a fluorometric detection system. This system is considerably more expensive and it has not yet proved consistently effective for culturing *M. bovis*from clinical specimens (Kubica*et al.,* 1984). Although, other techniques are still in use such as: 1) bacillary morphology and growth rates; 2) enhancement by glycerol of growth of *M. tuberculosis* isolates and suppression of *M. bovis*isolates; (3) niacin synthesis by *M. tuberculosis* but not by *M. bovis*isolates; (4) enhancement by pyruvate for growth of *M. bovis; (*5) oxygen preference-*M. bovis*is microaerophilic, whereas *M. tuberculosis* is aerobic; (6) growth characteristics-both *M. bovis*and *M. tuberculosis* fail to grow at 25^0C and 42^0C and hence are strict mesophiles; (7) colony pigmentation-neither *M. bovis*nor *M. tuberculosis* colonies are pigmented; (8) nitrate is reduced by *M. tuberculosis* but not by *M. bovis*isolates; (9) sensitivity to drugs-resistance to pyrazinamide of *M. bovis*isolates and sensitivity of *M. tuberculosis* isolates; (10) susceptibility to thiophene-2-carboxylic acid hydrazide by *M. bovis*but not by *M. tuberculosis* isolates(Pritchard,1988). Research is ongoing for alternative methods that are rapid, cheap and easy to perform. Recent studies using the mycobacterial antigen

(MPB64) revealed discrimination of MTC members from mycobacteria other than tuberculosis (Abe *et al,* 1999) while Hasegawa *et al,* 2002. reported using lateral flow immune chromatographic assay (ICA) to detect MPB64 with anti-MPB64 monoclonal antibody, with 100% specificity and sensitivity in differentiating MTC bacteria from MOTT (mycobacteria other than tuberculosis). Other modern laboratory methods can be applied to discriminate mycobacterial isolates on the basis of the genomic characteristics of the organism.

However, these methods are expensive for most developing countries with limited financial and technical resources (Hasegawa *et al*. 2002)

2.7.4.4 Molecular diagnosis

More recently, however, through international collaborative efforts, attention has been directed at *M. bovis*as a significant animal pathogen with zoonotic potential (Collins, 1999).

The intracellular nature and impermeability of mycobacterial cell walls, together with the presence of polymerase chain reaction (PCR) inhibitors in clinical specimens, limit the efficiency of PCR in the detection of infection with *M.bovis*. This can be problematic and impact on the potential uses of PCR in infected animals with certain clinical specimens and in particular with specimens taken from tuberculous cattle, where low numbers of bacilli are common. Although amplification-based detection kits are commercially available, a recent review indicates that detection of *M. bovis*in animals has focused primarily on 'in house' amplification-based systems (Collins, 1999).

Molecular biology techniques have now provided a means to detect and differentiate *M. bovis*isolates. Such differentiation enables systematic epidemiological surveys to be conducted and, potentially, the origins of infection to be traced. Fingerprinting of *M. bovis*has been extremely effective using the restriction fragment analysis technique referred to as REA (Collins, 1999). However, widespread adoption of the method has been curtailed by issues such as potential cost and difficulties in technology transfer. A modified method of pulse field gel electrophoresis (PFGE) for *M. bovis*has had little uptake generally, as most methods now have advanced to exploiting the repetitive DNA sequences that occur in the mycobacterial genomes. Two types of repetitive DNA are known in bacterial genomes-dispersed repeats and tandem repeats. Dispersed repeats include mobile genetic elements, including insertion sequences (ISs) and prophages (Gordon*et al.*1999). Most of these ISs are located in the same genome position in members of the MTC. However, there are significant differences between species that are useful for diagnostics. The insertion element IS*6110*, which belongs to the IS3 family, is the most abundant and best-characterized. The IS*6110* copy number varies from 0 to 25 in MTC bacteria, whereas in *M. bovis*isolates the IS*6110* copy number is low, often occurring singly, especially in isolates from cattle. Interestingly, and usefully exploitable, the IS*6110* copy number is often higher in *M. bovis*isolates from more 'exotic' animal species (Collins, 1999). Finding repetitive DNA in the genomes of MTC bacteria enabled an array of typing or fingerprinting techniques to be developed based on DNA amplification or Southern blotting, or a combination of the two. DNA polymorphism in MTC is found in *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. bovis*BCG, *M. bovis*subsp. caprae, *M. microti*and *M. canettii*(Kremer *et al*., 1999). Strain typing of *M. tuberculosis* isolates by IS*6110*-RFLP (restriction fragment length polymorphism) is now

internationally accepted and is used to monitor the efficacy of human TB control programmes and to detect point source outbreaks. However, for *M. bovis*isolates from cattle, because the IS*6110* copy number is low, additional probes are often required in RFLP typing to improve discrimination. It is widely accepted that the RFLP techniques are highly discriminating, but they are also cumbersome and inconvenient to use. Other methods such as the spacer-oligotyping (spoligotyping) technique, which employs PCR amplification of the DNA sequence of the highly polymorphic direct repeat (DR) locus and reverse-cross blot hybridization to detect the presence or absence of spacer DNA sequences, mostly from *M. tuberculosis* have been developed.The polymorphism is carried by these spacers, which are variable in length (Anon, 1997). Spoligotyping is easily executed and produces a simple digital pattern, but it is less discriminating than REA or RFLP (Roring*et al.*1998). Further examination of *M. tuberculosis* and *M. bovis*isolates has revealed additional spacer sequences that may further improve discrimination (VanEmbden*et al.,* 2000). Spoligotyping is possibly the most widely used typing method for differentiation of isolates belonging to the MTC. Because of its simplicity, it is becoming a favoured method for typing *M. bovis*isolates. The method has been employed for simultaneous diagnosis and typing of mycobacterial isolates. The availability of the complete genome sequences for *M. tuberculosis* and *M. bovis*, together with advances in bioinformatics, has revealed novel information about DNA repeat sequences that has been employed to develop new typing methods for discriminating isolates. One example is the variable number of tandem repeat (VNTR) typing method, which through amplification of PCR products has exploited the VNTR loci comprising repeat units of varying sizes and copy number. This method has the advantage of having the potential for automation and is hence useful. (Gordon *et al.,* 1999)

2.7.4.5 Control and Prevention

Cousins (2001) suggested that there are various reasons for attempting to eradicate bovine TB and they include the risk of infection to the human population, loss in productivity due to infected animals and animal market restrictions set by countries with advanced eradication programmes. The priority they are given will vary depending on factors specific to the country in question. In Africa, the economic losses associated with livestock contracting bovine TB has either never been studied or has not been examined sufficiently.

However, data can be obtained from other countries where bovine TB is prevalent. In Argentina, for example, the annual losses due to bovine TB are approximately US$63 million. Based on these estimates, it can be inferred that bovine TB may pose a serious economic risk to Africa. Moreover, preliminary reports submitted by member states to the OIE indicate that the disease occurs almost everywhere on the African continent, with inevitable economic and public health implications (Cousins, 2001).

