SURVEY OF BOVINE TUBERCULOSIS IN SLAUGHTERED CATTLE IN GUSAU ABATTOIR, ZAMFARA STATE, NIGERIA

BY

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DECLARATION

I declare that the work in this Dissertation titled **"Survey of Bovine Tuberculosis in Slaughtered Cattle in Gusau Abattoir, Zamfara State Nigeria"** has been carried out by me in the Department of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

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CERTIFICATION

This Dissertation titled **"SURVEY OF BOVINE TUBERCULOSIS IN SLAUGHTERED CATTLE IN GUSAU ABATTOIR ZAMFARA STATE, NIGERIA"** by Ibrahim AHMAD meets the regulations governing the award of the degree of Master of Science in Veterinary Medicine of the Ahmadu Bello University Zaria, Nigeria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to my unborn kids. It serves both as ‘substance’ testimony, and the greatest legacy I can ever leave unto my successors. The history of how it felt to have both combined efforts and long spent moments translated into words.

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"O my Lord! If it is delayed, I complain because of manly eagerness and ignorance of that how You designed tomorrow; and perhaps the delay is better for me, for You know the ends of the matters…I have not found a generous guardian more patient than You towards an always-grateful servant like me, O my Lord!"

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ABSTRACT

Bovine tuberculosis (bTB) is a wide spread disease of cattle and other animals with both economic and public health significance, but it has received little attention in Nigeria; particularly in some parts such as Zamfara State. This study aimed at detecting and speciation of *Mycobacteria* in cattle slaughteredin Gusau abattoir Zamfara State, Nigeria. The study was a cross-sectional abattoir-based which examined atotal of 3690 cattle slaughtered between the months of April to June 2014. The samples of lesions suggestive of bTB collected during participatory meat inspection were processed and examined for the presence of acid-fast bacilli (AFB) *Mycobcateria* by using microscopic technique (Ziehl-Neelsen staining and microscopy: ZN). Detected *Mycobacteria*were identified to species level using region of difference (RD) deletion typing, a multiplex polymerase chain reaction (PCR)-based method (involving RD1, RD4, RD9 and RD12 loci). Of the 226 animals observed with lesions compatible with bTB, 37 (16.4%) turned out to be acid-fast bacilli (AFB) positive.15 (6.6%) of the lungs samples were AFB positive out of which 14 (37.8%) were further tested positive for RD deletion typing. Animal level data on age, sex and breed were recorded. The individual animal prevalence was significantly affected by both age (ZN: $\chi^2 = 8.324$, $p= 0.004$; RD deletion typing: $\chi^2 = 6.68$, $p= 0.010$) and sex (ZN: $\chi^2 = 3.46$, $p= 0.063$; RD deletion typing: $\chi^2 = 3.63$, $p= 0.057$).Moreover, there was weak statistical association between age groups (OR= 0.254, $p= 0.004$) in ZN positive samples, and for RD deletion typing positive samples amongst ages (OR= 0.287, $p= 0.010$). While no statistically significant association was recorded between sex (OR= 4.58, $p= 0.063$) of the cattle positive for ZN, and sex groups (OR= 0.432, $p= 0.057$) in cattle positive for RD deletion typing. Based on RD deletion typing, 37 tissue samples were analyzed for molecular characteristics:band-patterns of 30 samples were identified to be *M. bovis*, 3 were *M.*

caprae and 1 was *M. tuberculosis*.Breed prevalence rates of the disease by ZN and RD deletion typing were found to be 25 (67.6%) and 22 (64.7%) for White Fulani, 8 (21.6%) and 8 (23.5%) Sokoto Gudali, and 4 (10.8%) and 4 (11.8%) Red Bororo respectively.Risk factors for transmission and exposure of bovine tuberculosis were assessed, 101 (67.3%) of 150 questionnaires administered were retrieved for analysis. Butchers made up of most of the respondents 34 (33.7%) compared to cattle traders 25 (24.8%), cattle handlers 21 (20.8%), meat sellers 8 (7.9%), cleaners 5 (5.0%), animal health scientists 3 (3.0%) and veterinarians 3 (3.0%).In conclusion, this study has for the first time recorded prevalence of bTB in the study area, and molecular identification of three different *Mycobacteria* species from thirty four 34 (92%) slaughtered cattle with caseous lesions suggests the presence of same or more species of *Mycobacteia* in circulation among other domestic animals in the State. These findings have raised concerns for both animal and public health considering the predominant extensive livestock production system (which is mixed: cattle, goat, sheep and camels combined), in-contact of humans with cattle, and the level at which unpasteurized milk and poorly processed meat are being consumed in the study area. This study approves of RD deletion typing to be useful for confirmation of bTB and an efficient tool in differentiating*Mycobacterium tuberculosis* complex (MTC) members, while it also considered Ziehl-Neelsen staining and microscopy as useful and accessible diagnostic method for detecting AFB from clinical specimen. Further research is needed to better understand bTB prevalence, genetic diversity of MTC and transmission in an extensive livestock production system of Zamfara State.

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LIST OF ABBREVIATIONS

AI Artificial Insemination

AIDS Acquired Immune Deficiency Syndrome

AFB Acid Fast Bacilli

Af1 African 1

AU/IBARAfrican Union/ Inter African Bureau on Animal Resourses

Btb Bovine Tuberculosis

CDC Center for Disease Control

CFT Caudal Fold Test

CIConfidence Interval

DNA Deoxyribonucleic Acid

DTH Delayed Type Hypersensitivity

DVR Direct Variant Repeat

DR Direct Repeat

E Ethambutol

ELISA Enzyme Linked Immunosorbent Assay

ETR Exact Tandem Repeat

FAO Food and Agricultural Organization

FMOH Federal Ministry of Health

FPA Fluorescence Polarization Assay

H Isoniazid

HIV Human Immunodeficiency Virus

LSPs Long Sequence Polymorphisms

MAHLD Ministry of Animal Health and Livestock Development

MAPIA Multiple Antigen Print Immunoassay

MIRU Mycobacterial Interspersed Repetitive Units

MTC *Mycobacterium tuberculosis* complex

NPC National Population Commission

NTM Non-Tuberculous *Mycobacteria*

OIE Office International des Epizootes

OR Odds Ratio

ORFs Open Reading Frames

PCR Polymerase Chain Reaction

PM Postmortem

PPD-A Purified Proteins Derivative Avian

PPD-B Purified Proteins Derivative Bovine

PZA Pyrazinamide

R Rifampicin

RD Region of Difference (Genomic)

RNA Ribonucleic Acid

SICCTTSingle Intradermal Comparative Cervical Tuberculin Test

SNPs Single Nucleotide Polymorphisms

Se Sensitivity

Sp Specificity

TB Tuberculosis

TST Tuberculin Skin Test

UK United Kingdom

USA United States of America

VNTR Variable Nucleotide Tandem Repeats

WAHID World Wide Animal Health Information Database

WHOWorld Health Organization

ZASIDEPZamfara State Integrated Development Project

ZNZiehl-Neelsen

INF-γ Gamma Interferon

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Bovine tuberculosis (bTB) is a chronic infectious and contagious disease of cattle, other domestic animals, certain wildlife populations, and humans (OIE, 2009). The disease is primarily caused by *Mycobacterium bovis* and, to a lesser extent, by *Mycobacterium caprae* (Alvarez *et al*., 2014), both are members of the *Mycobacterium tuberculosis* complex (MTC); in addition to *Mycobacterium bovis* and *Mycobacterium caprae,* this group comprises of: *Mycobacterium tuberculosis*, *Mycobacterium canetti*, *Mycobacterium africanum*, *Mycobacterium pinnipedii*, *Mycobacterium microti* and *Mycobacterium mungi* (Alexander *et al*., 2010; Rodriguez-Campos *et al*., 2014). This genetically related group of bacteria causes TB with comparable pathology in a wide variety of hosts (Wirth *et al*., 2008; Comas *et al*., 2013); however, *Mycobacterium bovis* has the widest host range among pathogenic mycobacteria, mainly spreads within and between animal species via aerosol and ingestion routes (O'Reilly and Daborn, 1995). Inhalation is the almost invariably the portal of entry in housed cattle, and even in those at pasture, it is considered the principal mode of transmission (Cadmus *et al*., 2004). However, infection by ingestion is obviously more likely at pasture, when faeces contaminate the feed, communal drinking water and feed troughs (Radostits *et al*., 1994; Theon and Steele, 1995; Ayele *et al*., 2004). While zoonotic transmission from cattle to man occurs mostly through consumption of unpasteurized milk and by close contact with the infected animals (Michel *et al*., 2010).

Bovine tuberculosis is often subclinical; when present, clinical signs are not specifically distinctive and are characterized by weakness, anorexia, emaciation, enlargement of lymph nodes, and cough, particularly in advanced tuberculosis (OIE, 2009). In cattle, infection due to *Mycobacterium tuberculosis* accounts for a small proportion of usually subclinical cases (Pritchard, 1988; Ameni and Erkihun, 2007). However, in diseased animals, the primary location of gross lesions depends on the route of infection but also subsequent dissemination of *Mycobacterium bovis* to other organs (Michel *et al*., 2010). It is characterized by the formation of granulomas in tissues and organs, more significantly in the lungs, lymph nodes, intestines, liver and kidneys (Shitaye *et al.*, 2007).

Mycobacterium bovis has a wide host range and causes significant economic hardship for livestock farmers, with estimates of more than 50 million cattle infected worldwide (Tuggle and Waters, 2015), while comprehensive econometric analyses place bovine TB as the fourth most important livestock disease worldwide (Perry *et al*., 2002). Losses in animals due to bovine TB can be estimated on the basis of condemnation of carcasses during meat inspection, payment of compensation to the cattle owners, shorter lifetime or death of animals, payment for veterinary services, sterility, reduced beef and milk production and restrictions in trade on live cattle and fresh cattle products (like: milk and beef) (Awah-Ndukum, 2012). Besides its direct impacts to the cattle industry, bTB is a zoonotic disease of great concern; for these reasons, regulatory restrictions in most countries are in place to prevent the trade of infected animals and their products within and between countries and to reduce the risk of spillover from the animal to the human compartment (Rossi *et al*., 2015).

Bovine tuberculosis is distributed throughout the world and in general, the geographic distribution of *Mycobacterium bovis* in livestock parallels the distribution of livestock throughout the world (Kaneene and Pfeiffer, 2006). As many parts of the world have no active surveillance programs and limited epidemiological studies, the prevalence and impact of bTB is likely to be underestimated (Grace *et al*., 2012). According to OIE database, during the period 1996-2011, the majority of African countries (78%) including Nigeria reported the existence of bovine TB in their livestock while only four countries reported the absence of the disease (DeGerine-Wichatitsky *et al*., 2013). Although great strides have been made in the control of bovine tuberculosis in most regions of the world, this costly and devastating disease still persists in Africa (Waters *et al*., 2012), particularly in Nigeria where independent reports have established its prevalence in different parts of the country (Okaiyeto *et al*., 2008; Cadmus *et al*., 2009; Damina *et al*., 2011; Nwata *et al*., 2011; Danbirni *et al*., 2012). However, the epidemiology and public health significance of bTB in Nigeria remain largely unknown; this may be due to few laboratories in the country that are capable of differentiating *Mycobacterium bovis* from *Mycobacterium tuberculosis* infectionsand that due to other members of the *Mycobacterium tuberculosis* complex (MTC) group (Cadmus *et al.*, 2004).

The diagnosis of bovine TB in Africa is carried out usually on the basis of postmortem examination during abattoir meat inspection, tuberculin skin test and rarely on bacteriological techniques (Ameni *et al*., 2000; Asseged *et al*., 2004; Shitaye *et al*., 2006; 2007). In Nigeria, identification of bTB lesions at meat inspection in abattoirs has been used to detect the disease (Cadmus and Adesokan, 2009).However, various other methods are being used in the diagnosis of bTB (McLernon *et al*., 2010). This includes region of difference (RD) deletion typing, a polymerase chain reaction (PCR)-based method that differentiates members of *Mycobacterium tuberculosis* complex (MTC) (Parsons *et al*., 2002; Warren *et al*., 2006); this method is cheap and less laborious (Malama *et al*., 2014).

1.2 Statement of Research Problem

In countries where a majority of poor rural pastoral communities depend on cattle for their livelihoods, bTB is reported to be a constant threat due to lack of pasteurization of milk (Cosivi *et al*., 1998; Ameni *et al*., 2003). It is known to be a major cause of human extra-pulmonary tuberculosis, and is of particular concern in developing countries where bovine milk is often not pasteurized (Sonnenberg *et al*., 2001). Unfortunately, in some parts of Nigeria, consumption of unpasteurized fresh or soured milk (nono) is being practiced (Abubakar, 2007); *Mycobacterium bovis* has been isolated in unpasteurized fresh and soured cattle milk (Abubakar, 2007; Ofukwu *et al*., 2008), and from abattoir samples (Cadmus *et al*., 2006; 2009). It has been also observed that the risk factors for *Mycobacterium bovis* infection in both animals and humans especially in many African settings where domestic animals are an integral part of human social life are close contact, and food hygiene practices like consumption of unprocessed dairy and meat products (Cosivi *et al*., 1998). These highlighted the potential risk for transmission of *Mycobacterium bovis* through fresh or soured unpasteurized milk (nono) which is often being used to prepare a common delicacy locally called 'Fura da Nono', and is being consumed by both rural and urban dwellers in the State. This is in addition to unprotected operational activities being carried out by butchers while handling animals and processing carcasses at the abattoir; they handle animals and process meat from both diseased and apparent healthy animals with their bare hands, exposing themselves to many infectious agents, apart from bovine tuberculosis.

In less developed countries, bovine TB due to *Mycobacterium bovis* has been identified in a wide variety of domesticated and non-domesticated animals (World Organization for Animal Health, 2009; Zinsstag *et al*., 2006). The fear of bovine tuberculosis infecting meat products is real and necessitates quick actions and immediate eradication policies; however, many factors affect transmission of the disease, one of such is via contaminated food which is a possible cause of spreading of bovine TB (Phenice *et al*., 2014). Consumption of undercooked or raw meat and meat products from tuberculous animals could represent a mechanism for human infection (Mamo, 2014). The risk poses by eating undercooked meat of tuberculous animals may be marginally greater in developing countries like Nigeria, where *Mycobacterium bovis* infection in animals is quite prevalent but veterinary controls (including meat inspection) are only sporadically applied (Cosivi *et al*., 1998; Ayele *et al*., 2004). Therefore, an increased risk of infection for humans exists in communities with close and repeated contact with livestock, and consumption of meat as part of their way of life (Unger *et al.,* 2003; Demelash *et al.*, 2009). Hence, this raises concerns about the potential risk for the transmission of zoonotic TB via consumption of poorly processed meat potentially contaminated/infected with *Mycobacterium bovis* by humans in the study area.

In an infected animal, *Mycobacterium bovis* can be excreted in all bodily fluids, meaning transmission is possible directly through close contact (aerosol transmission) or indirectly via contact with fluids or excreta in the environment (Neill *et al*., 2001; Radostits *et al*., 2007). In extensive animal husbandry system such as that of pastoral system, where animal movement from one area to another and feeding of animals on open grazing pasture are common practices, possibility of contamination of grazing fields by infective bacilli is the most likely condition and results in transmission of *Mycobacterium bovis* infection through oral route (Mamo, 2014). Cattle management practices of free and mixed grazing as well as using common water sources and free livestock movement are likely factors that can facilitate the expansion of bovine TB infection in a given area (Mengistu *et al*., 2015). However, in Nigeria, livestock production is predominantly by extensive pastoral system, cattle are often being grazed mixed with other animals sharing both water sources and grazing fields; most livestock reared in Northern Nigeria roam freely between neighborhoods and in most cases they are being moved to faraway locations in search of grazing pasture.

Bovine tuberculosis bears a zoonotic potential and is therefore of public health concern (Cosivi *et al*., 1998; Renwick *et al*., 2007) even though the degree of zoonotic transmission of tuberculosis from animals to humans is unknown (Cadmus *et al*., 2006). Nonetheless, the high risk of transmission of bTB is more among HIV/AIDS infected humans since immune-suppression increases their susceptibility to the infection (Ayele *et al.,* 2004), and it is considered a problem as FMOH (2010) reported 2.1-4.0% HIV/AIDS prevalence in Zamfara State, and tuberculosis has been the most frequent opportunistic disease associated with HIV/AIDS infection (Daborn and Grange, 1993; Cosivi *et al.,* 1998; Corbett *et al.,* 2006; Thoen *et al.,* 2009; Awah-Ndukum *et al.,* 2010). However, HIV-positive individuals have a 10% risk *per year* of life of contracting TB, and the HIV epidemic has been a main reason for the reversal of the steep decline in the prevalence of TB in places with effectively functioning TB control programs, and it has exacerbated the condition in high HIV prevalence areas in sub-Saharan Africa (Godfrey-Faussett and Ayles, 2003).

Furthermore, as many parts of the world have no active surveillance programs and limited epidemiological studies, the prevalence and impact of bTB is likely to be underestimated (Grace *et al*., 2012). Molecular epidemiological technique such as RD deletion typing has been used to differentiate infections caused by members of *Mycobacterium tuberculosis* complex, and to provide information on transmission of mycobacterial diseases between animals and humans (Warren *et al*., 2006). However, due to the lack of adequate expertise, this technique is not commonly used in most developing nations such as Nigeria where TB is endemic (Cadmus *et al*., 2006).