In industrialized countries, control and eradication of bovine TB has been successfully carried out by regular testing and removal of infected animals under mandatory national bovine TB programmes. Such programmes have been successful in many European Union member states and in seven central European countries between 1953 and 1980 (Pavlik*et al*, 2002). In developing countries, however, bovine TB remains a major animal health problem, mainly because these countries cannot shoulder the financial burden required to implement a control programme and compensate for slaughtered animals. Limited access to education, poor information networks and lack of disease surveillance are other factors that limit the implementation of any such programme. Bacilli Calmette-

Guerin (BCG), an attenuated strain produced by continuous subculture of a wild-type *M. bovis*isolate from cattle, has played a crucial role in controlling human TB, particularly in children (WHO, 1959). However, its use for bovine TB is less effective. Using BCG vaccination to control bovine TB is an option that has been considered in European countries, North America and some African countries. However, because of its limited effectiveness, the Joint WHO/FAO Expert Committee on Zoonoses stated in its second report that 'The committee is of the opinion that vaccination has no place in the eradication of bovine TB in cattle' (WHO, 1959). Absence of protective immunity in BCG-vaccinated cattle, possibly linked to immune responses developed to environmental mycobacteria, although it might have been expected that exposure to the shared antigens of environmental mycobacteria would provide acquired protection from bovine TB (Pavlik *et al*, 2002). Recently the efficacy of vaccination was examined with BCG alone and a DNA prime-BCG boost regimen in cattle challenged with virulent *M. bovis.* The prime-boost regimen significantly enhanced protection in six parameters compared to significant enhancement of protection in only two parameters for BCG alone. This was demonstrated by fewer animals with severe lung lesions, fewer lymph nodes with lesions per animal, a smaller proportion of animals with lesions, lower mean lung and lymph node lesion scores and less *M. bovis*isolated from retropharyngeal and thoracic lymph nodes compared to non-vaccinated challenge animals (Corner *et al.*2002). A vaccination strategy employing BCG would obviously necessitate developing differential diagnostic assays to distinguish vaccinates from non-vaccinates, as BCG vaccination causes sensitivity to tuberculin, the PPD routinely used in skin testing tuberculous cattle. Because of this and the varying efficacy of BCG in cattle, recent research capitalizing on advances immunology and molecular biology has focused on alternatives to BCG,

including novel attenuated *M. bovis*strains, sub-unit vaccines and recombinant DNA vaccines. The recently available *M. bovis*genome sequence should have a significant impact on new generation vaccine candidates (Garnier*et al.,* 2001). Eradication of bovine TB using compulsory test and slaughter strategies has proven difficult even in industrialized countries, where cattle movement can usually be controlled. In some of these countries, where natural reservoirs of the disease in wild animals pose a serious risk of transmission to domestic livestock, the problems have been exacerbated, and eradication programmes remain unsuccessful (Garnier*et al.* 2001). In most African countries, controlling free movement of animals within a country is difficult and movement between countries cannot be regulated, primarily due to a lack of border controls. In addition, spread of bovine TB amongst wildlife in game parks in Africa is increasingly being recognized as a serious problem, with consequences for domestic animals. African countries may therefore find advantages in vaccinating susceptible animals (Garnier*et al.* 2001).

Daborn and Grange (1993) have suggested strategic vaccination of susceptible domestic animals in endemic are as a feasible option for Africa, where control of bovine TB is a much more acceptable and practical measure and eradication is not the objective. Vaccination could potentially be used to control bovine TB in countries where wildlife reservoirs exist and in those that cannot afford conventional control procedures. Development and production of an effective vaccine with appropriate methods and strategies for delivery could therefore contribute to bovine TB control in Africa. This is obviously a task best undertaken at a global level and applied locally in appropriate scenarios (Daborn*et al.* 1993)

2.8 Factors Conducive for the Persistence of Tb In Africa

2.8.1 Culture and customs

In developing countries, particularly Africa, patients' beliefs and cultural factors are obstacles to TB control strategies (Edginton*et al*., 2002). TB is stigmatized in many cultures and TB control may be further complicated by patients hiding their TB status due to discriminatory views about TB sufferers. A recent study in a rural area of South Africa reported the strong local community belief that TB results from breaking cultural rules and that the disease can be treated by traditional healers (Edginton*et al.,* 2002). In addition to the use of traditional healing, factors such as stigma, difficulties in accessing health services, long waits at health centres and the sometimes negative attitudes of health workers can adversely influence patient adherence to allopathic treatment. Patients claimed to be unaware of the need to take a full course of treatment, and that the red colour of the urine due to rifampicin treatment adversely affected their partners, resulting in sexual abstention during treatment (Edginton*et al.,* 2002).

Social discrimination based on TB status is thus more of a matter of stigma than of appropriate public health precautions. Risk factor assessment and identification of this infectious agent in both humans and animals will be the first step towards adopting dependable preventive, therapeutic and control measures. (Edginton*et al.,* 2002)

In Nigeria the pastoralists have limited access to Veterinary services and are the main source of milk and milk products for most rural/urban communities (Abubakar, 2007). Consumption of unpasteurized milk and other products ("fura da nono") which are

associated risk factors for the transmission of *M. bovis* from infected cattle to humans is being practiced (Abubakar, 2007).

Another, yet unsolved social problem in most rural communities of Africa is illiteracy. Inability to read and write, failure to utilize modern methods of communication, and limited knowledge of the community related to the epidemiology of Btb infection makes prevention and control programmes difficult and often impossible to apply. (Anon, 1994b).

2.8.2 Demography, eating habits, living and socio-economic status of families

For most urban dwellers, milk is considered the main vehicle for infection by bovine TB, while farmers and abattoir workers are mostly exposed to aerosol infection by close contact with infected animals. For most rural African populations, consumption of raw milk and milk products and close association between animals and farmers are common, and encourage exposure to both alimentary and extra pulmonary and pulmonary infection by *M. bovis*(Anon, 1994a).

The following factors contribute to the acquisition of infection in farmers and urban dwellers (1) family ownership of cattle (2) previous livestock ownership (3) history of working with animals (4) living with a relative who owns cattle and (5) consumption of unpasteurized milk and raw or poorly cooked meat (Pavlik *et al.,* 2002). Occupations related to acquisition of infection are: (1) abattoir workers, veterinarians and laboratory technicians; (2) animal caretakers in zoos; and (3) workers in animal reservations and national parks. (Grange and Yate, 1994, Pavlik*et al.,* 2002).

Demographic factors also contribute to the epidemiology of bovine TB. These include income, education, age, number of families per dwelling, number of individuals per m^2 in a dwelling, sanitation. Families in low-income countries such as Africa often experience malnutrition. This, associated with the burden of HIV/AIDS infection, increases susceptibility to various infectious diseases such as TB by impairing the immune system, particularly lymphocyte function, which plays an important role in containing mycobacterial infections. Infants are more vulnerable to food-borne *M. bovis*infection, whereas in older individuals overt TB may occur as a result of endogenous reactivation. Poor sanitation, lack of access to clean water, crowding, poor housing and the absence of health care play an important role in the epidemiology of TB in developing countries (Theon *et al,* 1995; Pavlik*et al.,* 2002)

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Kaduna state, with an estimated land area of about 46,063 km² (KDSG, 2008). The State lies between longitude 7º and 44º East of the Greenwich meridian and between latitude 6º and 11º North of the Equator. Kaduna state occupies part of the central position of the Northern part of Nigeria (with Kaduna as its capital) and shares common borders with Zamfara, Katsina, Niger, Kano, Bauchi and Plateau States and Federal Capital Territory, Abuja.Kaduna state has a total amount of annual rainfall varying between 1,000 mm and 1,500 mm and a rainy season, which is between 120 and 150 days long. The northern part of the State is semi-arid, but further South, as the rain levels increase, the climate becomes sub-humid. The extreme South of the State is marked by rocky hills.(National Livestock Reports, 1992). According to the National Population Census (2006), Kaduna has a human population of more than 6 million (KDSG, 2008). Zaria (11° 3' N; 7° 42' E) is located about 83km North of Kaduna, along the Kaduna-Kano highway (Mortimore, 1970).

Nigeria has one of the largest livestock populations in Africa and Kaduna state is the centre of trade in livestock and livestock feed, attracting investors from neighboring states and countries. The state currently has 11 large livestock markets that make up 2.9% of Gross state production (Anon, 2010). According to the report from Kaduna state policy on Livestock (2005) Kaduna state has a total population of 1.9 million cattle, 1.6million goats and 1.3million sheep.Kaduna state is essentially an agrarian society with about 75% engaging in farming and it also has potentials for livestock industry.



Figure 3.1 Kaduna State Map

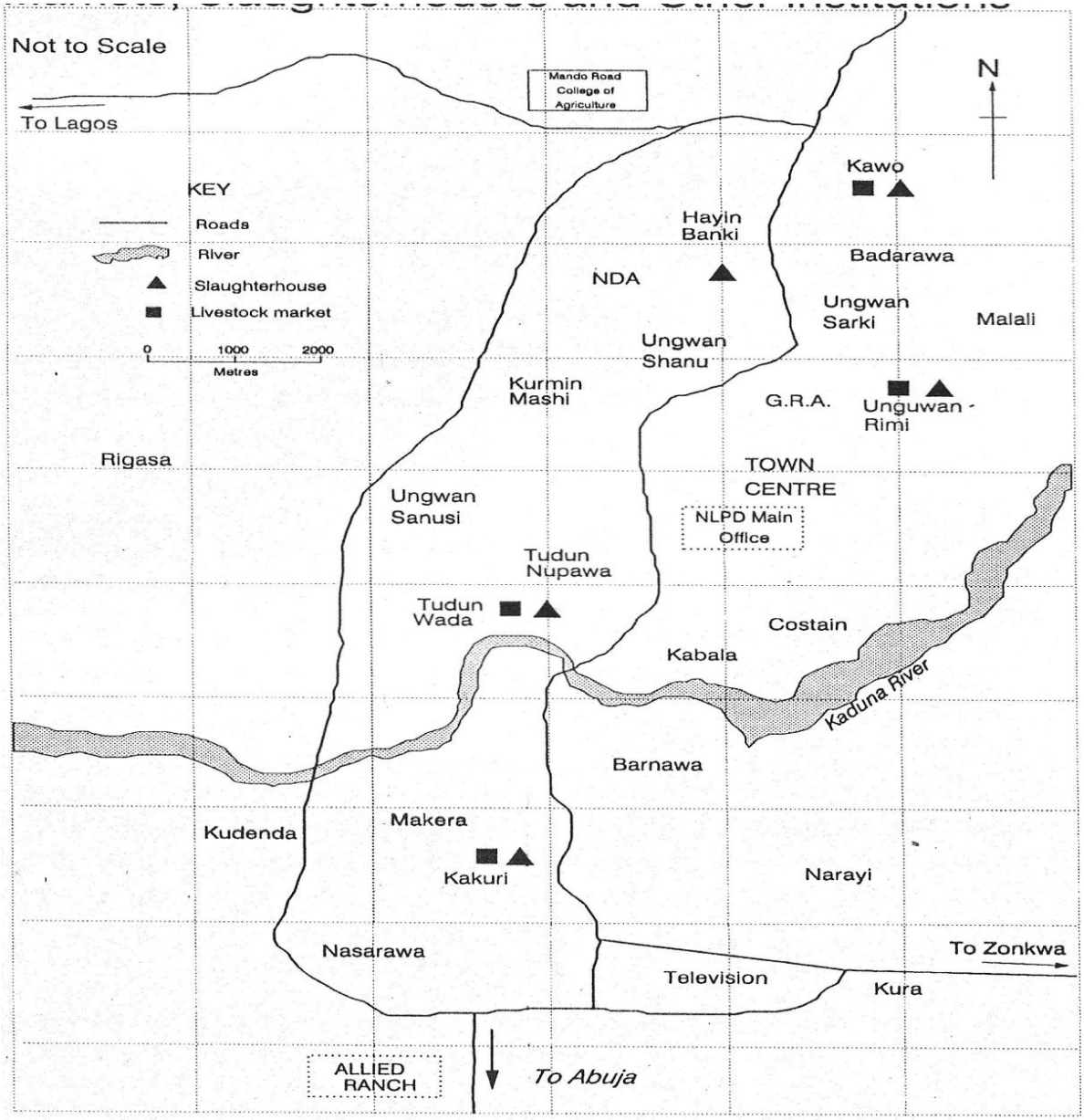


Figure 3.2: Showing the Kaduna metropolis and the distribution of slaughter houses and livestock markets. (National Livestock Report, 1992)

3.1.1 Sample sites

Cattle and goats from Zango abattoir (Zaria) Tudunwada and Kawo abattoirs were studied.Theseabattoirs were selected because of the large number of animals slaughtered routinely and due to their proximity to livestock markets.The Zango abattoir has a range of 20-40 cattle slaughtered daily, TudunWada has 120-140 cattle slaughter and Kawo abattoir has 100-120 goats slaughter daily respectively. Visits were made 2 times a week, on days with the highest slaughter which has been found to be Mondays and Saturdays for a period of 3 months.

3.2 Sampling

Sera from 500 cattle and 200 goats were collected using convenient sampling method. The samples from cattle were obtained from Zango abattoir, Zaria and Tudun Wada abattoir in Kaduna, while samples from goats were obtained only from Kawo abattoir Kaduna. All the samples were tested for *M. bovis*antibodies using immunochromotography assay test. Also suspected tuberculous lymphnodes from different organs of the animals sampled were tested for acid-fast organism using Ziehl-Neelsen staining technique as described by Kazwala*et al. (*1998). Physical characteristics such as coat colour shape of head and ears, position of hump were used to determine the breed of cattle tested. (Tawah and Rege, 1996).The ages were determined by examination of the teeth and the use of the cornual ring markers(Cole,1978).

3.2.1 Blood and tissue sample collection

Blood samples were collected from the jugular veins directly during slaughter usingproperly labeled sample bottles without anticoagulant and were allowed to stand for some hours at room temperature for a separation between the clotted blood and the serum. Sera were decanted into clean labeled serum bottles which were transported to the public health laboratory of the Department of Veterinary public Health in ice packed boxes and stored at 4^0C, until used.

Lymphnodes examined at meat inspection for the presence of suspected lesions suggestive of tuberculosis were collected, and placed in sterile screw capped, leak-proof specimen containers, containing 0.9% normal saline which was transported on ice to the Microbiology laboratory of the Department of Veterinary Medicine ABU Zaria for processing and staining using Ziehl- Neelsen

3.3 Laboratory Procedure

3.3.1 Description of the IMAT test kit

Anigen® rapid bovine tuberculosis test kit was obtained from Anigen Animal Genetic Incorporated in South Korea and was used for the detection of *M.bovis*antibodies in the sera collected. The test kit contains Anigen® rapid bovine tuberculosis antibodies test devices and specimen droppers. The rapid test was based upon the detection of antibodies in infected animals to a set of recombinant*M.bovis* antigens. The procedure used was immunochromatography assay test that uses a drop of blood serum and gives a visual result within 15-20 minutes (Greenwald *et al*, 2003).

3.3.2 Immunochromatography Assay Test (IMAT)

The kit was stored at 4^0C according to the manufacturer's instruction;

The test kit was brought out from the foil pouch and placed on a flat, dry surface at room temperature and then properly labeled

After allowing the sera to thaw at room temperature, a drop of the serum was slowly added to the sample holes with specimen dropper, after which 3 drops of developing buffer were added, reactions were observed after 20 minutes each.

Positive Reaction

The presence of two red color bands (T and C band) within the result window within 20minutes indicated positive reaction.

Negative Reaction

The presence of only one redcolour band (C band) within 20minutes in the result window indicated negative reaction.