1.3Justification of the Study

The impacts of bTB infection are manifold, including significant economic and social effects due to the slaughter of infected animals, compensatory payments to producers, continual surveillance programs, and disruption to agricultural trade and productivity (Zinsstag *et al*., 2008). Bovine TB is being acknowledgedworldwide as a disease with both economic and public health implications (Cosivi *et al*., 1998; OIE, 2009; Boukary *et al*., 2011). However, in Nigeria, there is no known active bTB surveillance program; and hence, the actual status of the disease in cattle country-wide is unknown. Therefore, in the light of the studies conducted at different parts of the country which have recorded varied prevalence rates of bovine tuberculosis (Okaiyeto *et al*., 2008; Cadmus *et al*., 2009; Damina *et al*., 2011; Nwata *et al*., 2011; Danbirni *et al*., 2012). Regarding also the identification of the aetiological agent(s) of bTB, no study of such has ever been conducted on tuberculosis in cattle in the study area; hence, there is the need to investigate the status of the disease in cattle, so as to provide a base-line information on the role cattle play in the maintenance of *Mycobacterium bovis* and also as a source of infection to other domestic animals and man. Therefore, postmortem examination followed by laboratory bacteriological examination of suspected lesions in cattle are important tools to confirm the presence of *Mycobacteria* (Corner, 1994), while molecular studies help to identify and characterize mycobacterial species that are involved (Haddad *et al*., 2004; Rasolofo Razanamparany *et al*., 2006). Although in circumstances where the prevalence of bovine tuberculosis is high, pathology can be relied on for the diagnosis (Corner, 1994), but lesions due to the infections caused by *Mycobacteria* other than tuberculosis can be mistaken for bovine tuberculosis; therefore, control programs are in need for definitive diagnosis using reliable detection techniques on all suspected cases (El Tigani *et al*., 2013).

The great majority of the cattle in Nigeria are owned by pastoralists, and even those owned by settled farmers are often managed by pastoralists for part of the year (Blench, 1999). However, amongst States in Nigeria, Zamfara has been widely known for its high population of farmers and pastoralists (who constitute over 80% of the people) (Anonymous, 2000), animals in this region are tended on extensively mixed and transhumant production systems with most herds composed of cattle, sheep, goats and scanty camels; they are being grazed together on natural pastures and farm by-products with rarely enriched feeds given to enhance production, and are given poor or none veterinary attention. Moreover, livestock production in this region forms parts of the natural beings of the populace and provides means of livelihood that, it is largely focused on production of food animals raised for slaughter and dairy purposes, both milk and meat yields are consumed at homes and sold at the markets. Hence, this mixed system of livestock farming favors an increasing contact between animals that; if infected, cattle can transmit infection to other species of animals and man, as Cadmus *et al*. (2009) detected *Mycobacterium bovis* in goats herded together with cattle, suggesting transmission of bovine tuberculosis infection from the primary host (cattle) to goats.

Moreover, cultural practices also exist that could facilitate transmission of bovine TB between animals and humans. For example, prior to sale, cattle are fattened in close proximity tofarmers' homes offering ample opportunity for zoonotic transmission of infection to man (Cadmus *et al*., 2006). More so, an outdoor farming favors contacts between animals of different herds, it serves as one of the pathways for bTB transmission (Rossi *et al*., 2015); in addition to between-farm movement of the live animals (Johnson *et al*., 2011), which have been the dominant practices in this region. Therefore, preventing contacts between cattle of different herds is considered an effective measure to the control of bTB transmission (Phillips *et al*., 2003; Johnson *et al*., 2011). This, when applied to the extensively mixed and transhumant production systems being predominantly operated in the region; will go a long way in minimizing the spread of bovine TB.

In Nigeria, identification of bTB lesions at meat inspection in abattoirs has been used to detect the disease (Cadmus and Adesokan, 2009); the diagnosis of tuberculosis in animals is faced with challenges that, it stops at the acid-fast stain for the treatment of clinical cases without really going further to differentiate species of the tubercle bacilli, this underscores the role of *Mycobacterium bovis* infected cattle in the transmission of tuberculosis (Danbirni *et al*., 2012).The Ziehl-Neelsen stain is simple and rapid, but cannot differentiate *Mycobacterium bovis* from nontuberculous mycobacteria, because incorrect identification of *Mycobacterium bovis* can have serious consequences, conventional or molecular tests to confirm the presence of this microorganism should be performed when microbiological findings are suggestive of *Mycobacterium bovis* (Byeon *et al*., 2015). However, Huard *et al*. (2003) investigated the RD profile of the MTC groupings, and based upon this information developed a rapid, simple, and reliable solely PCR-based MTC typing method that made use of MTC chromosomal region-of-difference (RD) deletion loci; they used PCR primer pairs to amplify specific loci which together formed an MTC PCR typing panel, and going further not only did the panel provides an advanced approach to determine subspecies of MTC but also differentiates them from clinically important nontuberculous *Mycobacteria* (NTM) species. The genomic comparisons across the MTC revealed a number of “regions of difference” (RD loci), with the presence or absence of these loci capable of differentiating the constituent strains of the MTC (Rue-Albrecht *et al*., 2014). For given the multifaceted issues surrounding bTB, it is likely that control and eradication will require additional control strategies (Bermingham *et al*., 2014). Hence, this study employed more sensitive and specific technique (RD deletion typing) that is extended to definitive approach of exploiting pathogens' genetic differences.

1.7 Aim of the Study

To determine the prevalence of TB due to *Mycobacterium bovis* in slaughtered cattle and establish risk factors to infection with *Mycobacterium bovis* at Gusau abattoir, Zamfara State.

1.8 Objectives of the Study

(a) To determine the prevalence of bovine tuberculosis in slaughtered cattle at Gusau abattoir using detection methods (Postmortem examination, Ziehl-Neelsen staining/microscopy and RD deletion typing).

(b) To determine the risk factors among Professionals working with animals and general public on bovine tuberculosis.

1.6 Research Questions

(a) What is the prevalence of *M. bovis* infection in cattle slaughtered in Gusau abattoir?

(b) Are the granulomatous lesions observed at Gusau abattoir during postmortem inspection due to *M. bovis* infection alone?

(c) Are there risk factors to exposure and transmission due to *M. bovis* infection among personnel working in the abattoir?

(d) How enlightened is the public about the zoonotic implications of bovine tuberculosis in the study area?

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical Perspective of Bovine Tuberculosis

It has been assumed for a long time that human tuberculosis had its origin as a zoonosis, with *M. tuberculosis* evolving from *Mycobacterium bovis* by specific adaptation of an animal pathogen to the human host at the time of cattle domestication 10,000 to 15,000 years ago (de la Rua-Domenech, 2006). However, deletion analysis of*Mycobacterium tuberculosis* complex(MTC) strains isolated in 30 countries and the recent sequencing of the *M. bovis* genome have shown the genome of *M. bovis* to be smaller than that of *M. tuberculosis* (Brosch *et al*., 2002; Garnier *et al*., 2003). It now appears that *M. bovis* is the final member of a separate lineage of bacteria (represented by *M. africanum*, *M. microti* and *M. bovis*) that branched from the common ancestor of the MTC when members of this complex were already human pathogens (de la Rua-Domenech, 2006). By contrast, modern strains of *M. tuberculosis* would constitute a separate lineage that emerged approximately 35,000 years ago, before the *M. bovis* lineage, and are thus philogenetically closer to the common progenitor of the MTC (Brosch *et al*., 2002; Garnier *et al*., 2003).

It is presumed that the genus *Mycobacterium* originated more than 150 million years ago (Daniel, 2006). *Mycobacterium tuberculosis* was thought to have co-evolved with early hominids in East Africa in about 3 million years ago, the modern members of *M. tuberculosis* complex seem to have originated from a common progenitor about 15,000-35,000 years ago (Gutierrez *et al*., 2005).Historically, taxonomic segregation of the *Mycobacterium tuberculosis* complex (MTC) has been based upon each species' unique combination of growth, morphological, physiological and biochemical characteristics (Niemann *et al*., 2000). Variation in geographical distribution, host preference, virulence, and relative human infectivity also differentiate the tubercle bacilli (Huard *et al*., 2003). The understanding of genetic variability within the MTC is ever increasing, and genetic criteria are now routinely used to identify veterinary isolates to the species level and fingerprint them (Barnes and Cave, 2003). The MTC is generally considered a family of "ecotypes'' of very closely related *Mycobacteria*, with each ecotype being adapted to cause tuberculosis disease in a specific host species or group, even though inter-species transmission can occur (Smith *et al*., 2006a). In contrast to the earlier hypothesis that tuberculosis has evolved from an originally animal disease to a human disease (Diamond, 2002), new findings indicated that, in fact, tuberculosis first emerged in humans and was subsequently transmitted to animals (Wirth *et al*., 2008). Recent studies suggest that the common ancestor of the *Mycobacterium tuberculosis* complex emerged from its progenitor perhaps 40,000 years ago in East Africa. Some 10,000 to 20,000 years later, two independent clades evolved, one resulting in *M. tuberculosis* lineages in humans, while the other spread from humans to animals, resulting in the diversification of its host spectrum and formation of other *M. tuberculosis* complex member species, including *Mycobacterium bovis* (Gutierrez *et al*., 2005; Wirth *et al*., 2008). This adaptation to animal hosts probably coincided with the domestication of livestock approximately 13,000 years ago (Michel *et al*., 2010).

Evidence in the form of skeletal lesions compatible with Port's disease and especially the use of PCR-based DNA techniques date the occurrence of early documented cases of tuberculosis in both humans and animals to at least 3000BC (Taylor *et al*., 2005; Taylor *et al*., 2007). Pathognomonic bone lesions indicative of tuberculosis in bovids were found in skeletons of ice-age representatives of this genus but the link with hominids is currently unclear (Rothschild and Martin, 2006).

In modern history, cattle served as principal reservoir species for *M. bovis*, hence the name bovine tuberculosis. This term is also commonly used to describe *M. bovis* infection in other species including wildlife and humans to demonstrate the bovine source of infection. Movement of cattle within and between countries and continents certainly facilitated the worldwide distribution of bovine tuberculosis, although the ultimate origin of *M. bovis* is unknown (Michel *et al*., 2010). However, progress has been made in the understanding of the population structure of *M. bovis* through the use of the PCR-based spoligotyping and variable nucleotide tandem repeat(VNTR) typing methods, which allowed the identification of clonal complexes of *M. bovis* dominant in larger geographic locations. Recently, a clonal complex of strains of *M. bovis* named African1 (Af1) that is geographically localized to the Central-West African region has been described (Müller*et al*., 2009). Strains of Af1 were very frequent in this region and appeared to have nearly reached fixation in some areas of Central-West Africa. The most likely explanation for this observation is an introduction of *M. bovis* into cows that were originally naïve to tuberculosis (Müller*et al*., 2009). Similarly, a clonal complex provisionally named Eu1 appears to be dominant in the British Isles (Michel *et al*., 2010). It can then be expected that other groups are likely to be geographically localized to other regions of the world (Müller*et al*., 2009). However, advances made in the understanding of the population structure of *M. bovis* notwithstanding, the actual origin of these clonal complexes remains unknown (Michel *et al*., 2010).

Historical data could suggest that bovine tuberculosis actually emerged in Europe and was distributed throughout the world mainly during the colonial period. Myers and Steel (1969) suggested that *M. bovis* emerged in Europe and spread from northern Italy to Western Europe and the UK. According to Webb (1939), *M. bovis* was thereafter distributed throughout the world through exportation of infected cattle from (mainly) the UK and the Netherlands to their former colonies (Renwick *et al*., 2007). *Mycobacterium bovis* was reported in Africa at the beginning of the 20th century (Ostertag and Kulenkampff, 1941). During colonial times, the emigration of European settlers and their livestock facilitated the large-scale inter-continental movement of infected cattle. In the post-colonial era, cattle were exported from Europe into many African countries, mainly to improve the dairy production in these countries. As a result, strains representative of *M. bovis* clades which had evolved through clonal expansion in a restricted geographical location were subsequently shared between geographically distinct countries with political and economic ties (Müller*et al*., 2009). Examples have been documented in Algeria, Mali and South Africa where VNTR typing revealed a link between local *M. bovis* isolates and those described in France and the United Kingdom, respectively (Michel *et al*., 2008; Sahraoui *et al*., 2009). Intensification of the dairy industry in combination with the movement of cattle (Gilbert *et al*., 2005) has contributed to the transmission of *M. bovis*, especially in the absence of suitable control measures. Cattle trade between neighboring countries and trading partners probably led to the regional dispersal of *M. bovis* and to the dominance of strains in large areas (Cadmus *et al*., 2006;Diguimbaye-Djaibe *et al*., 2006; Müller*et al*., 2008).

2.2The Importance of Bovine Tuberculosis

2.2.1 General overview

Bovine tuberculosis (bTB), as an important zoonotic disease of cattle and humans, has been controlled in most developed countries. However, the same cannot be said for developing countries (Amanfu, 2006). The disease is important not only because of its economic effects on animal production but also for its public health implications (Radostits *et al*., 2007).

2.2.2 Bovine tuberculosis as a zoonosis

Tuberculosis in humans due to *M. bovis* is both clinically and pathologically indistinguishable from cases caused by *M. tuberculosis* (Wedlock *et al*., 2002). As for animals the primary location of lesions depends on the route of infection but also on subsequent dissemination of *M. bovis* to other organs, transmission of tuberculosis from cattle to humans mostly occurs through the consumption of unpasteurized milk and close contact to infected animals (Michel *et al*., 2010).The epidemiological link between tuberculosis in cattle and in humans, especially children, has long been recognized even before Robert Koch identified the tubercle bacillus in 1882 (Michel *et al*., 2010). It appears that in previous centuries the easily noticeable so-called "TB grapes" in slaughter cattle, caused by nodular tuberculous lesions on the pleura or mesentery, were considered harmful to human health and were later associated with a living infectious agent "contagium vivum" transmitted to humans from cattle (Orland, 2003). Emil von Behring and leading paediatricians in the early 20th century thought of human tuberculosis caused by the bovine tubercle bacillus as an infectious disease, which was in many cases acquired in early childhood and remains latent before causing pulmonary disease in adults (Zeiss and Bieling, 1940). This hypothesis received new support decades later when the rapid success in combating cattle tuberculosis was not immediately parallel by a decline in human *M. bovis* cases, especially in adults (Meissner, 1974).

During the first half of the 20th century bovine tuberculosis was considered as one of the largest veterinary public health problems in Central Europe (Michel *et al*., 2010). Before the implementation of the eradication scheme 90% of the cattle herds in Germany were infected (Meissner, 1974). A significant percentage of tuberculosis cases in humans were thought to be caused by *M. bovis*, especially in children and cattletending persons in rural areas (Schmiedel, 1968). The breakthrough in the eradication of bovine and zoonotic tuberculosis in developed countries was achieved through mandated tuberculin testing of livestock and removal of positive reactors and compulsory pasteurization of milk (Michel *et al*., 2010). In contrast, it has been speculated that up to 7000 new human cases may occur in Latin America each year (de Kantor and Ritacco, 2006). Despite the fact that most of the human *M. bovis* cases in Europe occurred in the United Kingdom where bovine tuberculosis remains problematic to control, only a few of these are attributed to recent cattle-to-human transmission. Most cases are believed to be rather due to reactivation of latent infections contracted before 1960 or infections contracted outside the UK (de la Rua-Domenech, 2006; Jalava *et al*., 2007). The latter has also been confirmed in the United States of America(USA), where the Center for Disease Control(CDC) reported 165 cases of bTB in humans out of 11,860 cases studied (Moonan *et al*., 2009), of which most, if not all, were attributed to consumption of unregulated and unpasteurized dairy products in foreign-born persons.

Human-to-human transmission of *M. bovis* as well as concurrent infection with *M. tuberculosis* is rare and quite probably occurs mostly in unusual cases (LoBue *et al*., 2001; Gibson *et al*., 2004; Sunder *et al*., 2009). Where the opportunity exists, transmission of *M. bovis* from humans back to cattle can occur and may, under these circumstances, complicate efforts to control bovine tuberculosis in cattle (Szewzyk *et al*., 1995; Fritsche *et al*., 2004). Immuno-suppression due to Human Immunodeficiency Virus-infection is a known complication in humans affected by *M. tuberculosis* and has recently emerged as an aggravating factor in *M. bovis* infection in humans at the livestock-human interface, mainly in nosocomial outbreaks, some of these were caused by multidrug-reistant *M. bovis* strains and caused complications in hospitalized HIV-infected patients (Cobo *et al*., 2001).

In developing countries, the conditions for *M. bovis* transmission to humans not only exist unchanged, but the human population has a greater vulnerability due to poverty, HIV and reduced access to health care (Ayele *et al*., 2004; WHO, 2009). The exact percentage of *M. bovis* in human tuberculosis cases is often difficult to determine, since generally the diagnosis of ''TB'' is made on the basis of sputum smears only (Theon *et al*., 2006). The WHO reported in 1998 that 3.1% of tuberculosis cases in humans worldwide are attributable to *M. bovis* and that 0.4-10% of sputum isolates from patients in African countries could be *M. bovis*. This is despite the fact that *M. bovis* is more often associated with extrapulmonary disease in humans (Cosivi *et al*., 1998).