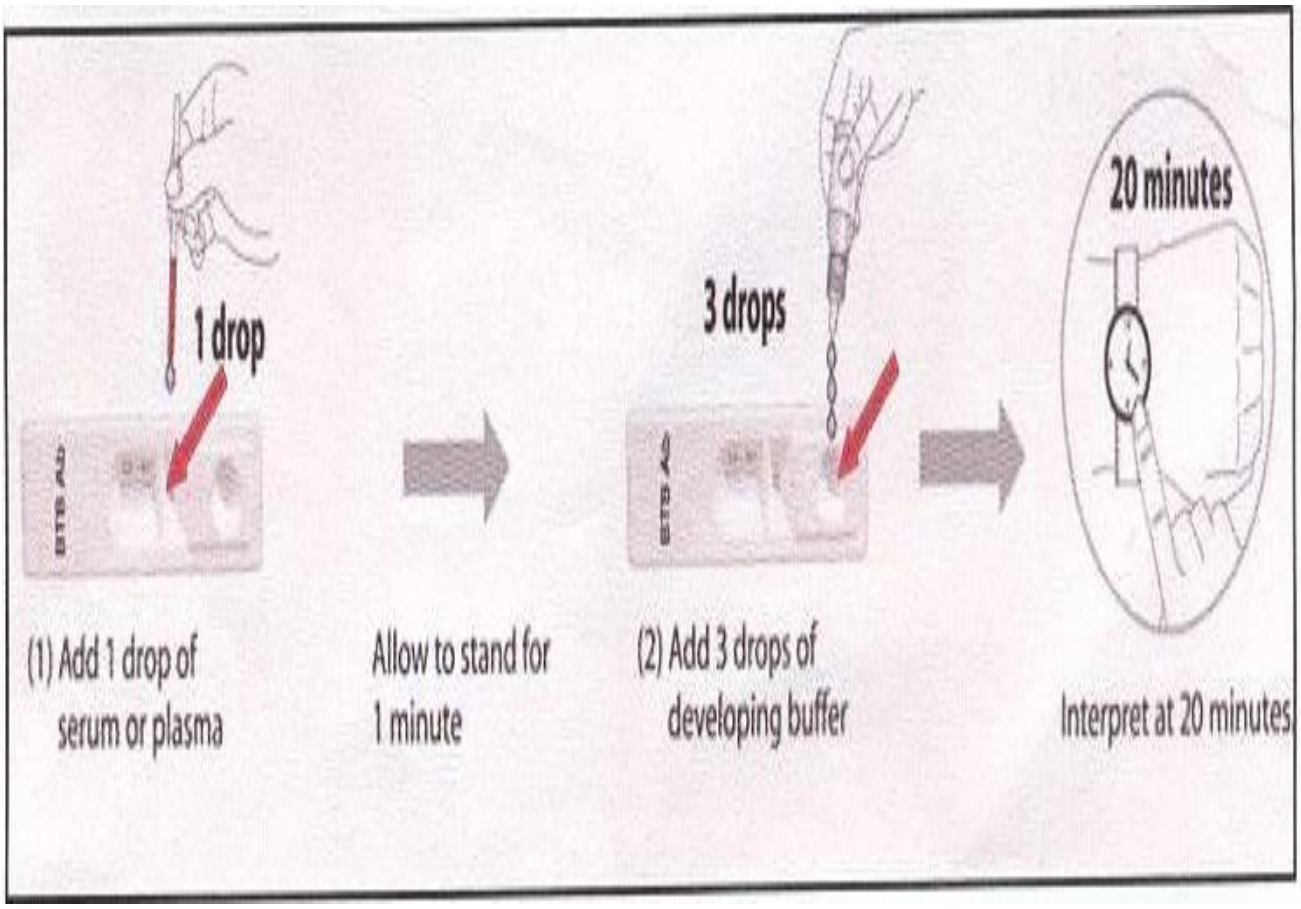


Figure 3.3: Immunochromatography assay test procedure as described by the manufacture



Figure 3.4: Evidence of Negative test result with immunochromatography assay test



Figure 3.5: Evidence of Positive test result with immunochromatography assay test

3.3.3 Tissue Preparation for ZiehlNeelsen technique

The suspected lymphnodes suggestive of Tuberculosis tuberculeswhich were placed in screw capped, leak-proof containers containing 0.9% normal saline were removed and put in a clean petri dish using a wooden applicator stick and properly crushed. The crushed tissues were used to make a thin smear on cleaned labeled grease free glass slides. The smear was evenly distributed keeping the area of the smear relatively small; smears were made on each glass slide.

3.3.3.1 Acid-fast StainingTechnique

Ziehl-Neelsen acid-fast staining procedure was used for staining the slides prepared based on procedures given by Kazwala*et al.* (1998) as follows:

The smeared slides were air dried and heat fixed by passing them gently over the flame of fire so as to fix the specimen to the slide in order to preserve the bacteria morphology.The slides were flooded with carbolfuchsin stain and steamed gently with flame from under side for 1minute.The slides were rinsed off with water and decolorized with 5% acid alcohol for 1-2 minutes until the red color disappeared.Theslides were counter stained with methylene blue for 1minute. Excess color was removed by additional rinsing with water and dried accordingly. The slides were examined under the microscope with oil immersion lens x 100.Positive bacilli stained red, straight rods occurring singly.

3.4 Data Presentation and Analysis

Graphadprism version 4.0 for windows (Graphad software San Diego California, USA) was used for the analysis. Chi-square and Fishers" exact test were used to determine association between ages, breed and sex with the presence of mycobacterium antibodies. Prevalence rate was determined using the formula:

$$Prevalence = \frac{Number\ of\ positive\ samples}{Total\ number\ of\ samples\ analysed} \times 100\%$$

A value of $P<0.05$ was considered significant.

CHAPTER FOUR

RESULTS

The results of immunochromatography assay test carried on the sera of 500 cattleand 200 goats showed a total prevalence of 16.57%. Of the 500 hundred cattle sampled, the prevalence obtained was91(18.2%)(Table 4.1).Breed specific prevalence was found to be 18.57% for the White Fulani while the SokotoGudali had 0% prevalence (Table 4.2). By sex thegeneral prevalence obtained was16.0% for males and 32% for females (Table 4.3). On closer look at the two abattoirs,the prevalence obtained by sex was 16% and 17% for males at Tudunwada and Zangonshanu respectively (Table 4.4).By age for the two abattoirs (Tudunwada and Zangonshanu) prevalence are given in Table 4.5and they include 18.2% and 14.5% for the cattle in the 3 to 4 years age bracket in Tudunwada and ZangonShanu abattoirs respectively while for those within the 5 to 6 years age bracket the prevalence were 18% and 21.2% respectively.There was no prevalence for the single animal within the over 6 years old bracket. Considering the female cattle the prevalence by age group indicated that female cattle within the age group of 3 to 4 years had a prevalence of 38.4% while those within the 5 to 6 years old had a prevalence of 30% and there was no female cattle above 6 years examined (Table 4.6). The Age relationship for prevalence for cattle with regard to the males, animals within the age group of 3 to 4 years had prevalence of 13% while those within 5 to 6 years had prevalence of 20%, (Table 4.7).

Of the 200 goats sampled the prevalence of12.8 %(Table 4.8) was obtained mainly in the Red Sokoto breed of goat. By sex the prevalence obtained were13% for males and9.6%for females (Table 4.9).By age8 out of 70 (11%)goats aged 6-24 months were

positive, while 17 (13.1%) goats of ages 25-36months were positive (Table 4.10). There was a significant association between the sex of cattle and detection of mycobacterium antibodies (Chi square, P=0.0022). None of the 200 goat tissues examined had lesions suggestive of tuberculosis. However, of the 500 cattle sampled, 2 had tissues suggestive of tuberculosis and subsequently were positive using acid fasttechnique.An overall prevalence of 0.4% was obtained for the tissues.

Table 4.1 Prevalence of *M.bovis* infection detected by Immunochromatography assay test in cattle and goats sampled in 3 selected abattoirs in Kaduna State.

Specie	No of animals tested	Number +ve	% +ve
Cattle	500	91	18.2
Goats	200	25	12.5
Total	700	116	16.57

Table 4.2: Prevalence of*M.bovis* infection in cattle breeds in 2 selectedabattoirs in Kaduna State using immunchromatography assay test.

Breed	No of animals tested	Number +ve	% +veP-value
WF	490	91	18.57P=0.2207
SG	10	0	0
Total	500	91	18.57

Table 4.3: Prevalence of *M .bovis* infection by sex in cattle in 2 selected abattoirs in Kaduna State using Immunochromatogaphy assay test.

Sex	No of animals tested	Number +ve	X^2	% +veP-value
Male	438	71	9.395	16P=0.0022
Female	62	20		32
Total	500	91		18.2

Table 4.4: Prevalence of *M .bovis* infection by sex in cattle in 2 selected abattoirs in Kaduna State using Immunochromatogaphy assay test.

ZangonShanu				
Sex	**No of Sera Tested**	**No. of Sera Positive**	**X^2**	**P-Value**
Male	212	35 (17%)	2.0999	0.1474
Female	38	10 (26.3%)		
Total	250	45 (43.3%)		
Tudun Wada				
Male	226	36 (16%)	9.572	0.002
Female	24	10 (42%)		
Total	250	46 (58%)		

Table 4.5Prevalence of *M .bovis* infection by age in cattle in 2 selected abattoirs in Kaduna State using Immunochromatogaphy assay test.

Tudun Wada				
Age (years)	**No of Sera Tested**	**No. of Sera Positive**	**X^2**	**P-Value**
3 – 4	99	18 (18.2%)	0.005197	0.9425
5 – 6	151	28 (18%)		
Total	250	46 (36.2%)		
ZangonShanu				
3 – 4	113	16 (14.6%)	2.061	0.1511
5 – 6	137	29 (21.2%)		
Total	250	45 (35.8%)		

Table 4.6: Prevalence of *M.bovis* by age among female cattle and different age groups

Age	No animals tested	number +ve	% +ve	X^2	P – value
3 – 4	13	5	38.4	0.2897	
5 – 6	49	15	30		
> 6	-	-	-		
Total	62	20	32.2		

Table 4.7: Prevalence of *M.bovis* in male cattle with regard to different age groups

Age	No animals tested	number +ve	% +ve	X^2	P – value
3 – 4	222	28	13	0.2897	0.5904
5 – 6	216	43	20		
Total	438	71	33		

Table 4.8Prevalence of *M .bovis* infection by breed in goats in Kawo abattoir in Kaduna State using Immunochromatogaphy assay test.

Breed	No of animals tested	Number +ve	% +veP-value
RS	194	25	12.8%P=1.00
KB	6	0	0
Total	200	25	12.5%

KEY:

RS-Red Sokoto

KB-Kano Brown

Table 4.9: Prevalence of *M .bovis* infection by sex in goats in Kawo abattoir in Kaduna State using Immunochromatogaphy assay test.

Sex	No of animals tested	Number +ve	% +veP-value
Male	169	22	13.P=0.7725
Female	31	3	9.6
Total	200	25	12.5

Table 4.10: Prevalence of *M .bovis* infection by age in goats in Kawo abattoir in Kaduna State using Immunochromatogaphy assay test.

Age range (months)	No of animals tested	Number +ve	X^2	% +veP-value
6-24	70	8	0.1130	11.0P=0.7367
25-36	130	17		13.1
Total	200	25		12.5

CHAPTER FIVE

DISCUSSION

From the study a prevalence of 16.57% for *M.bovis* in cattle and goats in Kaduna State was demonstrated. This shows that the infection due to *M.bovis*is increasing.Abubakar*et al,* (2007),who compared the trend for the infection in the last 30 years due to *M.bovis* showed a range from 2.5% in 1976 to 14% in 2007.This could be because there has not been any established program for the control of the disease in addition to poor and inadequate implementation of animal disease control, unrestricted movement of animals, increasing interaction among others.

The study further shows that goats had prevalence rate of 12.5% which means that goats can come down with *M.bovis* infection. This could probably be because livestock owners in Nigeria graze both cattle and other animals together which posses a high risk of transmission. Furthermore,Kazwala*et al. (*1998) andAyele,*et al. (*2004) have reported thatshared housing among animals especially goats could be a source of infection.

Mycobacteriumbovis has been known to cause tuberculosis in goats, although the importance of the disease varies depending on the country. Infection occurs as a direct result of contact with infected cattle. Infection in several herds of goats in India between 1941 and 1942 was associated with goats grazing along with heavily infected cattle (Lau, 1969). In one case where common grazing had occurred for four years 24.7% of 417 goats gave a positive result to the tuberculin tests and 90% of these had tuberculosis lesions. In Australia where goats are rarely kept with cattle, a single case of *M. bovis* infection in a goat has been reported; a low prevalence of tuberculosis in goats has been reported in Taipei china Lu *et al.*(1992).

Females from the study have higher prevalence than males even though more males were slaughtered in the abattoirs. This could be because most females that are brought to the abattoirs might have stayed longer in the farms compared to males that are fattened for slaughter. The fact that pregnancy results in compromising their immune system, makes them more susceptible to the infection. This agrees with the work ofKazwala*et al.*2001 who stated that females have higher life expectancy and stress due to pregnancy and milk production that depress their immunity making them susceptible to the disease. Furthermore, Kazwala*et al. (2001*)showed that more cows tested positive to the skin test than males even though in this work, IMAT kit was used. If such female cattle are brought to the abattoir this could be a source of infection to butchers, who by practice, do not use gloves and clothing and to consumers who do not properly cook TB infected offals. A newly introduced TB infected animal into a herd could also spread the infection in the herd. This agrees with the findings of Cadmus *et al,* (2006).

Another reason could be that farmers rarely require few males in their herds since such adult males could fight during the stage of oestrus by cows in an attempt to breed them. The fact that males do not add to herd size could be a contributory factor for seeing more males at abattoirs than females.

White Fulani breed of cattle and Red Sokoto breed of goats had higher rate of reactors than other breeds sampled, this could be because from records more of these breeds mentioned are the abundant breeds in the area,and about 90% of cattle are the white Fulani (KSPSL,2005).

Of all the factors, breed,age and sex,only the sex of cattle showed a significant association with the mycobacterial antibodies using IMAT.$p<0.05$.This means that sex plays an important role in the susceptibility of cattle to Mycobacterium infection.

Older cattle were reactors and this could likely be because the disease is chronic and manifests more as the cattle gets older.This agrees with the work of Cleaveland*et al.,*(2007) who showed that older cattle come down with mycobacterial infection more than younger ones. It has been suggested that the increase in the incidence of TB in older animals can be explained by waning of protective capability in aging animals as experimentally confirmed in murine system.(O'Reilly *et al*, 1995)

Only2 suspected tissues were found and all tested positive using acid fast staining technique even though a higher number of the sampled animals tested positive without any gross lesions.This could be because the disease is chronic and lesions are mostly seen at the chronic stage but IMAT kit that was used is very sensitive and can pick antibodies at an early stage. The work of Assegede*t al*, 2004, Teklu*et al*, 2004 stated that not all cattle infected with *M.bovis* have visible lesions at slaughter.

With the result from this study it shows that with the total prevalence of 16.57% attention should be drawn btb infection especially in our slaughtered houses.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The study was carried out in three selected abattoirs in Kaduna State, Nigeria. A total of 500 cattle and 200 goats were used for the study. The study was set out to determine the prevalence of *M.bovis* in these animals using immunochromatography assay test and ZeilhNeelsen Acid fast stain on Lymphnode samples from the chest of the sampled animals. An overall seroprevalence rate of 16.57% was obtained. Furthermore, a seroprevalence rate of 18.2% and 12.5% were obtained for both cattle and goats respectively.

Among cattle the seroprevalence for the female and male cattle were 32% and 16% respectively. Among the different age groups of animals in the 3 – 4 years old bracket had the highest seroprevalence of 22% while those in the 5 – 6 year old bracket had 16.50% seroprevalence.

Among goats 22 (13%) of the 169 males were seropositive with only 3 (9.6%) of the 31 female goats seropositive for *M.bovis*. In terms of age, goats within the 6 to 24 months old bracket had 11% seroprevalence while those between 25 old 36 months old had 13.1% seropositive.