More detailed data including strain characterization have recently been presented and confirmed that the occurrence of *M. bovis* in the human population is a persistent, though insufficiently quantified feature in developing countries. The isolation rate of *M. bovis* from symptomatic human patients in specific studies was 13.8% in Mexico (Perez-Guerrero *et al*., 2008), 6.9% in Uganda (Oloya *et al*., 2008), 5% in Nigeria (Cadmus *et al*., 2006), 0.5% in Taiwan (Jou *et al*., 2008) and between 0 and 2.5% in 10 Latin American countries (de Kantor *et al*., 2008). In some noteworthy studies in Tanzania and Uganda, *M. bovis* accounted for 18 to 30% of all *M. tuberculosis* complex strains isolated from human patients, in rural settings (Kazwala *et al*., 2001; Mfinanga *et al*., 2004; Cleaveland *et al*., 2007) whereas low prevalence rates of *M. bovis* infections were found in urban populations (Asiimwe *et al*., 2008). It is to be expected that the incidence of zoonotic tuberculosis in developing countries is heterogeneously distributed and that the livestock producing rural populations are mostly affected by *M. bovis* infections. However, there are only very few studies that have investigated the prevalence of zoonotic tuberculosis in rural communities of developing countries (Michel *et al*., 2010).

2.2.3 Bovine tuberculosis in cattle

Bovine tuberculosis is a disease caused mostly by *Mycobacterium bovis* (Smith *et al*., 2006a).*Mycobacterium caprae* (Brosch *et al*., 2002), and also*Mycobacterium tuberculosis* have been reported as the cause of tuberculosis in cattle (Cadmus *et al*., 2006) as well as other members of the *Mycobacterium tuberculosis* complex (Cadmus *et al*.,2009; Jenkins *et al*., 2011).The disease is characterized by the formation of granulomas mostly in the thoracic cavity (Shitaye *et al*., 2006) and other parts of the body including genitalia. The disease is distributed worldwide, with Africa and Asian countries ranking highest in terms of the disease burden (WHO, 2004). In developed countries, bovine tuberculosis had been contolled through test and slaughter methods (Cosivi *et al*., 1998; Ayele *et al*., 2004; Amanfu, 2006; Smith *et al*., 2006a; Theon *et al*., 2006), but among industrialized nations bovine tuberculosis is still endemic in Australia and Caribbean Island (Tweddle and Livingstone, 1994).

The increasing incidence of bovine tuberculosis in the United Kingdom has been attributed to the presence of European badgers which act as reservoirs of the causative agent (*M. bovis*) in the wild (Gallagher and Clifton-Hadley, 2000). Wherever there are cattle there is bovine tuberculosis (Smith *et al*., 2006a). The disease has been reported in all the continents of the world and for most countries where burden of bovine tuberculosis is high, neither surveillance nor control programmes exist (Theon *et al*., 2006).

2.2.4*Mycobacterium bovis* infection in cattle

Mycobacterium bovis is the causative agent of tuberculosis in a range of animal species and man, with worldwide annual losses to agriculture of 3 billion dollars (Cosivi *et al*., 1998). It is the agent responsible for bovine tuberculosis. However, it can also cause the disease in humans if infected animal products are consumed (Cadmus *et al*., 2006).It can be transmitted by inhalation of aerosol droplets, ingestion or through break in the skin. The routes vary among animal species (OIE, 2009). Venereal transmission through artificial insemination (AI) had been documented (Wentink *et al*., 2000).

The pathology caused by *M. bovis* is similar to that caused by*M. tuberculosis* in humans, causing chronic debilitation, coughing and further spread to other organs (Taylor *et al*., 2007). In the cow from which *M. bovis* AF2122/97 was isolated suffered from necrotic lesions in lung and bronchomediasternal lymph nodes (Garnier *et al*., 2003). Infected cows produce mycobacterial mastitis that causes the shedding of the bacteria in milk, leading to transmission to humans if the milk is consumed unpasteurized (Garnier *et al*., 2003).

2.2.5 *Mycbacterium tuberculosis* infection in cattle

Infection with *M. tuberculosis* occurs most frequently in animals living in close contact with humans and has therefore been one of the most frequently recorded infectious diseases of captive wildlife (Kovalev, 1980). In domestic animals, infection with the human tubercle bacillus has been known since the beginning of the previous century, when typical lesions were found in livestock exposed to garbage and effluents from tuberculosis hospitals (Kraus, 1942). The number of documented cases of *M. tuberculosis* in cattle appears to have increased in recent years (Cadmus *et al*., 2006; Srivastava *et al*., 2008; Berg *et al*., 2009; Chen *et al*., 2009), and this may be due to improved diagnosis by molecular tools and/or an actual increase in transmission from humans to cattle in these countries. According to information released by the WHO on the global tuberculosis burden, the incidence has increased in sub-Saharan Africa and in someparts of Asia (http://www.who.int/vaccine_research/disease/ari/en/index4.html).

At the same time many developing countries have intensified their livestock production to meet the growing demand for food security, which has led to a higher risk of transmission for both *M. tuberculosis* as well as *M. bovis* at the human-livestock interface (Michel *et al*., 2010).

The isolation of *M. tuberculosis* from cattle raises a number of questions relating to the role of humans as a source of infection to cattle. It may be speculated that in some countries more cattle contract tuberculosis from humans than vice versa. The possible existence of cattle adapted *M. tuberculosis* strains and subsequent cattle-to-cattle transmission still needs clarification. The pathological changes in cattle do not appear to support disease transmission, since *M. tuberculosis* infection usually does not progress beyond the development of small granulomas in several different lymph nodes (Cousins *et al*., 2004). On the other hand, Srivastava *et al*. (2008) were able to isolate *M. tuberculosis* from milk samples in India, suggesting that in rare cases transmission to humans may occur through unpasteurized milk.

2.2.6 *Mycobacterium caprae* infection in cattle

In 1999, strains from MTC isolated from goats in Spain were described as *Mycobacterium tuberculosis* subsp *caprae* (Aranaz *et al*., 1999). The strains share common phenotypic properties with *M. bovis*, although they were susceptible to pyrazinamide, other property that differentiates these strains from other members of the MTC. The isolates were subsequently characterized as *Mycobacterium bovis* subsp. *caprae* (Niemann *et al*., 2000) and finally as a new species; *M. caprae* (Aranaz *et al*., 1999).

2.2.7 Cattle and other maintenance hosts of *M. bovis*

Only in a small number of maintenance or reservoir host species is *M. bovis* infection self-sustaining from generation to generation without input from other species (Morris *et al*., 1994 Cousins, 2001; de Lisle *et al*., 2001). Domestic cattle are considered the true natural host of the bacterium and the principal reservoir of infection for other animals and man (de la Rua-Domenech, 2006). Additionally, in the UK and Ireland, the Eurasian badger (*Meles meles*) is the maintenance host of *M. bovis* and acts as a significant reservoir of infection for cattle (Krebs *et al*., 1997; Gallagher and Clifton-Hadley, 2000; Delahay *et al*., 2002; Phillips *et al*., 2003; Griffin *et al*., 2005). Amongst domestic animals, the captive (farmed) deer and occasionally, goats can act as the maintenance hosts of *M.bovis* (Cousins, 2001). Amongst free-living mammals, the brushtail possum (*Trichosus vulpecula*) and the ferret (*Mustela furo*) in New Zealand, the Cape buffalo (*Syncerus caffer*) in parts of South Africa, Kafue lechwe antelope (*Kobus leche kafuensis*) and other *Bovidae* in Africa, the North American bison (Bison bison) in parts of Canada and the white-tailed deer (*Odocoileus virginianus*) in Michigan, USA, are also considered maintenance hosts of *M. bovis* (de Lisle *et al*., 2001).

Goats are quite susceptible to *M. bovis* and can develop both pulmonary TB and tuberculous mastitis, with the shedding of the bacillus in the milk of lactating goats (O'Reilly and Daborn, 1995; Seva *et al*., 2002). Clinical TB due to *M. bovis* infection has been reported in individuals in contact with domestic goat herds in Spain (Gutierrez *et al*., 1997), a country where this species is considered a maintenance host of *M. bovis* alongside cattle (Bernabe *et al*., 1990; Aranaz *et al*., 2003).

2.2.8 Animal spill-over hosts of *M. bovis*

In all other animal species *M. bovis* infection is self-limiting and occurs only as long as a maintenance host is present in their ecosystem. Such species are referred to as 'spill-over' hosts (Morris *et al*., 1994; de Lisle *et al*., 2001). Spill-over hosts are usually 'dead-end' hosts, in which the incidence and pathology of the disease indicates that they play no significant role in its onward transmission (de la Rua-Domenech, 2006). Spill-over hosts may also be 'amplifier' hosts if they act as incidental sources of TB for livestock or other species (de Lisle *et al*., 2001). Among domestic animals, bovine tuberculosis is particularly rare (but not unheard of) in horses and sheep and these animals species should be considered true dead-end hosts (de la Rua-Domenech, 2006). Pigs, farmed wild boar, dogs, cats and camelids should, on the other hand, be treated as potential amplifier hosts of *M. bovis*, which means that, if infected, these species may act as a source of infection for other animals and man (de la Rua-Domenech, 2006).Infected humans can also act as amplifier hosts of bovine TB, representing a potential source of *M. bovis* for animal and human contacts (de la Rua-Domenech, 2006).

2.3 Aetiology and Pathogenesis of Bovine Tuberculosis

2.3.1 Aetiology

Mycobacterium bovis is the causative agent of the vast majority of cases of tuberculosis in cattle (bovine TB) and a large number of domesticated and wild mammal species, in which it causes chronic, progressive and principally respiratory disease (Francis, 1947; O'Reilly and Daborn, 1995; Cousins, 2001; de Lisle *et al*., 2001).*Mycobacterium bovis* is a close relative of *M. tuberculosis*, and both share genetic identity over 99% at the whole genome level (Garnier *et al*., 2003). Different studies have supported the fact that, although slight differences are found in the genome sequence of *M. tuberculosis* and *M. bovis*, the physiology and host range spectrum are different (Golby *et al*., 2007; Rehren *et al*., 2007).Acoording to novel taxonomy,*M. bovis* was divided to *M. bovis* subsp. *bovis* and *M. bovis* subsp. *caprae*; the currently accepted taxonomy for these two members of the MTC is *M.bovis* and *M. caprae* (Aranaz *et al*., 2003).They have been differentiated on the basis of antimycobacterial sensitivity as *M. bovis* subsp.

*bovis*shows resistance to pyrazinamide (PZA), while*M. bovis* subsp. *caprae* is sensitive to pyrazinamide (PZA) (Wayne and Kubica, 1986).

The typical *Mycobacterium tuberculosis* complex organisms such as: *M. tuberculosis, M. bovis, M. africanum, M. microti,* and *M. canett*i; can cause tuberculosis in an exceptionally wide range of domestic and wild mammalian hosts including humans (O'Reilly and Daborn, 1995; van Soolingen *et al*., 1997; Kremer *et al*., 1998; van Soolingen*et al*., 1998; Harris and Barletta, 2001; Aranaz *et al*., 2003; Biet *et al*., 2005; Bruday e*t al*., 2006; Une and Mori, 2007; Theon *et al*;, 2009).

Members of the *Mycobacterium tuberculosis* complex have slow growth rates and do not grow outside of a host except when cultured on selective laboratory media; they are resistant to acid decolorizing stains and appear microscopically as slender rods (tubercle bacilli) that are 0.3-0.6µm in diameter and 1.5-3.0µm in length (Theon *et al*., 2009). They cannot tolerate harsh environments such as prolonged exposure to heat, direct sunlight and dry conditions but can survive for long periods under cold, dark, and moist conditions (Goodchild and Clifton-Hadley, 2001; Phillips *et al*., 2003).

Mycobacteria are structurally more closely related to Gram-positive bacteria. However, they do not fit into the Gram-positive category as the molecules attached to the cell wall are distinctively lipids rather than proteins or polysaccharides, they do not retain the crystal violet and appear as "ghost" after Gram Staining (Metchock *et al*., 2005). The waxy cell wall of mycobacteria is impermeable to aniline and other commonly used dyes unless these are combined with phenol (Metchock *et al*., 2005). The expression "acid-fastness" describes the resistance of certain microorganisms like mycobacteria to decolorization with acid-alcohol solutions after staining with arylmethane dyes such as Carbol Fuchsin (Madison, 2001).

The members of*Mycobacterium tuberculosis* complex are characterized by 99.9% similarity based on the nucleotide level and identical 16S RNA sequence (Taylor *et al*., 2003). The evolution and adaptation of the various members of the *Mycobacterium tuberculosis* complex has been linked to a combination of discrete acquired single-nucleotide polymorphisms (SNPs) (Gutacker *et al*., 2002) as well as by way of gross gene alteration such as deletions, invasions, insertions and duplication (collectively) known as region of difference (RD) loci (Brosch *et al*., 2002; Huard *et al*., 2006). The RDs are some regions of the genome that are present in *M. tuberculosis* complex but absent in *M. bovis* BCG sub-strains and severeral non-tuberculous mycobacteria (NTM) (Parkash *et al*., 2009). These regions of difference (RD) encompasse 80kb genomic DNA of *M. tuberculosis* and are predicted to have 89 open reading frames (ORFs) capable of encoding an equal number of proteins (Behr *et al*., 1999; Mustafa, 2002).

The regions of difference contain genes that encode potential virulence factors such as prophage (RD3, RD11) phospholipases C (RD5), invasins (RD7) and expolysaccharide biosystem (RD4) (Cole, 2002). Region of Difference 1(RD1) is the only region that appears to be missing from the vaccine strains BCG and *M. microti* but is present in all virulent members of the MTC (Cole, 2002). The RDs have helped to establish the evolutionary relationship of the MTC (Brosch *et al*., 2002). Brosch *et al*. (2002) concluded that, the region of difference, primarily RD9 and TbD1 but also RD1, RD2, RD4, RD7, RD8, RD10, RD12 and RD13 represent very interesting candidate for development of powerful diagnostic tools for rapid and unambiguous identification of members of the *Mycobacterium tuberculosis* complex.

2.3.2 Pathogenesis

Tuberculosis in humans may develop as a result of reactivation of a latent focus of a previous infection, primary progressive disease or reinfection. Pathology from latent infections can occur years or decades after an initial infection and this reactivation is often due to a change in the immune status of the individual (Hernandez-Pando *et al*., 2000).

At the cellular level in cattle, once inside the host, *M. bovis* is ingested by macrophages and their activation aids limitation of or destruction of infecting mycobacteria (Dannenberg, 1989; Pollock *et al*., 2006). Moreover, differences in the antibacterial capacity of bovine macrophages, depending upon microenvironment and organ-specific factors or genetic background, may exist (Qureshi *et al*., 1995). Experimental data suggests that an excessive immune response or a high virulence of the bacilli favour the pathogenesis of bTB (Wedlock *et al*., 1999; Waters *et al*., 2006). In susceptible hosts, the interaction of *M. bovis* with cells from the innate and adaptive immune responses in the respiratory tissue and lymph nodes result in colonization and infection with the development of several-stage lesions leading to the production of granulomas (Palmer *et al*., 2007). Granulomas represent the focal expression of the inflammatory response aimed at preventing mycobacterial growth, thus constituting a means whereby bacteria could potentially be directed or primed to enter a persistent state. During this interaction, T-cells are among the first cells involved in early granuloma formation (Cassidy *et al*., 2001). All of the main T-cell subsets ($\gamma\delta$ T-cells, $CD4^+$ and $CD8^+$ $\alpha\beta$ T-cells) have shown to be involved in the anti-mycobacterial immune response in cattle (Pollock *et al*., 2005). Therefore, both $CD4^+$ T-cells and $CD8^+$ T-cells have an important involvement in controlling infected cells (Pollock *et al*., 2005). Interestingly, $CD8^+$ cells may play a deleterious role by contributing to the immunopathology of bTB(Villareal-Ramos *et al*., 2003). Within granulomas, there is a balance between mycobacterial killing and survival (Saunders and Britton, 2007) that results in eradication, containment (persistence, latency) or progression of the infection, according to which side of the immune response is favoured (Pollock *et al*., 2005; Pollock *et al*., 2006).

2.4 Sources of Infection, Routes of Transmission and Infective Dose

Bovine TB in naturally infected cattle presents predominantly as a disease of the respiratory tract (Francis, 1947; Corner, 1994; Neill *et al*., 1994a). It is, therefore, not surprising that the bacilli excreted in aerosol form, particularly from the lungs, represent the most widely recognised and efficient source of bovine TB infectivity (Francis, 1947; Pritchard, 1988; Neill *et al*., 2001; Phillips *et al*., 2003). Infection by aerosols generated after excretion of *M. bovis* in the environment is also a significant mechanism of transmission for cattle at pasture or feeding from troughs contaminated with faeces or urine of the infected animals (Goodchild and Clifton-Hadley, 2001; Phillips *et al*., 2003). Experimental studies conducted have shown that the majority of natural infections of cattle are caused by aerosol infection and that as little as a single colony-forming unit of *M. bovis* (6-10 bacilli) delivered by the intratracheal route can result in TB pathology in cattle, while infection of cattle via the alimentary route is thought to be a rare occurrence and, compared with the respiratory route of infection, it requires infective doses several orders of magnitude greater (Francis, 1947; Neill *et al*., 2001). Natural transmission of *M. bovis* can occur between domestic and wild animals of the same or different species, from animals to humans, more rarely, from humans to animals or among humans (Collins, 2000).