6.2 Conclusion

The prevalence of bovine tuberculosis obtained in this study (16.57%) showed that the disease is on the increase with more animals getting exposed. The prevalence in goats in particular, indicates that other species, apart from the primary host, cattle, are getting

exposed to *M.bovis.* This is of great epidemiological and public health significance and it requires urgent attention by all stakeholders .Therefore, a multidisciplinary approach is needed to address the disease.

6.3 Recommendations

1. Ante mortem inspection before slaughter should be introduced to reduce slaughtering reactive animals.
2. Detailed meat inspection should be implemented in abattoirs, to avoid consumption of suspected tissues or organs.
3. Education of all stakeholders and the general population on the potential health impact of bovine tb infection should be carried out with immediate effect.
4. Detailed epidemiological studies to determine the true extent of bovine tb infection in the State and Nigeria as a whole soonest is required.

REFERENCES

Abubakar, I.A. (2007). Molecular epidemiology of human and bovine tuberculosis in Federal Capital Territory and Kaduna State, Nigeria.Ph.D. Thesis, Plymouth University, U.K.

Adams, L. G. (2001). *In vivo* and *in vitro* diagnosis of *Mycobacterium bovis* infection In: *Review. Science tech. Office of International Epizootics,* 20 (1): 304 – 324.

Atsanda, N.N. and Agbede S.A. (1990). A survey for brucellosis,tuberculosis and cysticercosis in cattle slaughtered in Ibadan and Maiduguri abattoirs.*Nigerian Veterinary Journal*,29(1):61-66

Ajogi,I,Uko,U.E.and Tahir,F.A.(1995).A retrospective (1990-1995)study of tuberculosis,cysticercosis and Hydatidosis in food animals slaughtered in Sokoto abattoirs,Nigeria.*Tropical Veterinarian,*13:81-85.

Alhaji, I. (1976).Bovine tuberculosis in four northern states of Nigeria.PhD. Thesis. Ahmadu Bello University Zaria, Nigeria. 236.

Alito, A.; McNair, J.; Girvin, R.M.; Zumarraga, M.; Bigi, F.; Pollock,J.M.andCataldi, A. (2003).Identification of *Mycobacterium bovis*antigensby analysis of bovine T-cell responses after infection with a virulentstrain.*Brazilian.Journal.Biology.Research.*36 (11), 1523-1531.

Amanfu W. (2006).The situation of tuberculosis and tuberculosis control in animals of economic interest.*Tuberculosis.*86:330.

Ameni, G. and Tibbo, A. (2003). Preliminary study on bovine tuberculosis in the peripheral blood of calves vaccinated with BCG. *Journal of Immunoassay and Immunochemistry* 23(2): 245-253.

Anon (1994a) Framework for effective tuberculosis control.Geneva,Switzerland: World Health Organization, 179 pp.

Anon (1994b).Zoonotic tuberculosis (*Mycobacterium bovis*): A memorandumfrom WHO (with participation of FAO). Bulletin ofWorld HealthOrganization; 72: 851–857pp.

Anonymous, (2010).Ministry of Agriculture Kaduna state Report. http://www.kadunastate_ng.com.htm. Retrieved 1/24/2011. 10:57pm.

Asseged B., Woldesenbet Z., Yimer E., Lemma E. (2004): Evaluation of abattoir inspection for the diagnosis of*Mycobacterium bovis*infection in cattle at Addis Ababaabattoir. Tropical Animal Health and Production, 36:537–546.

Aranaz, A., Liebana, E., Enrique, G., Juan, C. G., Debby, C., Arturo, O., Jesus Blazquez, F.B., Ana, Mateos, Guilermo, S., and Lucas, D. (1999). *Mycobacterium tuberculosis subsp. caprae subsp. nov.*: A taxonomic study of a new member of the Mycobacterium tuberculosis complex isolated from goats in Spain. *InternationalJournal of systematic bacteriology.*49,1263-1273.

Ayele, W.Y., Neil, S.D., Zinsstag, J., Weiss, M.G. and Pavlik, I. (2004). Bovine tuberculosis L and old disease but new threat to Africa: Review article. International Journal of Tuberculosis and Lung Disease, 8(8): 924-937.

Biet, F., Boschiroli, M.L., Thorel, M.F. andGuilloteau, L.A. (2005).Zoonotic aspects of *Mycobacterium bovis* and *Mycobacterium avium-intracellulare* complex (MAC).*Veterinary Research. 36(3): 411-436.*

Blood D C,andRadostis O M, eds. (1989) Disease caused by mycobacteria—IV. In: Veterinary Medicine. 7th edition. London, UK: BailliereTindall,; 7: 710–740.

Brooks, Geo., Carroll, Karen C., Butel, Janet s., Morse, Stephen A. (2007). Jawetz, Melnick and Adelberg's Medical Microbiology 24th Edition. McGraw-Hill Companies, Inc., Pp. 320-326

Cadmus, S.I.B., Palmer, S. Okker., M. Dale J. Gover. Smith, N. Jahans, K. Hewinson, R.G. and Gordon, S.V. (2006): molecular analysis of human and bovine tubercle bacilli from a local setting in Nigeria. *Journal of Clinical Microbiology.* 44. 129 – 34.

Chadwick, Maureen V. (1982). *Mycobacteria*.Institute of Medical Laboratory Sciences Monographgs. John Wright & Sons Ltd, at Stonebridge Press, Bristol BS4 5NU, Pp. 1-19.

Cleaveland S., Shaw D.J., Mfinanga S.G., Shirima F., Kazwala R.R., EblateE.and Sharp M.(2007) *Mycobacterium bovis* in rural Tanzania: risk factors for infection in human and cattle populations, Tuberculosis87:30-43.

Cole, B. V.Sc; V.G. (1978) Beef production guide Macarthur press Parramatta. ISBN 0-959-9973-8.

Collins C. H. and Grange J. M. (1983).The bovine tubercle bacillus.*Journal of Applied Bacteriology*. 55; 13-29.

Collins D M.(1999) DNA typing of *Mycobacterium bovis*strainsfrom the Castlepoint area of the Wairarapa. *N Z Veterinary Journal*; 47: 207–209.

Corner L.A (1994).Post mortem diagnosis of *Mycobacterium bovis*infection in cattle. *Veterinary microbiology*, 40(1-2): 53-63.

Corner L A L, Norton S, Buddle B M, and Morris R S.(2002) The efficacyof bacilli Calmette-Guerin vaccine in wild brushtail possums(*Trichosurusvulpecula*). *Research Veterinary Science;* 73: 145–152.

Cosivi O, Grange J M, Daborn C J (1998)Zoonotic tuberculosisdue to *Mycobacterium bovis*in developing countries.*Emerging Infectious Disease* 4: 59–70.

Cosivi O, Meslin F X, Daborn C J, Grange J M. (1995) Epidemiologyof *Mycobacterium bovis*infection in animals and humans, with particular reference to Africa.*Revised Science Technology Office International Epizootic* 14: 733–746.

Cosivi,O., Grange J.M., Darborn, C.J., Ravigilione, M.C., Fujikura T., Cousins D., Robinson R.A., Huchzermeyer H.F., Dekantor I. and Meslin, F.X. (1998). Zoonotic tuberculosis due to *Mycobacteriumbovis* in developing countries.*Emerging infectious disease.* 4, 1-17.

Cousins D V. (2001)*Mycobacterium bovis*infection and control indomestic livestock. *Revised Science Technology Office International Epizootic;* 20: 71–85.

Daborn C J, Grange J M, and Kazwala R R.(1996) The bovine tuberculosis cycle-an African perspective. *Journal Applied Bacteriology*; 81: S27–S32.

Daborn C J and Grange J M.(1993) HIV/AIDS and its implications for the control of animal tuberculosis.*Britain Veterinary Journal;* 149: 405–417.