Compared with *M. tuberculosis*, *M. bovis* has much broader host range (de la Rua-Domenech, 2006). In fact, all terrestrial mammals are susceptible to the infection to a degree determined by the exposure level, innate resistance, predominant immunological pathways, characteristics pathology, type of husbandry and ecology (Morris *et al*., 1994). Human-to-cattle transmission of *M. bovis* has been documented, although case reports in the medical literature are scarce (Sjogren and Hillerdal, 1978; O'Reilly and Daborn, 1995). Cattle infection of human origin was usually associated with farmers suffering from pulmonary or genitor-urinary TB (Pritchard, 1988;Danker *et al*., 1993; Grange and Yates, 1994;Gallagher and Jenkins, 1998;O'Reilly and Daborn, 1995;Ayele *et al*., 2004). Several incidents have been recorded in the literature of animals contracting TB from owners, farm workers and zookeepers infected with *M. tuberculosis* (O'Reilly and Daborn, 1995; Montali *et al*., 2001). *M. tuberculosis* usually causes minor, non-progressive infections in cattle, although these may result in temporary sensitization to bovine tuberculin, the antigen used for routine *ante mortem* diagnosis of TB in cattle and other animals (Steele, 1980;Gallagher and Jenkins, 1998; Wedlock *et al*., 2002; Pavlik *et al*., 2003).

2.4.1 Excretion of *M. bovis* and survival in the environment

Information on survival of *M. bovis* outside living animals is controversial (Humblet *et al*., 2009). Menzies and Neill (2009) stated that *M. bovis* would be able to survive in the environment for only a few weeks under natural conditions, considering that environmental contamination is a less effective means of disease transmission for cattle. Infection of cattle through environmental contamination is probably negligible, because the required infective dose is high; up to 10^7 bacilli would be necessary for oral transmission (O'Reilly and Daborn, 1995), whereas for aerosol transmission only one bacillus was shown to infect 4-7 month old calves (Neill *et al*., 1988). Morris *et al*. in

1994 concluded that contaminated feed and pasture play a negligible role in the transmission of the disease to cattle because *M. bovis* does not survive long enough on formites to be infective, but also because animals are not commonly exposed to a high enough dose to become infected orally (Morris *et al*., 1994).

The survival of time of *M. bovis* in the soil has been estimated in different studies (Humblet *et al*., 2009). Sunlight causes desiccation, and ultraviolet light may strongly influence survival in tropical areas. Furthermore, adequate levels of moisture are necessary for survival (Wray, 1975). The major factors influencing survival of *M. bovis* in the soil and on pasture are temperature, moisture, and p^H, exposure to sunlight, dissolved oxygen, presence of naturally occurring antibiotics in the soil, natural microflora and types of microfloral associations (Kelly and Collins, 1978). Soil ecology is also of major importance in the environmental survival of *M. bovis* (Artoise*et al*., 2004). *M. bovis* is expected to persist in slurry-treated soil for up to two years (Kelly and Collins, 1978). *M. bovis* survived for four weeks in non-sterile dry and moist soils under 80% shade, in darkness and in the laboratory and for a similar time in the sterile moist soil kept in the shade and in darkness (Duffield and Young, 1985).

As demonstrated, excreting animals infected with *M. bovis* can contaminate running water directly (Phillips *et al*., 2003); when cattle drink, splashing from those waters could favour the entry of bacilli into the respiratory tract through inhalation of contaminated droplets (Phillips *et al*., 2003). This may pose a particular risk in Africa where most herds share water points: an excreting animal has thus a high infective potential (Cleaveland *et al*., 2007). In Africa, flooding has also been suggested as a propagating factor of *M. bovis* in the environment (Cleaveland *et al*., 2007). The survival period of *M. bovis* in the environment was shown to be inversely proportional to mean daily temperatures (Jackson *et al*., 1995), temperatures above 0^0C and a strong hygrometry are favourable for *M. bovis* survival; hot and dry weather do not allow a long-time survival of *M. bovis* in the environment (Artoise*et al*., 2004).

2.4.2 Understanding how cattle to cattle transmission occurs

Aerosolized bacteria are the most common source of infectious *M. bovis* in cattle, and the primary site of natural infection is the respiratory tract (Costello *et al*., 1998; Cassidy, 2006). In bTB, airborne infection is considered the most common route of transmission, and more than 19% of cattle with bTB shed the mycobacteria (de Kantor and Roswurm, 1978; Vitale *et al*., 1998), mainly early during infection (Neill *et al*., 1989; Neill *et al*., 1994b). The intermittent nature of the bacilli shedding from infected animals, after a short initial post-infection period has been documented (Neill *et al*., 1989; Menzies and Neill, 2000). Several studies indicate that between 8-26% of naturally infected animals release mycobacteria in clinical samples, but the precise duration of the secretion period is unknown (Vitale *et al*., 1998; Romero *et al*., 1999;Palmer and Waters, 2006).

Cattle can become infected in many ways, with animal age and behaviour, existing environment and climate, and prevailing farm practices having significant influence (Neill *et al*., 2005). Inhaling *M. bovis* is the most probable and important route, as lesion distribution and pathology in field cases show predominant involvement of the upper and lower respiratory tract and associated lymph nodes (Pritchard, 1988; Crews, 1991; Corner, 1994; Neill *et al*., 1994a; Whipple *et al*., 1996). Ingestion of *M. bovis* directly, say from swallowing infected milk or from contaminated pastures, water or formites is considered secondary to respiratory spread (Menzies and Neill, 2000). Genital transmission can occur also if the reproductive organs are infected, but this remains extremely rare (Neill *et al*., 1994a), as are congenital and vertical transmitted infections (Neill *et al*., 2005). For faeco-oral route of transmission, cattle do not usually graze where other cattle deposit faeces, and thus *M. bovis* infection is unlikely to be acquired directly from eating grasses contaminated by other cattle (Phillips *et al*., 2003). Nevertheless, mycobacteria are not frequently and regularly excreted in cattle faeces, even by a heavily infected animal as demonstrated in the study conducted in three groups of five calves (4 to 7 months of age) infected intra-nasally with *M. bovis*: two animals presented intermittent excretion over several months (Neill *et al*., 1988).

2.5Clinical Signs

Bovine tuberculosis in cattle is a chronic and wasting (weight loss) disease and other non specific clinical signs include anorexia, drop in production (e.g: drop in milk yield), chronic intermittent cough (which may be productive), dyspnoea and enlarged regional lymph nodeswhich may rupture in advanced cases (Blood and Radostits, 1989; OIE, 2009). In cattle, signs usually become visible at the advanced stage of the disease (Corner, 1994; Shitaye *et al*., 2006) and mainly in adult or old animals (Oloya *et al*., 2006). Some infected livestock are apparently in healthy condition showing no evidence of infection but lesions may be found during meat inspection (Murray *et al*., 1991; Shitaye *et al*., 2006). Bovine tuberculosis (bTB) is often subclinical; when present, clinical signs are not specifically distinctive (OIE, 2009), and it is difficult to be diagnosed based only on clinical manifestations due to similarities in clinical manifestations with other conditions such as: Contagious Bovine Pleuropneumonia (CBPP), *Pasteurella*or *Corynebacterium pyogenes*pneumonia, traumatic pericarditis and chronic aberrant liver fluke infestation (Blood and Radostits, 1989; Gracey and Collins, 1992; Grist, 2008) which are often differentially diagnosed for bovine TB in cattle.

Mycobacterium bovis infected cattle do not present two well-defined clinical (active or latent) phases, nor does the immune reactivity test used nowadays (tuberculin skin test, TST) has the power to discern between these two likely states in infected bovine. Asymptomatic TST$^+$ cattle have been found infected with *M. bovis* as well (Srivastava*et al*., 2008). Persistence (latent bTB) could perhaps be defined as the existence of a positive skin or interferon-gamma test in cattle in which no lesions can be detected or when a culture is positive in the absence of lesions (Alvarez *et al*., 2009). Should latent bTB in cattle be present, reactivation could be associated with changes in cell-mediated immunity in tuberculous cattle as well as with viral immunosuppression, metabolic unbalance, corticosteroids and postpartum period (Doherty *et al*., 1995; Shafer-Weaver and Sordillo, 1997;Pollock and Neill, 2002; Piccinini *et al*., 2006).

2.6 Gross Lesions/Pathology

The routes of transmission of TB agents determine the distribution pattern of the lesions in the infected hosts (Biet *et al*., 2005; Palmer and Waters, 2006). Bovine tuberculosis is characterized by progressive development of granulomatous lesions or tubercles in affected tissues/organs (Blood and Radostits, 1989; McAdams *et al*., 1995; Cassidy, 2006; Liebana *et al*., 2008). Lesions have been reported to be distributed mostly in the respiratory tract and associated lymph nodes of naturally infected cattle (Francis, 1971; Collins and Grange, 1983; Blood and Radostits, 1989; McAadams *et al*., 1995; Cassidy *et al*., 1998; Cassidy, 2006; Palmer and Waters, 2006; Liebana *et al*., 2008), particularly in proportions of the lungs close to the pleural surface (Cassidy, 2006). Gross lesions may be extensive, involving entire organs of one or both body cavities. However, the anatomical sites of the the lesions, the extent of pathological involvement and the consistency of nodular formations with some caseous necrosis are often present before unthriftness is often apparent (Theon *et al*., 1995).

2.7 Epidemiology/Distribution of*M. bovis*

The epidemiology and public health significance of bovine TB in Africa remains largely unknown for politicoeconomic reasons, including the high cost of a testing programme, social unrest due to political instability, ethnic wars resulting in displacement of large numbers of people and animals, and a lack of veterinary expertise and communication networks (Ameni *et al*., 2010). In addition, there are few or no laboratories that are capable of isolating and characterizing mycobacteria from animals and humans (Ameni *et al*., 2010). Bovine tuberculosis has been on the increase in developing countries and continues to occur in developed countries (Cosivi *et al*., 1998;Grange, 2001; Gilbert *et al*., 2005). Infections occur in diverse groups of mammals, which include farm animals of economic importance, wildlife, and humans (Grange *et al*., 1990;Grange, 2001; Pavlik *et al*., 2002a).Risk factors contributing to difficulties in controlling bovine tuberculosis in cattle across continents can have their origin at farm-level, for example cattle breed, age, behaviour, and nutrition of the animals have been shown to play role in the development of the disease (Menzies and Neill, 2000; Ameni and ErKihun, 2007). However, host independent factors are considered more important in most cases and include, amongst others, production types and management practices (Carrique-Mas *et al*, 2008; Elias *et al*., 2008), cattle movement (Green *et al*., 2008), existence of a wildlife reservoir (Porphyre *et al*., 2008), and possibly mycobacterial strain related differences (Andreevskaia *et al*., 2007) and survival of *M. bovis* (Tanner and Michel, 1999).

Bovine tuberculosis is widespread in cattle throughout the globe. According to information on the worldwide animal health information database of the OIE (WAHID Interface, http://www.oie.int/wahis/public.php?page=home), 128 out of 155 countries reported the presence of *M. bovis* infection and/or clinical disease in their cattle population during the period between 2005 and 2008. In developed countries, the driving forces for the control and eradication of bovine tuberculosis from the national domestic herd are indisputably of economic and socio-political nature, based mainly on the negative economic impact of the disease (Reviriego Gordejo and Vermeersch, 2006). In large parts of the developed world, policies regulating the control of bovine tuberculosis are aimed at complete eradication of the disease from its livestock populations as part of an integrated approach to food safety. These policies follow an expensive test-and-slaughter strategy for the control of bovine tuberculosis and significant successes have been achieved in many countries (Radunz, 2006; Reviriego Gordejo and Vermeersch, 2006). On the other hand, the benefit and the sustainability of such costly programmes have been increasingly questioned in the light of the rising economic burden and social impacts on and reduced acceptance by farmers (Bennett, 2009; Torgerson and Torgerson, 2009). However, in general, with the exception of a few countries with a wildlife reservoir of *M. bovis*, the prevalence of bovine tuberculosis has reached very low levels, in most developed countries (EurosurveillanceEditorialTeam,2005.http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2712).

The situation is profoundly different in developing countries, which are in general unable to apply expensive test-and-slaughter schemes for the control of animal tuberculosis. Although in parts of the Latin American and Caribbean countries there has been significant progress in bovine tuberculosis control and infection rates under 1% have been reported for 30% of the region’s cattle, 70% of cattle are kept in areas where rates of infection are higher and where herd prevalence of up to 56% have been reported (de Kantor and Ritacco, 2006; de Kantor *et al*., 2008). On the African continent, more than 80% of the human population co-exists with cattle in the absence of any organized control of bovine tuberculosis (Cosivi *et al*., 1998). In recent years a growing awareness of neglected zoonoses, including bovine tuberculosis, has led to initiatives supported by the WHO/FAO/OIE to investigate, calculate and mitigate the unknown risk from these animal diseases on livestock productivity, human health and livelihoods (WHO, 2009). Overall, the presence and extent of bovine tuberculosis in the developing world has been poorly investigated in the past, but a number of recent studies have revealed new data confirming the presence of *M. bovis* in cattle (Diguimbaye *et al*., 2004; Oloya *et al*., 2007; Srivastava *et al*., 2008; de Kantor *et al*., 2008) and moreover providing insights into the specific risk factors associated with tuberculosis in cattle in different countries and regions. In Africa, high prevalence rates of bovine tuberculosis (up to 50% at herd level) were reported in areas of Zambia where cattle and Kafue lechwe shared grazing and water as well as in areas where the traditional management of livestock in transhumant herds prevailed (Oloya *et al*., 2007; Munyeme *et al*., 2009). Under these often nomadic conditions, the risk of exposure to *M. bovis* was increased significantly by creating multiple herd contacts and increasing the total herd size. The latter has also been suggested as a driver of the disease prevalence in Ethiopia and Ecuador (Ameni *et al*., 2003; Proano-Perez *et al*., 2006). On the other hand, in countries with a rapidly increasing livestock production and intensification of production systems, the propagation and insufficient detection of circulating *M. bovis* strains may be the most important contributor to increasing economic losses from bovine tuberculosis, rather than the importation of infected cattle, as previously suggested (Tadayon *et al*, 2008).

Most importantly, in the mainly rural livestock production areas of developing countries, bovine tuberculosis can have devastating impacts on the livelihood of millions of the world’s most vulnerable communities as the disease compromises their sustainable food supply, income and social status (http://www.who.int/zoonoses/Report_Sept06.pdf).

At present, cases of *M. bovis* infection have been reported in more than 40 free-ranging wild animal species. Despite significant variations in size, appearance and distribution of the tuberculous lesions in different species, in the majority of affected wildlife species lesions closely resemble those in cattle (Zanella *et al*., 2008; Drewe *et al*., 2009).It is difficult to quantify the extent of disease in this large variety of animals, but measurement in predators as a surrogate to measure extent of disease in prey can possibly be considered (VerCauteren *et al*., 2008). Tuberculosis in wildlife can pose serious difficulties for bovine tuberculosis control and eradication. Particularly noteworthy is the case of the British Isles, where the European badger represents an important and well-documented disease reservoir (Smith *et al*., 2006a). In several industrialized countries that have adopted animal tuberculosis control programmes and in which wildlife has been involved, control programmes were designed to exclusively benefit the livestock sector with less importance given to wildlife conservation or protection (Radunz, 2006; Porphyre *et al*., 2008). This is mostly due to the fact that many of the wildlife maintenance host species, with the exception of badgers in United Kingdom and Ireland, score a low priority on their national wildlife conservation listings and enjoy, at best, the status of valued, sought-after hunting trophies (Rudolph *et al*., 2006). In some cases these reservoir species are even classified as alien or feral with well-documented examples being the brushtailed possums in New Zealand and feral water buffaloes in Australia (Radunz, 2006; Porphyre *et al*., 2008).

In sharp contrast to the spectrum of scenarios found in developed countries worldwide, bovine tuberculosis is an endemic disease in livestock in many African countries. In South Africa and other African countries, *M. bovis* has been transmitted from livestock to wildlife reservoirs in free-ranging ecosystems with potentially far reaching direct and indirect implications on wildlife, livestock and human populations (Michel *et al.*, 2006).

2.7.1 Burden of bovine tuberculosis in the world

Bovine tuberculosis is distributed throughout the world and has been reported in every continent except Antarctica; in general where there are cattle there is, or has been, bovine tuberculosis (Smith *et al.*, 2006a). The exceptions are those countries that successfully used a test and slaughter programme to identify and cull infected cattle. In Australia, some Caribbean islands (including Cuba) and parts of South America, eradication has been achieved (Tweddle and Livingstone, 1994; de Kantor and Ritacco, 2006). In other countries, notably the USA, Canada, South Africa and most of the European Union (EU) with the exception of the British Isles- bovine tuberculosis in cattle has been reduced to negligible levels, although sporadic, and sometimes persistent outbreaks associated with the import of infected cattle or wildlife reservoirs of the disease have been reported (Smith *et al.*, 2006a).