Dalovisio, J.R., Stetter, M., Mikota-Wells, S. (1992). Rhinoceros' rhinorrhea: cause of an outbreak of infection due to airborne *Mycobacterium bovis* in zookeepers. *Journal ofClinical Infectious Dis*eases; 14:598–600.

Edginton M E, Sekatane C S, Goldstein S J (2002). Patients' beliefs: dothey affect tuberculosis control? A study in a rural district ofSouth Africa.*International Journal Tuberculosis Lung Disease;* 6: 1075–1082.

Falkinham, J. (1996).Epidemiology of infection by non tuberculous*Mycobacteria*.*Clinical Microbiology Review* 9(2): 177-215.

Fhanning, A.and Edwards, S. (1991). *Mycobacterium bovis* infection in human beings in contactwith elk (*Cervuselephus*) in Alberta, Canada.*Lancet*; 338:1253–1255.

Federal Ministry of Health. (2006) .A technical report on the 2005 National HIV/Syphilis sero-prevalence Sentinel Survey among pregnant Women attending Ante-natal clinics in Nigeria. FMOH, Abuja.

Fifis, T.; Rothel, J.S.; Wood, P.R. (1994). Soluble *Mycobacteriumbovis*protein antigens: Studies on their purification and immunologicalevaluation. *Veterinary Microbiology.*40 (1-2): 65-81.

Francis J. (1947). Bovine tuberculosis including a contrast with humantuberculosis. London, UK: Staples Press Limited: 220 pp.

Garnier T, Eiglmeier K, Camus J C,(2001).The complete genome sequence of *Mycobacterium bovis.*ProcNational Academic Science USA; 100: 7877–7882.

Gibbons W.J. (1963). In: Diseases of Cattle Revised Second Edition pp. 628.

Goodchild, de la RuaDomenech, R.; A.T.; Vordemeier, H.M.;Hewinson, R.G.; Christiansen, K.H.andClifon-Hadley, R.S. (2006). Antemortem diagnosis in cattle: A review of the tuberculin tests, g-interferon assay and other ancillary diagnostic techniques. *Research Veterinary Science.*81 (2), 190-210.

Goodchild,A.V. and Clifton-Hadley,R.S. (2001).Cattle to cattle transmission of *Mycobacterium bovis*.*Tuberculosis* 81(1-2): 23-24.

Gordon S V, Heym B, Parkhill J, Barrell B,and Cole S T.(1999) New insertionsequences and a novel repeated sequence in the genome of*Mycobacterium bovis*H37*Revised Microbiology*; 145: 881–892.

Grange J M(1996).The future and the challenge.*Mycobacteria* andhuman disease.2nd edition. London, UK: Arnold; 2: 7–10.

Grange J M, and Yates M D. (1994) Zoonotic aspects of *Mycobacteriumbovis*infection. *Veterinary Microbiology* 40: 137–151.

Grange, J.M. (1995). Human aspect of *Mycobacterium bovis* infection in: C.O. Theo and J.H. Steel (eds). *Mycobacterium bovis* infection in animals and human, lowa state university press, ames, lowa, pp. 29 – 46.

Grange.J.M(2001).*Mycobacterium bovis* infection in human beings,*Tuberculosis* 81:71-77.

Greenwald, R., Esfandiari, J., Lesellier, S., Houghton, R., Pollock, J., Aaguard, C., Anderson, P., Hewinson, R.G., Chambers, M and Lyaschenko K. (2003). Improved serodetection of*M.bovis* infection in badgers(melesmeles) using multiantigen test formats.Diagnostic microbiology and infectious disease,46:197-203.

Guerrero A, Cobo J, Fortun J (1997). Nosocomial transmissionof *Mycobacterium bovis*resistant to 11 drugs in people withadvanced HIV-1 infection.Lancet; 350: 1738–1742.

Hasegawa N, Miura T, Ishii K, (2002). New simple and rapid testfor culture confirmation of *Mycobacterium bovis*complex: amulticenter study. *Journal of Clinical Microbiology*; 40: 908–912.

Ibrahim, S. (2007).The epidemiology of human and bovine tuberculosis in JigawaState Unpublished M.Sc thesis,Ahmadu Bello University,Zaria.Pp.45-69.

Igbokwe,I.O,Madaki,I.Y,Danburan,S.Ameh,J.A,Aliyu,M.M,andNwosu,C.O(2001).Prevalence of pulmonary Tuberculosis Lesions in Cattle Slaughtered in Northeastern Nigeria.*Revue d"elevage et de medicine veterinaire des pays tropicaux,*54(3-40:191-195

Idigbe, E.O., Ayiwo, C.E. and Onwujekwe, D.I. (1986).Human pulmonary infections with bovine and atypical mycobacteria in Lagos, Nigeria.*Journal of Tropical Medicine Hygeine.*89:143 –148.

Kaduna State Government (KDSG) (2008)Kaduna State Achievements in: Data on EstimatedAnnual Animal Population and Fish Investment Opportunities in Kaduna State Ed,pp 16-18.

Kazwala R R, Daborn C J, Kusiluka L J, Jiwa S F, Sharp J M, and Kambarage D M.(1998) Isolation of *Mycobacterium* species fromraw milk of pastoral cattle of Southern Highlands of Tanzania.*Tropical Animal Health Production;* 30: 233–239.

Kazwala R.R., Kambarage D/M., Daborn C.J., Nyange J., Jiwa S.F.H., and Sharp J.M. (2001), Risk factors associated with the occurrence of bovine tuberculosis in cattle in the Southern Highlands of Tanzania, Veterinary.*ResearchCommunity*. 25: 609-614.

Kremer K, van Soolingen D,and Frothingham R,(1999). Comparisonof methods based on different molecular epidemiologicalmarkers for typing of *Mycobacterium bovis*complex strains:inter-laboratory study of discriminatory power and reproducibility.*Journal Clinical Microbiology*; 37: 2607–2618.

Kubica G P and, Wayne L G(1984), eds. In: The mycobacteria, a source book, Part A. New York, NY:Marcel Dekker, Clinical microbiology. 680.

Lau J.M (1969). Tuberculosis among animals in India *Veterinary Bulletin,* 39(6), 385 – 390.

Lilenbaum, W.; Fonseca, L.S andPessolani; M.C.V. (2006).The use of Ag85complex as antigen in ELISA for the diagnosis of bovine tuberculosis indairy cows in Brazil.*Journal Veterinary Medicine* 48 (3), 161-166.

Lilenbaum, W.; Schettini, J.C.; Ferreira, M.A.S.; Souza, G.N.; Ribeiro,E.R.; Moreira, E.C.and Fonseca, L.S. (1999).Evaluation of an ELISA – PPDfor the diagnosis of bovine tuberculosis in field trials in Brazil.*Research Veterinary Science.*66: 191-195.

Liu, S.; Guo, S.; Wang, C.; Shao, M.; Zhang, X.; Guo,Y.andGong, Q.A.(2007).Novel fusion protein-based indirect enzyme-linkedimmunosorbent assay for the detection of bovine tuberculosis.*Tuberculosis* 87 (3), 212–217

Lu Y., Tsei H.J., Tsung C.S., Chen C.C., Leeh L., Hung H.H. and Lee S.H. (1992) – Goat tuberculosis in Taiwan. Taiwan *Journal. Veterinary Medicine Husbandry,* 59, 61-68.

McNair, J.; Corbett, D.M.; Girvin, R.M.; Mackie, D.P.and Pollock, J.M.(2001). Characterization of the early antibody response in bovinetuberculosis: MPB 83 is an early target with diagnostic potential. *Scandinevia.Journal Immunology*. 53, 365-371.

Morris P J, Thoen C O,and Legendre A M.(1996) Pulmonary tuberculosisin an African lion (*Pantheraleo*). *JournalZoo Wild life Medicine*; 27: 392–396.

Mortimore MJ (1970). Zaria and its Regions. Annals of the Association of America Geographers, 60(1):73-80.