In New Zealand, the control of bovine tuberculosis in both cattle and farmed deer has proved more difficult, and a wildlife reservoir of brush-tailed possums has been implicated. The eradication programme for bovine tuberculosis in New Zealand, which is successfully reducing the incidence of disease in cattle, consist of a test and slaughter program for cattle in concert with eliminating tuberculosis from wildlife (Ryan *et al.*, 2006). The combination of cattle surveillance and controlling bovine tuberculosis in local wildlife has also managed to limit a persistent outbreak of bovine tuberculosis associated with white-tailed deer in Michigan, in the United States (O’Brien *et al.*, 2006). The problem of bovine tuberculosis in Michigan (Ryan *et al.*, 2006), Canada (Nishi *et al.*, 2006) and Kruger National Park in South Africa (Michel *et al.*, 2006) is notable in that the suspected wildlife reservoir of the disease is itself of economic or social value. This constraint means that social issues and public approval could be important factors in eliminating the disease, and has strong resonance for the British Isles, where the badger, a protected species, has been implicated as a reservoir host (Hewinson *et al*., 2006).

2.7.2 Burden of bovine tuberculosis in Africa

The extent of *M. bovis* involvement in the global TB burden in Africa is still largely unknown (Tschopp *et al*., 2009). This can be partly explained by the fact that, TB due to *M. bovis* is indistinguishable from that due to *M. tuberculosis* in terms of clinical signs and pathological features (Grange, 2001). In addition, most laboratories in sub-Saharan Africa do not have the capability to differentiate *M. bovis* from *M. tuberculosis* (Ayele *et al*., 2004).Bovine tuberculosis represents a potential health hazard to both animals and human populations in Africa; and as in most developing countries it remains largely under-estimated and under-investigated (Awah-Ndukum, 2012). Reported data are largely insufficient to determine the true regional epidemiological imformation of the disease (Ayele *et al*., 2004; Zinsstag *et al*., 2006). However, there are sufficient evidences to indicate that bovine tuberculosis is present in almost all African countries (WHO 1994; Daborn *et al*., 1996; Benkirane, 1998; Cosivi *et al*., 1998; Ayele *et al*., 2004;Cadmus *et al*., 2006; Zinsstag *et al*.,2006;Cadmus *et al*., 2008; Cadmus *et al*., 2009) and often at high prevalence rates in domestic and wild animals (Awah-Ndukum, 2012).

In developing countries, especially in Africa, where *M. bovis* infection is present in various animal species, there is substantial lack of knowledge of the distribution, epidemiological patterns, and zoonotic implication of this pathogen (Daborn *et al*., 1996). Factors such as economic constraints, lack of adequate legistilation or failure to implement that which does exist, are the reasons for failure to control and eradicate bovine tuberculosis in Africa (AU/IBAR, 2006; Biffa *et al*., 2009), and also limited diagnostic capacity and high costs of a sustainable testing program (Daborn *et al*., 1996; Ayele *et al*., 2004; Zinsstag *et al*., 2006; Anaelom*et al*., 2010). Other limiting factors include lack of veterinary expertise, inadequate diagnostic facilities, poor communication networks, insufficient collaboration between neighboring countries, lack of quarantine facilities and policies, free movement and smuggling of live animals across state boundries (Daborn *et al*., 1996; Ayele *et al*., 2004; AU/IBAR, 2006; Ameni *et al*., 2010). Furthermore, various national animal disease control preferences are often absorbed by actions against the high incidence of other acute and fatal animal diseases such as contagious bovine pleuropneumonia, foot and mouth disease, various arthropod-borne diseases and other parasitic diseases (Ayele *et al*., 2004).

Furthermore, various studies have further recorded various bovine TB prevalence rates in Ghana, Mali, Nigeria, Cameroon and Chad of West and Central Africa (Dusai and Abdullahi, 1994; Ankugah, 2002; Awah-Ndukum *et al*., 2005; Cadmus *et al*., 2006; Diguimbaye-Djaibe *et al*., 2006; Zinsstag *et al*., 2006; Muller*et al*., 2008; Okaiyeto *et al*., 2008; Ngandolo *et al*., 2009) and in Eritrea, Ehiopia, Uganda, Tanzania, Zambia and South Africa of Eastern and South Africa (Cook *et al*., 1996; Weinhaupl *et al*., 2000; Omer *et al*., 2001; Ameni *et al*., 2003; Ameni and Wudie, 2003; Teklu *et al*., 2004; Bernard *et al*., 2005; Kazwala *et al*., 2006; Oloya *et al*., 2006; Swai *et al*., 2006; Shitaye *et al*, 2007; Inangolet *et al*., 2008; Regassa *et al*., 2008; Durnez *et al*., 2009; Fetene and Kebede, 2009; Munyeme *et al*., 2009). These reports confirmed that bovine tuberculosis is prevalent in the four geopolitical regions of sub-Saharan Africa, but definitive regional prevalence status cannot be estimated due to the inadequate data from several key countries (Awah-Ndukum, 2012).

2.7.3 Epidemiology/burden of *M. bovis* infection in cattle

The epidemiology of *M. bovis* was described by numerous authors in extensive detailed reviews; however, they tend to focus mainly on the experiences from industrialized countries, where control and/or eradication programmes have been implemented since a long time (O'Reilly and Daborn, 1995; Cosivi *et al*., 1998; Pollock and Neill, 2002; Wedlock *et al*., 2002; Phillips *et al*., 2003; Neill *et al*., 2005). Although cattle are considered to be the main hosts of *M. bovis*, the disease has been reported in many other species, including humans, other domesticated animals and wildlife (de Lisle *et al*., 2002). Infectious animals shed *M. bovis* via milk, saliva, faeces/urine and discharging lesions (Phillips *et al*., 2003). In cattle, the main route of infection is through aerosol exposure, facilitated by close contact between animals (Neill *et al*., 1991), also ingestion of contaminated products (e.g.: pasture and water) is generally considered to be a secondary, less important route of transmission (Menzies and Neill, 2000). Publications from Africa also suggest that ingestion of *M. bovis* might be an important mode of disease transmission in cattle, since mesenteric lymph nodes were shown to be more affected than mediasternal lymph nodes (Cleaveland *et al*., 2007; Ameni *et al*., 2007). The primary factors that promote tuberculosis transmission within a herd are direct contact between animals, particularly dairy animals and animals raised in semi-intensive systems or feedlots, a carrier animal, a constant *M. bovis* source in the environment, and mycobacterial resistance to unfavourable environmental conditions (MAPA, 2006).

Bovine TB negatively affects animal welfare and productivity worldwide with significant economic losses in some countries (Pollock and Neill, 2002). Infected cattle are important sources of infection for healthy cattle, but wildlife reservoirs of infection have also been reported in many regions (Wedlock *et al*., 2002; Phillips *et al*., 2003, Kaneene and Pfeiffer, 2006; Theon *et al*., 2009), thereby complicating the epidemiological picture (Awah-Ndukum, 2012). Bovine TB is an FAO-OIE “List B” disease because of its important socio-economic and public health impact (Benkirane, 1998; OIE, 2008).The economic loss to the cattle industry due to tuberculosis manifests as reduced milk and meat production, infertility, and carcass condemnation (Oliveira *et al*., 2007). It affects the economy of farming communities and public health in societies where animals and humans live in close contact (Neill *et al*., 1994a). *M. bovis* has no geographical boundaries (Kazwala *et al*., 2006), the epidemiology in developed and developing countries differ, owing to differences in the implementation of preventive measures (WHO, 1999).

2.7.4 Burden and risk of zoonotic tuberculosis due to *M. bovis*

Tuberculosis caused by *M. bovis* is an important human zoonosis associated with consumption of dairy products contaminated with the bacilli, and to labour risk (such as direct contact or droplet transmission) in farms or slaughter houses (Grange, 2001;de Kantor and Ritacco, 2006;Une and Mori, 2007; Srivastava *et al*., 2008).*M. bovis* can enter human hosts by ingestion, aerosol inhalation or direct contact with the mucous membranes and skin abrasions (Grange and Yates, 1994; Ashford *et al*., 2001). However, amongst livestock industry workers and home dwellers, consumption of raw cows’ milk or milk products and airborne occupational exposure to infected animals (or their carcases) are still regarded as the most common routes of *M. bovis* transmission from cattle to man (O’Reilly and Daborn, 1995; Gallagher and Jenkins, 1998; Collins, 2000). Whatever the routes of infection, the precise infectious dose of *M. bovis* for humans remains unknown (de la Rua-Domenech, 2006). This dose has been estimated to be in the order of tens to hundreds of organisms by the respiratory route and millions by the oral/gastrointestinal route (Francis, 1971).

Eating undercooked or raw meat and meat products from tuberculous animals could present a mechanism for human infection (de la Rua-Domenech, 2006). Therefore, controls in meat and inspection of cattle carcases for TB were instigated by early studies of the pathogenesis of TB in animals, aesthetic and religious objections and the need to emphasise the public health importance of the disease (Francis, 1973). The risk posed by eating undercooked meat of tuberculous animals is marginally greater in developing countries where *M. bovis* infection in animals is quite prevalent but veterinary controls (including meat inspection) are only sporadically applied (Cosivi *et al*., 1998; Ayele *et al*., 2004).

Unpasteurised milk and milk products continue to be regarded as the main vehicles for transmission of *M. bovis* in countries where bovine TB is prevalent and eradication programmes are apatchy or non-existent (Ashford *et al*., 2001). In those regions, milk-borne *M. bovis* infection is the principal cause of cervical lympadenopathy (‘scrofula’) and other non-pulmonary forms of human TB(Moda *et al*., 1996; Cosivi *et al*., 1998). Therefore, the consumption of unpasteurized cows’ milk poses a public health risk, in relation not only to *M. bovis*, but many other zoonotic infections as well (Holsinger *et al*., 1997; Casemore, 2001; Kavanagh,2002; Anon, 2003). Although *M. bovis* will not multiply in milk or will do so very slowly (Lake *et al*., 2002), the large number of bacteria excreted by a single cow with tuberculous mastitis is generally sufficient to render infective the milk pooled from 100 milking cows (Pritchard, 1988). *M. bovis* survives well in cows’ milk, viable bacilli can be found in yoghurt and cream cheese made from unpasteurized milk for up 14 days after preparation and in butter for up to 100 days (Kleeberg, 1984).

Animal-to-animal transmission of *M. bovis* is most commonly by the respiratory route (McFadyean, 1910; Francis, 1947;Morris *et al*., 1994; Neill *et al*., 1994a; O’Reilly and

Daborn, 1995; Goodchild and Clifton-Hadley, 2001; Neill *et al*., 2001). Therefore, a potential risk of contracting bovine TB via the respiratory route also exists for people regularly handling animal reservoirs of *M. bovis* or their carcases, i.e. cattle farmers, veterinarians, meat inspectors, slaughterhouse operatives, TB diagnostic and research laboratory personnel (Collins, 1983; Kovalyov, 1989;Danker *et al*., 1993; Grange and Yates, 1994; O'Reilly and Daborn, 1995; Gallagher and Jenkins, 1998; Cousins and Dowson, 1999). Several reports support the view that bovine TB is an occupational zoonosis in those professions and that exposure to *M. bovis* aerosols from infected animals is a mechanism for human infection(de la Rua-Domenech, 2006).

Also, cutaneous or mucosal transmission of *M. bovis* is an occasional source of localized skin, tendon and lymph node lesions, otitis and conjunctivitis in milkers, those regularly dressing carcasses of tuberculous animals and veterinarians exposed during surgical interventions or necropsies (Pritchard, 1988;Kovalyov, 1989; Grange and Yates, 1994;Moda *et al*., 1996; Ashford *et al*., 2001). Occupational infection with *M. bovis* is most likely to occur as a result of aerosol inhalation or by hand-to-mouth contact after the handling of infected animals (de la Rua-Domenech, 2006).

2.7.5 Current status of bovine tuberculosis in Nigeria

Zoonotic tuberculosis is a chronic, infectious and contagious debilitating disease caused by *Mycobacterium bovis* that has become a resurgent problem in animals and humans in Nigeria (WHO, 2002;Cadmus, 2003; Ofukwu, 2006). Nigeria ranks fifth among the 22 high TB burden countries in the world (Chukwu *et al*., 2013). One of the available reports in Nigeria on diagnosis of bovine tuberculosis by bacterial culture from abattoir specimens of lung samples and associated lymph nodes showed that *M. bovis* was isolated in 62.5% of the cases, *M. tuberculosis* in 14.0% and *M. avium* and unclassified *Mycobacteria* in 9.5% (Igbokwe *et al*., 2001).Although studies have provided insights into the significance of zoonotic tuberculosis in developing countries in Africa, like Nigeria (Cadmus *et al*., 2006), the extent to which zoonotic transmission contributes to the burden of human tuberculosis in these areas is still largely unknown (Michel *et al*., 2010). Data on the bovine tuberculosis in Africa and particularly in Nigeria are limited; reports, however, showed that it is endemic in Nigeria (Amanfu, 2006; Okaiyeto *et al*., 2008; Cadmus *et al*., 2004; 2009).

The incidence rates of bovine tuberculosis in cattle have continued to increase from 0.3% in 1976 to as high as 7.3% in 2003 (Ofukwu, 2006). In cattle, this may be primarily due to a move towards sedentarism, importation of infected foreign breeds, and lack of sustained preventive measures (Cosivi *et al*., 1995).In Nigeria, most of the studies were based on pathological examination at abattoirs (Igbokwe *et al*., 2001; Ameen *et al*., 2008; Aliyu *et al*., 2009; Damina *et al*., 2011) and tuberculin skin testing in cattle herds (Yohanna *et al*., 2008; Danbirni *et al*., 2012; Ibrahim *et al*., 2012), with very few works on mycobacterial isolation and molecular characterization (Alhaji, 1976; Cadmus *et al*., 2004; 2006; 2009; Jenkins *et al*., 2011). Cadmus *et al*. (2011) showed that strains of *M. bovis* with the spoligotype pattern SBO944 were dominant in cattle slaughtered in Ibadan (South-Western Nigeria). They also have demonstrated that this molecular type is identical to *M. bovis* isolated from cattle in neighboring countries; hence it indicates the possibility of Trans-border transmission.The disease (bTB) could have a detrimental effect on animal production and the economic losses from TB in Nigeria and its environs was estimated to cause an annual loss (due to organs/carcass condemnation in cattle) of about 14 to 24 million Naira (Raufu and Ameh, 2010).

2.8 Diagnosis and Treatment of Bovine Tuberculosis

2.8.1 Diagnosis: general overview

Despite extensive knowledge on tuberculosis, disease diagnosis and the identification of the infective mycobacterial species is not yet a simple matter. Species identification is indispensable for the study of the transmission of *Mycobacteria*. Morever, diagnosis and species identification play a major role in tuberculosis surveillance and control (Michel *et al*., 2010).The detection of bovine tuberculosis in Africa is carried out usually on the basis of postmortem examination during abattoir meat inspection, tuberculin skin test and rarely on bacteriological techniques (Ameni *et al*, 2000; Asseged *et al*., 2004; Shitaye *et al*., 2006;2007), though the consistency of using a particular test varies among countries (Awah-Ndukum, 2012).

2.8.2 Clinical signs

Bovine tuberculosis (bTB) is often subclinical. When present, clinical signs are not specifically distinctive and are characterized by weakness, anorexia, emaciation, enlargement of lymph nodes, and cough, particularly in advanced tuberculosis (OIE, 2009).It is difficult to be diagnosed based only on clinical manifestations due to similarities in clinical manifestations with other conditions such as contagious bovine pleuropneumonia (CBPP), *Pasteurella* or *Corynebacterium pyogenes* pneumonia, traumatic pericarditis and chronic aberrant liver fluke infestation which are often differentially diagnosed for bovine TB in cattle(Blood and Radostits, 1989; Gracey and Collins, 1992; Grist, 2008).

2.8.3 Postmortem examination

A more direct method for tuberculosis diagnosis is based on the detection of the lesions suspicious of being tuberculosis at postmortem inspection, from tuberculous tissues/organ lesions (generally in animals). In Nigeria, identification of TB lesions at meat inspection in abattoirs has been used to detect the disease (Cadmus and Adesokan, 2009). Meat inspection provides very significant insight into the prevalence of many animal diseases (FAO, 1994; Hinton and Green, 1997; Grist, 2008) including TB (Awah-Ndukum, 2012). However, diagnosis of bovine tuberculosis based on postmortem examination at meat inspection may be confused with lesions caused by *Nocardia*, *Corynebacterium* and other granuloma causing organisms (Blood and Radostits, 1989; Gracy and Collins; 1992; FAO, 1994; Grist, 2008). The procedures to employ during postmortem meat examination include visual examination, palpation and incision of the suspected tissues lungs, liver and kidneys; lymph nodes of the thoracic and head regions; the mesenteric lymph nodes, other lymph nodes and tissues/organs of the body (FAO, 1994).