National Livestock Reports (1992). *NigerianLivestock Resources.* Vol.111: State reports: Kaduna state. Pp. 2-22.

Neill S D, Bryson D G, Pollock J M (2001). Pathogenesis of tuberculosisin cattle.*Tuberculosis*; 81: 79–86.

Neill S D, Cassidy J, Hanna J,(1994). Detection of *Mycobacteriumbovis*infection in skin test-negative cattle with an assayfor bovine interferon-gamma. *Veterinary Record*; 135: 134–135.

Neill S D, O'Brien J J, Hanna J.(1991) A mathematical model for*Mycobacterium bovis*excretion from tuberculous cattle. *Veterinary Microbiology*; 28: 103–109.

Okaiyeto, S.O,Allam,E.and Sabo G.(2008).Investigation of prevalence of bovine tuberculosis in dairy farm in Kaduna State, *Nigeria.Research Journal of Dairy Sciences,*2(1):27-29.

O'Reilly, L.M. and Darborn, C.J. (1995).The Epidemiology of *Mycobacterium bovis* infection in animals and man.*Tubercle and Lung Disease*.76:1 – 46.

Office International des Epizooties.World Animal Health.PartI. Paris, France: OIE, 1992: 263–264.

Pavlik I, Ayele W Y, ParmovaI,andMelicharek (2002)l. Incidence of bovine tuberculosis in cattle in seven Central European countries duringthe years 1990–1999. *Veterinary Medicine* Czech; 47: 45–51.

Pavlik I, Ayele W Y, Parmova I,(2002). *Mycobacteriumbovis*in animal and human populations in six CentralEuropean countries during 1990–1999. *Veterinary Medicine-Czech;* 48: 83–89.

Pollock, J.M. & Neill, S.D. (2002). *Mycobacterium bovis*infection andtuberculosis in cattle.*Veterinary Journal.*163 (2), 115-127.

Pritchard D G (1988). A century of bovine tuberculosis - conquest and controversy.*JournalComp Pathology;* 99: 357–399.

Quinn P. J., Carter, M. E., Markey, Y. B. and Carter, G. R. (1994).*Clinical Veterinary Microbiology,* Mosby Year Book Europe Ltd. London. Pp. 156-169

Radotsits, O, M., Blood, D.C., Gay, C.C and Keneath, W.H. (2000). Veterinary Medicine: In: A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses.(9th edition.). Harcourt Publisher Ltd., London.Pp. 909- 919.

RIM (Resource Inventory and Management). 1990. *Livestock resource survey*.

Roring S, Brittain D, and BunschotenA E, (1998). Spacer oligotypingof *Mycobacterium bovis*isolates compared to typing by restrictionfragment length polymorphism using PGRS, DR and IS*6110* probes. *Veterinary Microbiology;* 61: 111–120.

Rose, N. R., and Barron, A. L. (1983). Microbiology: basic principles and clinical applications. Macmillan Publishing Company, New York. Pp. 223-234.

RosembergJ,Tarantino,A,B,,Paula,A.(1990).Tuberculose.*In*:Tarantino,A,B(ed)*Doencas pulmonares*.3.ed.,GuanabaraKoogen,Riode Janeiro,p233-297)

Shitaye, J.E.; Parmova, L; Matlova, L.; Dvorska, L.; Horvathova, A.; Vrabas, V. and Pavlik, I. (2006). *Mycobacterial* and*Rhabdococcusequi* infections in pigs in the Czech Republic between the years 1996 and 2004: The causal factors and distribution of infections in the tissues. *Veterinarian Medicina*. 51: 497-511

Tawah, C.L. and Rege, J.E.O. (1996). Gudali cattle of west and central Africa. FAO *Animal Genetic Resources Information Bulletin.* 17: 159 – 170.

Teklu A., Aseeged B., Yimer E., GebeyehuM.andWoldesenbet Z. (2004): Tuberculous lesions not detected by routine abattoir inspection: the experience of the Hossanamunicipal abattoir, Southern Ethiopia. Review of Science and Technology, Office International des Epizooties,23, 957–964.

Thoen, C.O., Hall, M.R., Harrington, R. and Pietz, D.E (1975): Application of modified ELISA for detecting mycobacterial antibodies in sera of cattle from a herd in which *M. bovis* was diagnosed. *Proceedings of 87th Annual Meeting of US Animal Health Association. Las Vegas,* pp 603 – 610.

Thoen C O, Hums E M.(1986) Pathogenesis of *Mycobacteriumbovis*infection.Program *Veterinary Microbiology Immunology* 2: 198–214.

Thoen C O, Steele J H, (1995). *Mycobacterium bovis*infection inanimals and humans.Arnes, IA: Iowa State University Press, 355 pp.

Thoen, C. and Barletta, R.G. (2006).Pathogenesis of *Mycobacterium bovis*.In*fection in animals and humans.*Blackwell Publishing Ltd.,Iowa USA. Pp. 18-33.

Thoen, C.O., Steel, J.H. and Glisdor, M.J. (2006). *Mycobateriumbovis* infection in animals and humans. 2nd edn. Blackwell publishing. Ames. USA. Pp 329.

Van Embden J D A, van Gorkom T, Kremer K, Jansen R, vanderZeijst B A M, Schouls L M.(2000) Genetic variation and evolutionaryorigin of the direct repeat locus of *Mycobacteriumbovis*complex bacteria. *Journal Bacteriology* 182: 2393–2401.

Van Soolingen D, De Haas P E W, and Haagsma J (1994).Use of variousgenetic markers in differentiation of *Mycobacterium bovis*strains from animals and humans and for studying epidemiologyof bovine tuberculosis.*Journal of ClinicalMicrobiology* 32: 2425–2433.

Van Soolingen D, Hoogenboezem T, De Haas P E W, (1997). Anovel pathogenic taxon of the *Mycobacterium bovis*complex,Canetti: characterization of an exceptional isolate from Africa.*International Journal System Bacteriology*; 47: 1236–1245.

Van Soolingen D. (2001) Molecular epidemiology of tuberculosis andother mycobacterial infections: main methodologies and achievements. *Journal International Medicine*; 249: 1–26

WHO (1994).Zoonotic tuberculosis (*Mycobacterium bovis*). Memoranda from WHO/FAO meeting bulletin of the WHO. 72(6): 851-857.

Wiker HG. (2009). MPB70 and MPB83--major antigens of*Mycobacterium bovisScandineviaJournal Immunology.*69(6), 492-9.

Wiker, G.H. &Harboe, M. (1992). The Antigen 85 Complex: a majorsecretion product of *Mycobacterium tuberculosis*. *Microbiology Revised.* 56 (4),648-661.

Wiker, H. G.; Lyashcenko, K.P.; Aksoy, A.M.; Lightbody, K.A.;Pollock, J.M.; Komissarenko, S.V.; Bobrovnik, S.O.; Kolesnikova, I.N.;Mykhalsky, L.O.; Gennaro, M.L and Harboe, M. (1998). Immunochemicalcharacterization of the MPB70/80 and MPB83 proteins of*Mycobacterium bovisInfection Immunology.*66 (4): 1445–1452

Wood, P.R and Rothel, J.S. (1994).*In vitro* immunodiagnostic assays forbovine tuberculosis. *Veterinary Microbiology.*40: (1-2), 125-135.

World Health Organization Technical Report Series. (1959) JointWHO/FAO Expert Committee on Zoonoses, Second Report.Geneva, Switzerland: WHO, 169 pp.

World Health Organization. Global Tuberculosis Control: Surveillance, Planning, Financing. WHO Report 2005 Geneva.

Wray C. (1975) Survival and spread of pathogenic bacteria of veterinaryimportance within the environment.*Veterinary Bulletin*; 45:543–550.

Zumla A, Squire S B, Chintu C, Grange J M. (1999). The tuberculosispandemic: implications for health in the tropics. *Trans RoyalSociety Tropical Medicine Hygiene;* 93: 113–117.