Postmortem findings for TB diagnosis could be due to the presence of tuberculous lesions such as abscess with yellowish pus and tubercles which may be caseous or sometimes 'gritty' calcification in carcasses and during slaughter/meat inspection in abattoirs (Grossklaus, 1987; Murray *et al*., 1991; Gracy and Collins, 1992; FAO, 1994; Grist, 2008). Detection of bovine tuberculosis in most African countries is based on the postmortem findings of tuberculous lesions (Asseged *et al*., 2004; Shitaye *et al*., 2006). However, not all infected animals present lesions at carcass inspection while gross visible lesions suggest that the disease is at an advanced or late stage (Corner, 1994; Shitaye *et al*., 2006).

2.8.4 Direct smears with ziehl-neelsen stain and microscopy

In most resource-limited countries, such as Nigeria, the Ziehl-Neelsen's (ZN) smear microscopy method is used in clinical laboratories and for detection of TB organisms (acid-fast bacilli) in biological samples (Chukwu *et al*., 2013). It is not very specific and sensitive because it requires 10^3 to 10^4 cells per ml of specimen such as tissue homogenate and it detects only acid fast bacilli (Ojo, 2009). This makes the ZN method less dependable than the other methods such as DNA-based PCR technique (Chukwu *et al*., 2013). The presence of Mycobacteria in a given sample can be assessed by Ziel-Neelsen staining followed by light microscopy or auramine O staining and fluorescence microscopy (Marais *et al*., 2008). An eseential requirement for controlling TB disease is early diagnosis. Currently the easiest and fastest methods for detection of *Mycobacteria* species are making smears and staining of cultures or direct samples for microscopy to identify acid-fast bacilli (Huggett *et al*., 2003). Presumptive findings base on microscopic demonstration of acid-fast bacilli (AFB) has been described (Chakravorty *et al*., 2005; Johnson *et al*., 2008). Direct smears from clinical samples or suspected tissues are stained with the Ziehl-Neelsen stain to demonstrate the acid-fast tubercle bacilli under the microscope (Strong and Kubica, 1985; WHO, 1998a).

Although direct microscopy is easy, rapid and cheap, it fails to differentiate mycobacterial strains and to distinguish live from dead bacilli (Kiraz *et al*., 2010). Moreover, the sensitivity of direct microscopy is affected by several factors including the type of specimen, techniques and stains used, along with the experience of the laboratory technician and the number of smears to be made at a given time (Marks, 1993; Metchock *et al*., 1999; Murray *et al*., 2005).

2.8.5 Mycobacterial culture

Culture is still regarded as the gold standard for tuberculosis diagnosis despite certain deficiencies. For example, the yield of bacteria from samples in the culture may be low. Bacterial culture is also time consuming and doesnot by itself allow the differentiation between distinct mycobacterial species. However, in many cases, culture is a prerequisite for further testing and characterization of *Mycobacteria* (Michel *et al*., 2010). The diagnosis of bovine tuberculosis is confirmed following growth and isolation of *M. bovis*on various selective media such as Lowenstein-Jensen media, Middlebrook broth along with the incubation period of up to twelve weeks for *M. bovis* (Strog and Kubica, 1985; WHO, 1998a; WHO, 1998b; OIE, 2009).

Culture is about 500 times more sensitive than microscopy and provides organisms for further investigations including drug sensitivity and genotyping. However, culture using solid media techniques is slow, routinely taking weeks for a culture to be confirmed (Davies *et al*., 1999). Solid media is increasingly being superseded by a range of commercially available liquid culture media systems (Watterson and Drobniewski, 2000).

2.8.6 Polymerase chain reaction (PCR) and molecular typing

2.8.6.1 General overview

The polymerase chain reaction (PCR) is a diagnostic method for making many copies of a specific DNA fragment(Saiki *et al*., 1988), is one of the most widely applied tools in modern molecular biology (Innis *et al*., 1999). PCR-based techniques are indispensable for the accurate differentiation of mycobacterial species and molecular investigations of tuberculosis transmission (Parson *et al*., 2002; Warren *et al*., 2006). It can be used for any sample material in theory, but has some problems of its own, as certain samples may contain PCR inhibitors, which could lead to false negative results. Conversely, the generation of a vast number of amplicons can quickly give rise to false positive results. Moreover, new methods have recently been developed, which allow for quick identification and differentiaton of a range of common pathogenic mycobacterial species and some common antibiotic resistance mutations (Barnard *et al*., 2008; Hoek *et al*., 2008; Song *et al*., 2009).

Despite the different host tropisms, the *M. tuberculosis* complex is characterized by 99.9% or greater similarity at the nucleotide level, and by virtually identical 16S rRNA sequences (Sreevatsan *et al*., 1997; Brosch *et al*., 2002; Huard *et al*., 2006). It is true that bovine-adapted *M. bovis* has a smaller genome extant strains of *M. tuberculosis*, and that no significant chromosomal regions are present in *M. bovis* and at the same time absent from all strains of *M. tuberculosis* (Garnier *et al*., 2003). It is not necessarily true, however, that *M. bovis* has more deletions than any other member of the *M. tuberculosis* complex (Smith *et al*., 2006a). As Brosch *et al.* (2002), out of necessity, identified deletions in *M. bovis* by comparison with the sequenced chromosome of *M. tuberculosis*, it is inevitable that *M. bovis* would be the terminal group in the lineage. However, whole-genome microarray analysis has shown that both *M. tuberculosis* and *M. bovis* have the propensity to lose segments of DNA from the genome (Inwald *et al*., 2002; Tsolaki *et al*., 2004), which might result in the loss of antigens that have been identified as immunodiagnostic reagents.

A number of amplification-based techniques have been developed to speed up detection and increase the sensitivity of TB detection (Huggett *et al*., 2003). Characterisation of the members of *Mycobacterium tuberculosis* complex to further comfirm diagnosis can be achieved by direct Polymerase Chain Reaction (PCR) based genomic deletion typing for the presence or absence of various regions of difference (Frothingham, 1995; Brosch *et al*., 2002; Parsons *et al*., 2002; Warren *et al*., 2006).

2.8.6.2 Genomic deletion typing/PCR for region of difference (RD)

The members of the *Mycobacterium tuberculosis* complex share 85% to 100% DNA-DNA relatedness (Imaeda, 1985). Moreover, differentiation among members of the *Mycobacterium tuberculosis* complex has been by amplification of mycobacterial specific sequences (Sinclair *et al*., 1995), and a series of deletions (regions of difference [RD]) within the *Mycobacterium tuberculosis* complex have been used to identify phylogenetic relationships between members of MTC (Brosch *et al*., 2002). One of the first attempts to perform genomic comparison across the MTC was by Mahairas *et al*. (1996) who used subtractive hybridization techniques to identify three large scale deletions, RD1-RD3, that were absent from *M. bovis* BCG but present in the genome of virulent *M. bovis*. Currently, regions of difference PCR are being used widely for the differentiation of the species of mycobacteria (Parsons *et al*., 2002). Region of difference (RD) deletion typing method is a PCR-based method that makes use of the MTC chromosomal regions of difference deletion loci (Huard *et al*., 2003). The regions of difference represents the loss of genetic material that arise due to errors in DNA replication, movement of mobile genetic elements, mycobacteriophage-mediated transduction, or recombination between adjacent homologous DNA fragments with loss of the intervening sequence (Cole, 2002).Several PCR primer pairs specific to the loci were used which include; 16S rRNA, Rv0577, Rv1510 (RD4), Rv1970 (RD7), Rv3877/8 (RD1), Rv3120 (RD12), Rv2073 (RD9), Rv1257 (RD13), IS1561 (MiD3) and TbD1 (Huard *et al*., 2003; Huard *et al*., 2006). Deletions include genes predicted to affect a range of metabolic pathways and putative virulence factors (Behr *et al*., 1999; Gordon *et al*., 1999), and it promises to be an important source of variability among MTC members (Mostowy *et al*., 2002). Because genomic deletions are expected to represent unidirectional genetic events (Behr *et al*., 1999; Kato-Maeda *et al*., 2001), their distribution suggests a phylogeny for the MTC (Mostowy *et al*., 2002). The following are the distribution of RDs among members of *Mycobacterium tuberculosis* complex:

Mycobacterium tuberculosis strains:*M. tuberculosis* strains are highly conserved with respect to RD1, RD2, RD4, RD7, RD8, RD9, RD10, RD12, RD13 and RD14, and that these RDs represent regions that can differentiate *M. tuberculosis* strains independently of their geographical origin and their typing characteristics from certain other members of the *M. tuberculosis* complex (Brosch *et al*., 2002). While regions RD3 and RD11, corresponding to the two prophages phiRv1 and phiRv2 of *M. tuberculosis* H37Rv (Cole *et al*., 1998), RD6, containing the insertion sequence IS1532, and RD5, that is flanked by a copy of IS6110 (Gordon *et al*., 1999), are absent in some strains of *M. tuberculosis*.

Mycobacterium canetti:In *M. canetti*, of the RD and RvD regions except the prophages (phiRv1, phiRv2) are present. Therefore, *M. canetti* represents a fascinating tubercle bacillus, whose detailed genomic analysis may reveal further insights into the evolution of the *M. tuberculosis complex* (Brosch *et al*., 2002).

Mycobacterium africanum:Analysis of the West African isolates of the *M. africanum* indicated that, it lacks prophage phiRv1 and RD9 regions containing *cobL*, while the east African isolates lack prophage phiRv1 (RD3) only (Brosch *et al*., 2002).

Mycobacterium microti:*M. microti* lacks regions RD7, RD8, RD9 and RD10. Furthermore, vole isolates missed part of the RD5 region, whereas this region is present in the human isolate (Brosch *et al*., 2002).

Mycobacterium bovis and *Mycobacterium bovis* BCG:*M. bovis* has a very large host spectrum infecting many mammalian species, including man. The distribution of RDs allows its differentiation into groups among the tested *M. bovis* strains. The first group (seals and human isolates) is formed by strains that lack RD7, RD8, RD9, and RD10. The oryx isolates form the second group that lacks parts of RD5 in addition to RD7, RD8, RD9 and RD10 and very closely resembles *M. microti* isolates (Brosch *et al*., 2002). Group three consists of goat isolates that lack regions RD5, RD7, RD8, RD9, RD10, RD12, and RD13, and except for the added RD4 deletion found in classical *M.bovis*, the goat isolate displays the same deletion as in classical *M. bovis* strains (Brosch *et al*., 2002), and the most common *M. bovis* strains is the classical *M. bovis* (van Soolingen *et al*., 1994) and it the one mostly isolated from cattle as well as humans (e.g., the multidrug resistant *M. bovis* from Spain; Samper *et al*., 1997), which shows the greatest number of RD deletions and seem to have undergone the greatest lost of DNA relative to other members of the *Mycobacterium tuberculosis* complex (Brosch *et al*., 2002). Analysis of *M. bovis* BCG strains indicates that BCG lacked the same RD regions as classical *M. bovis* strains, in addition to RD1, RD2 and RD14, which apparently occurred during and after the attenuation process (Behr *et al*., 1999; Mahairas *et al*., 1996).

Other detailed molecular typing or genetic fingerprinting techniques that can be used to further differentiate, characterize and geographically map different strains of *Mycobacteria species* (Sreevatsan *et al*., 1997; Frothingham and Meeker-O'Connell, 1998; van Soolingen *et al*., 1998; Frothingham *et al*., 1999; Sritharan and Sritharan, 2000; Watterson and Drobniewski, 2000; Drobniewski *et al*., 2003; Chakravorty *et al*., 2005) are as follows:

2.8.6.3 Single-nucleotide polymorphisms (SNPs) and IS6110 RFLP typing

Single Nucleotide Polymorphisms (SNPs) within the MTC are a well documented means of acquired drug resistance, but for reasons that remain to be determined, very few spontaneous unselected mutations become fixed within the clonal population structure of the MTC, despite their having a mutation frequency believed to be in the range of most other bacteria (Sreevatsan *et al*., 1997). In fact, the degree of synonomous (genetically neutral) nucleotide substitution has been estimated to be as low as 1 in 10,000, whereas nonsynonomous nucleotide changes within the MTC genomes have been estimated to outnumber synonomous SNPs nearly 2 to 1 (Sreevatsan *et al*., 1997; Fleischmann *et al*., 2002; Gutacker *et al*., 2002); such proportions support the assumption that positive selection is driving diversity. Once established in a clonal lineage, SNPs in the MTC organisms are believed to be relatively stable over time (Musser *et al*., 2000), and frequently identified SNPs in certain genes have been used, alone or in combination, as either specific markers for a given MTC species or to categorize isolates into defined MTC phylogenies (Brosch *et al*., 2002; Huard *et al*., 2003; Mostowy *et al*., 2002).

In large-scale evaluations of expansive data sets of synanomous SNPs differentially present in the MTC (first identified by in silico genome-wide cross-comparisons), the population structure of *M. tuberculosis* was found to consist of up to nine major clusters, with *M. bovis* terminating a separate evolutionary offshoot bridged by the remaining MTC species (Gutacker *et al*., 2002; Filliol *et al*., 2006; Gutacker *et al*., 2006). Clearly, comprehensive SNPs informatics is a powerful tool to reconstruct familial interrelationships within the MTC, and, with the addition of newly identified SNPs, the branches of the MTC phylogenetic tree will continue to be refined (Huard *et al*., 2006). However, it is the acquisition of long-sequence polymorphisms (LSPs) rather than single-nucleotide changes that is now emerging as possibly the key mechanism of adaption in mycobacteria (Cole, 2002; Howard *et al*., 2002; Semret *et al*, 2004) and a significant source of MTC interstrain genetic variability.

IS6110 restriction-fragment length polymorphism (RFLP) typing identifies polymorphisms associated with the RFLP pattern of multiple copies of IS6110, and is the technique most frequently used for molecular typing and epidemiological studies of *Mycobacterium tuberculosis* (Kremer *et al*., 1999).

2.8.6.4 Spoligotyping

Spoligotyping is a PCR and hybridization technique commonly used to genotype isolates of the *Mycobacterium tuberculosis* complex and measures polymorphism in the presence or absence of multiple short spacer units found in the Direct Repeat (DR) region of the chromosome (Hermans *et al*., 1991; Kamerbeek *et al*., 1997). The unique spacer units are interspersed with similar sized, virtually identical, repeat units which are the target for the initial PCR reaction (Smith and Upton, 2012). Although over 60 spacer units may reside in the DR region, 43 spacers were selected to be the target for the internationally standardized spoligotyping technique (Kamerbeek *et al*., 1997). The order of the 43 spacer units in the chromosome is apparently conserved and the spacers are numbered from 1 to 43; in practice, a spoligotype pattern for an isolate resembles a commercial product bar-code (Groenen *et al*., 1993). Spacers 3, 9, 16 and 39-43, for example, are always absent from spoligotype patterns of *M. bovis*, *M. caprae*, *M. microti* and *M. pinnipedii* presumably because these spacers were absent from the most recent common ancestors of these clades and cannot be regained by recombination (Smith *et al*., 2006b). Spoligotyping has been used to genotype strains of the animal-adapted lineage throughout the world and in combination with VNTR typing can provide a reasonably robust genotype (combination of spoligotype and VNTR type) for each isolate (Smith and Upton, 2012).

The most common epidemiological molecular-typing method applied to *M. bovis* is spoligotyping (Durr *et al*., 2000). This method identifies polymorphisms in the spacer units in the direct repeat (DR) region of the chromosome (Kamerbeek *et al*., 1997). The

DR region comprises of multiple, virtually identical, 36bp regions interspersed with DNA spacer sequences of a similar size (direct variant repeat (DVR) units). The position of a spacer sequence in the DR region is conserved relative to the other spacer-repeat sequences (van Embden *et al*., 2000; Warren *et al*., 2002). The DR region can contain >60 DVR units. However, 43 of the spacer units were selected from the spacer sequences of *M. tuberculosis* reference strain H37RV and *M. bovis* BCG strain P3 and are used in the standard application of spoligotyping to strains of the *M. tuberculosis* complex (Groenem *et al*., 1993; Kamerbeek *et al*., 1997). Each spoligotype pattern is given a local identifier and is also assigned an international name by Mbovis.org. For bacteria that frequently exchange DNA, makers at one locus provide little information about the alleles present on the rest of the chromosome; however, for clonal bacteria such as *M. bovis*, the spoligotype pattern can be used as a proxy, within limits, for the complete genotype of the cell (Smith *et al*., 2006a).

Spoligotyping measures the variation in the number of unique spacer sequences located in the direct repeat region, and in practice, the spoligotype pattern for a strain looks similar to the bar code that is commonly used to identify and track merchandise (Smith *et al*., 2006a). As the spacer sequences are not found anywhere else on the *M. bovis* chromosome, the loss of the spacer sequences can be used as phylogenetic markers in the same way as the deletions identified by Brosch *et al*. (2002).

2.8.6.5 VNTR typing

Variable nucleotide tandem repeat (VNTR) typing is the equivalent of mini-satellite typing and measures variation in the number of repeats at a series of loci dispersed throughout the genome (Jeffreys *et al*., 1985). The loci used in VNTR typing of mycobacterial isolates have been named as exact tandem repeat (ETR) loci and mycobacterial interspersed repetitive units (MIRU) loci. Although there are subtle differences between the loci identified by these different nomenclatures, they are not exclusive; some chromosomal regions are both ETR and MIRU loci, as well as being known as VNTR loci (Frothingham and Meeker-O'Connell, 1998).

In contrast to spoligotyping, VNTR typing has the virtue of being a multilocus technique; however, this is of less consequence in a purely clonal organism where polymorphism at any single locus marks the entire chromosome. The number of repeats at a mycobacterial VNTR locus can increase or decrease and the rate of variation is locus- and genotype-dependent (Kremer *et al*., 2005). These attributes have the effect of making of VNTR typing, in the absence of any other molecular typing, highly homoplastic; unrelated clones can have the same VNTR type (Gibson *et al*., 2005; Filliol *et al*., 2006), although this problem can be ameliorated by using multiple VNTR loci or by spoligotyping the strains. More troubling, some VNTR loci are located within transcription units (Frothingham and Meeker-O'Connell, 1998). Variation in the number of repeats at these loci could be driven by selection and these loci tend to converge in unrelated clones. These caveats aside, there is no doubt that VNTR typing can identify more genotypes than spoligotyping alone (Gutacker *et al*., 2002, Smith *et al*., 2003; Filliol *et al*., 2006).

2.8.6.6 Sensitivity and specificity of molecular diagnostic assays

The sensitivity and specificity values of PCR-based assays differ with respect to the technique, type of specimen and smear positivity or negativity (Woods, 2001; Singh *et al*., 2002). Hence, nucleic acid-based tests rely on the amplification of specific nucleic acid sequences of *Mycobacteria* and provide rapid diagnosis (Kiraz *et al*., 2010). The majority of molecular TB detection protocols are based on the Polymerase Chain

Reaction (PCR). PCR is sensitive, easy to use and a rapid technique. Although its adoption in the clinical laboratory has been relatively slow despite a substantial body of published methods. This is partly due to restrictive licensing agreements, leading to the development of competing, alternative technologies but also the result of early problems with cross-contamination and false positivity as a result of a poor assay design (Gillespie and McHugh, 1997). Sensitivity can be reduced by the presence of inhibitors which can cause false negative results. This can be tackled by improving the extraction protocol and controlled for by using positive samples to determine assay efficiency. Laboratory practice is another aspect that must not be overlooked as the different assays have been shown to be less variable than the laboratories performing them (Noordhoek *et al*., 1996).

Molecular amplification techniques are incredibly powerful and capable of detecting very low numbers of the DNA target, thus very low level of contamination can lead to false positivity. However, there are steps that can be used to reduce contamination; the most important is to establish a strict four room strategy with unidirectional work flow (McHugh, 2000). Further approaches include the use of UTP in the amplification reactions instead of TTP and then treatment of the reagents with uracyl-*n*-glycosylase (UNG) at the beginning of a PCR reaction to destroy any templates that have been generated by previous reactions (Huggett *et al*., 2003).

Another factor that will affect specificity is the sequence of the primer or probe used in the assay. Care must be taken in the selection and design of amplification primers, if they are not unique to the target sequence then the risk of mis-diagnosis is increased. An example of this is some sets of primers selected for IS6110 amplification which have been shown to amplify mycobacteria other than tuberculosis (McHugh *et al*., 1997).

2.8.7 Detection of cell-mediated immune response

The first and best-known test for tuberculosis diagnosis is the tuberculin skin test. The same principle is used for testing in both animals and humans and although imperfect, the tuberculin test has not yet been replaced by any other more accurate or satisfactory method. Some of the main deficiencies of the test are its inability to differentiate between distinct species of *M. tuberculosis* complex, failure to distinguish between latent stages of infection and disease, and also failure to distinguish between vaccinated and infected individuals (de la- Rua-Domenech *et al*., 2006).The two most commonly used test formats are the caudal fold test (CFT) and the single intradermal comparative cervical tuberculin (SICCT) test (Monaghan *et al*., 1994). For both tests, skin induration reactions are read 72 hours following tuberculin injection. This being a classic manifestation of a cell mediated delayed-type hypersensitivity (DTH) response is characterized in cattle by the predominant infiltration of $CD4^+$ and $CD8^+$ T-cells and macrophages (Doherty *et al*., 1996). The SICCT is the more specific test format since the comparative use of PPD-A allows for the measurement of non-specific sensitization to environmental mycobacteria which can cross-react with antigens present in PPD-B (Stenius, 1938).

In addition, anergy, exposure to environmental *Mycobacteria*and operator errors can lead to false results (de la- Rua-Domenech *et al*., 2006). Two recent studies also suggested that the cut-off generally used for positive test interpretation (>4mm) may not be applicable for at least some countries in sub-Saharan Africa and that a cut-off >2mm could be more appropriate in some settings (Ameni *et al*., 2008; Ngandolo *et al*., 2009).Paradoxically, the proteins used as antigens in tuberculin skin test are produced by mycobacteria under conditions not experimentally defined as persistent. In spite of this fact, their use is considered in some developed countries as a valuable means of diagnosing both clinical (active) and subclinical (latent) TB (Abebe *et al*., 2007; Andersen *et al*., 2007; Cehovin *et al*., 2007). It was not until after the new tests started to emerge for determining TB infection status (Andersen *et al*., 2007; Cho, 2007). These new tests are based on the detection of INF-γ in blood samples after in vitro stimulation of blood cells with the specific antigens ESAT-6 and CFP-10 (Andersen *et al*., 2000).

The more recently developed Bovigam® test for cattle, the QuantiFERON®-Tb-test, detects the production of interferon-gamma in (in vitro) stimulated blood samples. Applied in both standard commercial and customized test formats, this assay has contributed significantly to the improved detection of early *M. bovis* infection in cattle as well as an increasing number of wildlife species (e.g. non-human primates, cervids) (Grobler *et al*., 2002; Morar *et al*., 2007; Denis *et al*., 2007; Lin *et al*., 2008; Waters *et al*., 2008). Recent improvements of the test include the use of two *M. tuberculosis* complex specific antigens, ESAT-6 and CFP-10, which has resulted in increased test specificity (Buddle *et al*., 2009). In contrast to the tuberculin skin test, the interferon-gamma test could also be used to differentiate between infected and vaccinated individuals. For diagnosis in animals in developing countries, this test however appears to be impractical as it requires sophisticated laboratory equipment and the need to quickly process the blood samples after collection (de la Rua-Domenech *et al*., 2006).There have been several studies investigating the influence of the skin-test on interferon-gamma responses in blood since measurement of interferon-gamma responses are being increasingly used as an adjunct to the skin-test to improve disease diagnosis (de la Rua-Domenech *et al*., 2006). Previous reports have shown that SICCT does not exert a significant effect on PPD stimulated interferon-gamma responses measured in blood from naturally infected cattle collected 3-10 days after the test (Doherty *et al*., 1995; Gormley *et al*., 2004; Coad *et al*., 2007). In contrast, it has been reported that CFT can boost interferon-gamma response immediately following the test (Whipple *et al*., 2001; Palmer *et al*., 2006).

Evidence suggesting that diagnostic assays (TST and INF-ɣ tests) can detect bTB in cattle soon after *M. bovis* infection regardless of the dose has been reported (Neill *et al*., 1994b; Dean *et al*., 2005). Thus, none of the tests currently available for the diagnosis of bTB allows a perfectly accurate determination of the *M. bovis* infection status of cattle (de la Rua-Domenech *et al*., 2006). During cow-to-cow *M. bovis* transmission, it takes about two months to present a TST^+ reaction (ranging from 8 to 65 days) (Goodchild and Clifton-Hadley, 2001), potentially increasing the number of false negative results. Moreover, reversion from a TST^+ result occurs at a 5% rate per year (Goodchild and Clifton-Hadley,2001), possibly indicating eradication of infection or simply waning of immune memory (Alvarez *et al*., 2009).

For Gamma interferon (IFN-ɣ) test: in this test, the release of a lymphokine Gamma interferon (IFN-ɣ) is measured in a whole-blood culture system, the assay is based on the release of IFN-ɣ from sensitized lymphocytes during a 16-24 hour incubation period with specific antigen (PPD-tuberculin) (Wood *et al*., 1990). The detection of bovine IFN-ɣ is carried out with a sandwich ELISA that uses two monoclonal antibodies to measure bovine Gamma-interferon, it is recommended that the blood samples be transported to the laboratory and the assay be conducted as soon as possible, but not later than the day after blood collection (Ryan *et al*., 2000; Coad *et al*., 2007).

2.8.8 Detection of humoral immune response

The tuberculin skin test and the interferon-gamma test are both based on the detection of the early cell-mediated immune response in tuberculosis infection. However, at late disease stages, the cell-mediated immune response can wane as opposed to a generally increasing humoral immune response and the tuberculin skin test or Bovigam® test can therefore give false negative results (de la Rua-Domenech *et al*., 2006). This is of importance for the diagnosis of bovine tuberculosis in settings where no or little disease control measures are applied and where the percentage of late stage diseased animals is believed to be high. Therefore, in developing countries, serological tests, which are based on the detection of humoral immune response, may be of particular use. Unfortunately, to date, no satisfactory serological test is available. Some of the problems related to the development of serological tests for tuberculosis diagnosis include; the observed highly variable antibody responses between individuals to mycobacterial antigens and antigenic variation between mycobacterial strains (Pheiffer *et al*., 2005). However, a recently developed lateral flow test that is based on the detection of more than one antigen has shown promising results for tuberculosis diagnosis in certain animal species like in elephant (Lyashchenko *et al*., 2008; Greeenwald *et al*., 2009). Another recently developed serological test for animals is based on antibody detection using fluorescence polarization but has shown variable effectiveness in different settings (Jolley *et al*., 2007; Ngandolo *et al*., 2009).

Classically following infection the animal mounts a cell mediated immune response which can with time wane to be replaced with an antibody response (Pollock and Neill, 2002; Welsh *et al*., 2005; de la Rua-Domenech *et al*., 2006). Importantly, however, the switch from cellular to antibody responses has been related to an advanced disease profile (Welsh *et al*., 2005). Indeed the antibody response may act as a marker for advancing disease or it may actually have a role in the development of a disease by down-regulating cell mediated control mechanisms (Hussain *et al*., 2001).

At any single time-point most infected animals will not be displaying a measurable specific antibody response (Plackett *et al*., 1989; Fifis *et al*., 1992). There is some evidence that SICCT performed on recently infected cattle can lead to a measurably increased response thereby potentially increasing the sensitivity of antibody testing(Thom *et al*., 2004; Waters *et al*., 2006). However, there has been a concerted effort by several research groups in trying to identify potential TB specific antigenic targets for antibody assays (Schiller *et al*., 2010). To date, the most promising antigens for serodiagnosis appear to be MPB70 and MPB83 (Lightbody *et al*., 1998; McNair *et al*., 2001; Wiker, 2009). However, it is increasingly clear that, to improve overall test sensitivity, it will be necessary to include additional antigens which individually are not recognized by many infected animals but bring overall improved sensitivity when included in the assays (Waters *et al*, 2006;Lyashchenko *et al*., 2008).

Currently, the serological testsin use include multiantigen print immunoassay (MAPIA) (Waters *et al*., 2006), fluorescence polarization assay (FPA) (Jolley*et al*., 2007), Immunochromatographic lateral flow test (Lyashchenko *et al*., 2006), Single antigen chemiluminescence (Green *et al*., 2009) and multiplex chemiluminescence immunoassay (Whelan *et al*., 2008).Tests of humoral immunity employing the Enzyme-linked immunosorbent assays (ELISAs), immunochrommatographic (lateral flow) assay and other serologic based tests may complement tests of cellular immunity in anergic hosts. However, anti-TB antibody titres are inconsistent and rise only in the late stages of infection while a limited cocktail of selected *Mycobacterium* antigens (e.g.:ESAT-6, MTSA-10, MPTS1, MPT63, MPB59, MPB64, MPB70, MPB83) are employed to detect circulating antibodies (Lyashchenko *et al*., 1998; Pollock *et al*., 2001; Banerjee *et al*., 2003; Cousins and Florrison, 2005; Lyashchenko *et al*., 2007; Lyashchenko *et al*., 2008).

2.8.9 Treatment

Mycobacterium bovis reacts very similarly to *Mycobacterium tuberculosis* in terms of sensitivity to antibiotics. In the case of *M. tuberculosis*, the standard therapy for antibiotic sensitive isolates consists of 2 months of isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z), followed by 4 months of H and R.*Mycobacterium bovis* is sensitive to all these first-line antibiotics except Z (Daly *et al*., 2006).

Furthermore, in the absence of acquired antibiotic resistance, wild-type *M. bovis* is sensitive to most, if not all, other antibiotics used to treat TB, these are streptomycin, ethambutol, ofloxacin and ethionamide (Parreiras *et al*., 2004; Romero *et al*., 2007).The treatment of tuberculosis in animals depends on the specific reaction of individual species to the various antibiotics and the logistics and ease of administration of medication. It has been done for a few rare animal species in captivity, but it is not really viable for a herd or free-ranging animals (Michel *et al*., 2010).

2.9 Control and Eradication of Bovine Tuberculosis

Bovine tuberculosis is recognized as being potentially important in many developingcountries throughout the world. However, only relatively few countries, such as those in Africa, have introduced effective control or eradication programmes (Chillaud, 1992). This can be attributed to many factors, amongst which politico-economic reasons are significant constraints on a sustainable testing programme often complicated by social unrest due to political instability and ethnic wars (Neill *et al*., 2005). There is often a lack of veterinary expertise and communication networks and insufficient collaborative operations with bordering countries and hence a lack of quarantine, associated with smuggling of live animals across state boundaries (Ayele *et* *al*., 2004).The test-and-slaughter policy is the basis for international bovine tuberculosis control and eradication programmes using tuberculin skin test (TST) to detect affected herds (and re-test) periodically and removing reacting cattle (Gilbert *et al*, 2005; Abernethy *et al*., 2006; Good, 2006), that may shed the infective organism (Awah-Ndukum, 2012). Control and eradication programmes for bTB due to *M. bovis* are based on early accurate detection and removal of infected animals (Abernethy *et al*., 2006; Good, 2006; Goodchild and Clifton-Hadley, 2006; Pavlik, 2006). However, although animal test-and-slaughter schemes have successfully reduced the prevalence of bovine tuberculosis in most industrialized countries, such expensive control programmes have been increasingly questioned considering their economic burden and increasing opposition by farmers (Bennett, 2009; Torgerson and Torgerson, 2009).

In many industriliased nations there is "effective" compulsory reporting of *M. bovis* infection of all animals, quarantine of infected herds, tracing and re-testing of animals in-contact with bovine tuberculin skin positive reactors, movement restrictions of cattle herds not yet tested for TB as well as control of animals movement out of the known TB infected herds and endemic areas (Citron, 1988; Gilbert *et al*., 2005; Abernethy *et al*., 2006; Good, 2006; Goodchild and Clifton-Hadley, 2006; Pavlik, 2006; OIE, 2008; 2009). However, the test-and-segregation programme, a modified form of the test-and-slaughter policy, may be more useful for developing countries, where the test-and-slaugter policy can not be practicable for the whole cattle population (WHO, 1994). Thus, interim measures to segregate herds and phase slaughter of reactors are done (Awah-Ndukum, 2012). In most countries with strict TB eradication programs, the test-and-segregation strategy made up the early stages followed by the test-and-slaughter methods in the final stage (CFSPH, 2009), and infected slaughter/meat cases during inspection are traced back to the originating farms (DEFRA, 2008). Informed farm management decisions such as proper sanitation and disinfection are also important to reduce the spread of *Mycobacterium* within and between herds, as well as the risks of exposure and transmission of bTB infection to humans (Wilsmore and Taylor, 2008).Also, culling to reduce wildlife reservoir hosts population density can decrease animal TB transmission, but the situation must be assessed carefully to avoid unanticipated effects such as the economic benefits and increase scattering members of the infected species (Donelly *et al*., 2007; Smith *et al*., 2007; CFSPH, 2009).

It is only Algeria, Burkina Faso, Cameroon, Morroco, Namibia and South Africa out of the 48 countries in Africa that apply a test-and-slaughter policy as a control measure and consider bovine tuberculosis as a notifiable disease (Cosivi *et al*., 1998), and this policy has successfully reduced the prevalence of bovine tuberculosis (Michel *et al*., 2009).The control of bovine tuberculosis in Nigeria is regulated by Federal Ministry of Agriculture. However, this control policy as stated in the Animal Disease (Control) Decree of 1988 is poorly or inadequately implemented in recent years. This is largely due to politico-economic reasons such as high cost of sustainable testing and slaughter, and the subsequent compensation to the farmers (Abubakar *et al*., 2011).

The development of TB vaccines for wildlife reservoirs (Hughes *et al*., 1996; Ayele *et al*., 2004) and use in situations where the test-and-slaughter policy is totally impracticable (WHO, 1994). Also, pasteurization of milk and milk products are components of bovine tuberculosis eradication programs (WHO, 1994; Ayele *et al*., 2004).According to Cross and Gertz (2006), vaccination could potentially control bovine tuberculosis. However, combining vaccination and culling of infected animals is a more attractive management option. But, vaccination of cattle against bovine tuberculosis or improvement in tuberculosis testing procedures will have no effect on wildlife in tuberculosis prevalence (Kao *et al*., 1997). The current TB vaccine, *M.*

*bovis*BCG provides little or no protection against pulmonary tuberculosis in cattle and man (Hogartha *et al*., 2005). Collins and Grange (1983) reported that ‘it is axiomatic that no control measures against transmissible diseases can be totally effective unless all reservoirs of the causative agent can be eliminated’.

CHAPTER THREE

MATERIALSAND METHODS

3.1 Study Area

Gusau abattoir is located in Gusau Local Government Area, capital of Zamfara State, and is one Kilometer Northwest of Gusau town. It is administered by the State Ministry of Animal Health and Livestock Development, and was established to provide slaughter and meat inspection services to local butcher shops in the town. It is the only source of inspected beef in town, and to the neighboring Local Government Areas like Bungudu, Maru and Tsafe. Gusau is located between latitude $11°53’^{N}$ and longitude $06°39’^{E}$, and occupies an area of $3{,}364km^2$ (Topographic-sheet, 1990). Based on the results of 2006 National Population Census, Gusau Local Government Area had a population of about 383,162 people (NPC, 2006).

Zamfara State comprises of fourteen (14) Local Government Areas and has an estimated population of 3,259,846 people according to 2006 Census figures from the National Population Commission(NPC, 2006; ZASIDEP, 2007). About 82% of the population in the State are engaged in Agriculture with most farming families also owning livestock (ZASIDEP, 2007). Zamfara State is bordered in the North by Niger Republic, to the East by Katsina State, to the West by Sokoto State, to the South by Kaduna, Niger and Kebbi States. It is located within latitudes $10°52’^{N}$ to $13°10’^{N}$ and longitudes $04°40’^{E}$ to $07°10’^{E}$ (ZASIDEP, 2007). The State has an estimated animal population of: 3,190,010 Cattle, 34,796 Camels, 5, 177,348 Goats and 4, 933,304 Sheep (MAHLD, 2011).

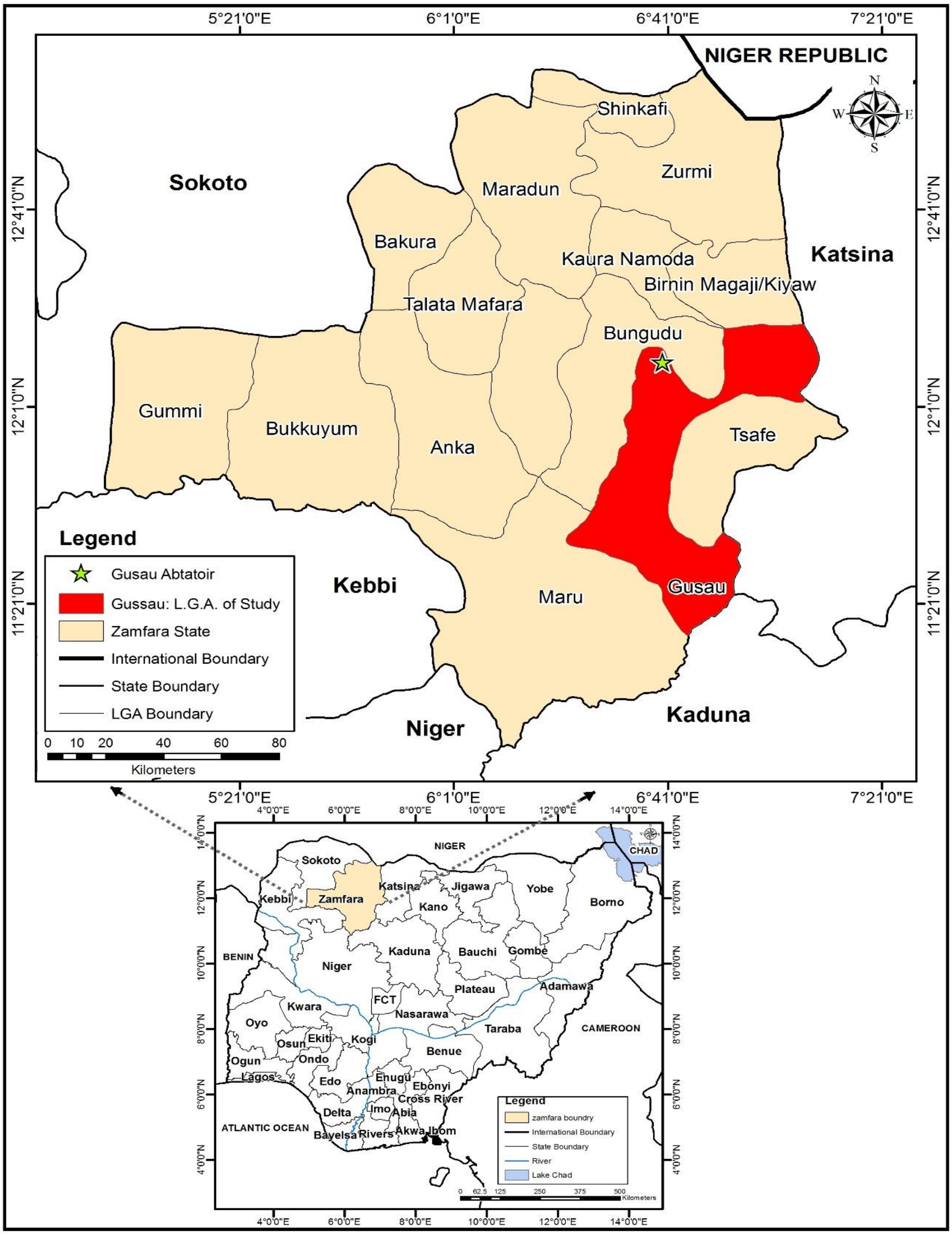

Figure 3.1: Map of Zamfara State showing Gusau L.G.A. and Gusau abattoir (Marked-star).

Source: Modified from the administrative map of Zamfara State.

3.1.1 Study design

A cross-sectional study was carried out in Gusau abattoir, Zamfara State Nigeria. All the cattle slaughtered daily between the months of April to June, 2014 were examined and that only those observed to have gross lesions typical of bovine tuberculosis were considered as cases. Tissue/organ sample each was collected from every animal identified as case during participatory meat inspection. Microscopic examination and RD deletion typing were used to determine the prevalence of bovine tuberculosis in the idenitified cases. Questionnaires were administered to assess risk factors due to exposure and transmission of bovine tuberculosisamong persons at the risk especially abattoir personnel and veterinarians, and also to determine public awareness and knowledge, attitude, and practices towards bovine TB.

3.4 Chemicals and Reagents

Ethanol, phenol, hydrochloric acid, methelyne blue powder, basic fuchsin, ethidium bromide, 1x TAE buffer and agarose powder were used in the study.

1- Primary stain (Ziehl's carbol-fuchsin solution) (0.3%):

Basic Fuchsin.............3g
95% alcohol................up to 100 ml.

A stock solution of 3% basic fuchsin was prepared by dissolving 3g basic fuchsin in 100ml ethanol. A stock solution of 5% phenol was prepared by adding 25ml molten phenol (put in a flask maintained at 60^0C in a water bath) to 475 ml distilled water. Carbol fuchsin (0.3%) solution was prepared by adding 10ml of 3% basic fuchsin solution to 90ml of 5% phenol solution (Selvakumar *et al*., 2005; IUATLD, 2000), and it was filtered before used.For ZN method, International Union Against Tuberculosis and Lung Disease (IUATLD) guidelines recommends the use of 0.3% Carbol fuchsin for staining of acid-fast bacilli (IUATLD, 2000). However, the filtered solution when put ina labelled amber bottle, it can be stored at room temperature for six to twelve months (Strong and Kubica, 1985; WHO, 1998a; Awah-Ndukum, 2012).

2-Decolourising agent (5% acid-alcohol):

Acid-alcohol solution

Alcohol 95%................950 ml

Concentrated (35%) hydrochloric acid......50 ml.

As described by Selvakumar *et al*. (2005) and IUATLD (2000) with modification (50 ml of concentrated hydrochloric acid was used instead of the prescribed 30 ml):50 ml of concentrated hydrochloric (technical grade) acid was carefullyadded to 950 ml of95% ethanol (technical grade) and stored in a labelled amber bottle.The preparation can be stored at room temperature for six to twelve months (Strong and Kubica, 1985; WHO, 1998a; Awah-Ndukum, 2012).

3. Counterstain(Methylene blue):

Methylene blue chloride (1g) was dissolved in distilled water (100ml) and stored in a labelled amber bottle (Selvakumar *et al*., 2005). The preparation can be stored at room temperature for six to twelve months (Strong and Kubica, 1985; WHO, 1998a; Awah-Ndukum, 2012).

4-Agarose Gel (3%):

Agarose powder weighing 3g was dissolved in 100ml of 1 X TAE buffer and boiled to melt the agarose, 5µl of ethidium bromide was added to the melted mixture after which it was poured onto the combs and allowed to solidify at room temperature (Warren *et al*., 2006).

3.3 Study Population

3.3.1 Study animals

A total of 3,690 cattle slaughtered in the abattoir between April and June, 2014 were examined for gross lesions of bovine tuberculosis; all were local zebu breeds.Cattle brought to the abattoir for slaughter and intended for human consumption were used for the study. As described by ZASIDEP (2007), in Zamfara State animals are managed under traditional husbandry system and depend entirely on natural pastures without extra-feeds supplements or proper veterinary care. The major sources of cattle to this abattoir were various markets that included Gusau, Kasuwar Daji, Talata Mafara, Shinkafi and Bungudu that were located in major towns in the various Local Government Areas within the State. Furthermore, trade cattle on transit to other parts of the country could be sold and brought to the abattoir.

3.3.1.1 Inclusion criteria

All cattle slaughtered at the abattoir at the time of sample collection were included.

3.3.1.2 Exclusion criteria

All animals slaughtered at the abattoir other than cattle at period of the study were excluded.

3.3.2 Human population

3.3.2.1 Inclusion criteria

All abattoir cleaners, animal health scientists, butchers, cattle handlers and traders, meat sellers and veterinarians present at the abattoir at the time of the study were included.

3.3.2.2 Exclusion criteria

All individuals at the abattoir at the time of the study who are not abattoir cleaners, animal health scientists, butchers, cattle handlers and traders, meat sellers and veterinarians were excluded.

3.4Sampling

3.4.1 Sample size determination

3.4.1.1 Animal sample size for postmortem examination

The number of cattle required to be sampled in this study to detect at least one infected cattle with 95% confidence interval, and a desired absolute precision of ≥5% was calculated using a previously described formula by Thrusfield (2007).

Thus: $$n = \frac{z^2 \text{ x} Pexp \text{ x } q}{d^2}$$

Where:

n = sample size

q = 1-*Pexp*

Pexp = expected prevalence

d = desired absolute precision = 0.05 (95% confidence interval)

Z = appropriate value for the standard normal deviation for the desired confidence = 1.96

Using abattoir based bovine TB prevalence of 12.27% reported by Aliyu*et al*. (2009) in Gombe State, Nigeria. Hence: sample size n

$$n = \frac{1.96^2 \text{ x } 0.12\ (1-0.12)}{0.05^2} = 165$$

The estimated size (n= 165) was increased for a better precision and was applied in a purposive sampling of only those cattle with visible bovine TB lesions.

3.4.1.2 Human sample size for risk factors assessment

$$n = \frac{z^2 x P_{exp} \times q}{d^2}$$

Where:

n= Minimum sample size

q = 1-*Pexp*

Pexp = proportion rate 28.1% (0.281) of individuals with knowledge of bTB reported by Hambolu *et al*.(2013) in an abattoir-based study, Lagos Nigeria.

d= desired absolute precision 8% (0.08)

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

$$n = \frac{1.96^2 \times 0.281\ (1-0.281)}{0.08^2} = 121$$

The estimated size (n = 121) was increased to 150 for a better precision and employed a purposive assessment on only those designated target respondents.

3.4.2 Sampling procedure and sample collection

Cattle carcasses were examinedduring participatory meat inspection for gross lesions typical of bovine tuberculosis.Idenitification of cases was purposive and based on the presence of lesions compatible with bovine tuberculosis. Examination of the carcasses was performed according to the methods of Corner (1994) and Aylate *et al*. (2013); thus, organs/tissues including the lungs, liver, kidneys, mammary tissue, intestines, heart, and lymph nodes of abdominal and thoracic cavities were examined both visually and by palpation, and also by incision where necessary.For each animal identified as a case, sample collected from it was labeled with the number that represented the carcass age, sex and cattle breed. Breeds were identified based on the descriptions by Blench (1999), whileage was determined by examining teeth eruption, development and wear according to the description of Whiting *et al*. (2013); however, cattle sampled were classified into two groups: ‘young’ if examined to be less than 6 years and ‘old’ if estimated to be more than 6years, following the procedure of Best (2014).

A total of 226 organ and tissue bearing lesionsfrom 226 carcasses (cases) were collectedinto sterile screw-capped universal containers (labeled) containing 5ml of0.9% normal saline solution, and then transported in an ice box to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine Ahmadu Bello University, Zaria, and stored in a freezer until processed. Stored samples were processed for Ziehl-Neelsen (ZN) staining technique and microscopy. Those positive for ZN stain were transported on ice to DNA-LABS Kaduna, Nigeria for RD deletion typing.However, samples from heart, kidneys, lymph nodes, liver, lungs, mammary glands, pleural sac, small intestines and spleen containing compatible bovine TB lesions were collected for laboratory diagnoses.

3.5Questionnaire Survey

Close-ended structured questionnaires were prepared to assess the risk factors for the exposure and transmission of bovine TB infection. They were administered in order to collate information on a range of variables relating to knowledge and awareness, attitude and practice towards zoonotic TB. The cattle professionals(butchers, cattle handlers, cattle traders and meat sellers) and abattoir cleaners were actively assessed through face-to-face interviews; while veterinarians and animal health scientists were served with the questionnaires and the outcome of their judgments were later retrieved.

The choice of respondents was based on compliance during the interview.The questionnaire survey covered the period of May, 2014 and June, 2014.

3.6Sample Processing

In the laboratory, frozen samples were thawed overnight at room temperature before processing (Awah-Ndukum, 2012). Fat and excess connective tissues were removed from the specimens, and individual tissues were macerated in sterile Petri dishes to obtain fine pieces and then crushed using pestle and mortar followed by rinsing with 25ml of 0.9% normal saline solution and homogenized for 10 min. The homogenate was decontaminated with an equal volume of 4% NaOH for 15 min, concentrated by centrifugation and neutralized with 1% (0.1 N) hydrochloric acid (HCl) using phenol red as an indicator as described by WHO (2002) and Aylate *et al*. (2013). The solution was said to be neutralized when the color changed from purple to yellow (Biru*et al.*, 2014). Supernatant was discarded; the sediment was reconstituted to 5 ml using sterile distilled water, and the resulting suspension from each whole-processed sample was used to prepare smears for microscopic examination.

3.7 Microscopic Demonstration of Acid-Fast Bacilli

Loop-full(s) of decontaminated homogenatewas gently smeared on a clean, grease-free microscopic slide. The smeared slide was air-dried, heat-fixed and then flooded with carbol fuchsin, steamed without boiling with the Bunsen burner flame from the underside of the slide for 5min. It was then rinsed gently with water until all the free stain was washed away. The slide was again flooded with 5% acid-alcohol decolorizing solution for 10 min, and then rinsed again with water and excess water was drained. The slide was also flooded with methylene blue counter stain for 1 min. It was then rinsed thoroughly with water, after which excess water was drained from the slide and the smear was then allowed to air dry. Each smeared-slide was systematically examined under a light microscope (100x oil immersion objective) for the presence of acid-fast bacilli. The presence of acid-fast bacilli (AFB) that retain the primary stain in 100x immersion fields was recorded positive, smears in which no acid-fast bacilli were seen were considered negative. Microscopic examination was performed following the procedure described by Awah-Ndukum (2012) with modifications. The differences to the above stated procedure were: concentration of an acid-alcohol was raised to 5% instead of 3% and decolourization time was extended from 5 to 10 min.

3.8 Genomic Typing

3.8.1Mycobacterial DNA extraction

All procedures involved in genomic analysis were conducted at DNA LABS, Kaduna Nigeria. The decontaminated suspensions from samples positive for AFB (n= 37) were transferred into sterile 1.5ml micro-centrifuge tubes. In each tube, the contents were dispersed in a mixture of 400µl lysis buffer and 25µl proteinase K already contained there-in and then vortexed; genomic DNA extraction from the lysate was performed by a series of alcohol precipitations protocol described by Janice *et al*. (1997). The tube was then incubated at 65^{0}C for 1 hour, during which it was vortexed at 15 min intervals throughout incubation. Furthermore, 400µl of phenol-chloroform was added to the lysate, vortexed for 15s and then centrifuged at 13,000 x g for 10 min.The supernatant (upper layer) was transferred into a new 1.5ml microcentrifuge tube, unto which 400µl of chloroform was added. It was then vortexed and centrifuged at 13,000 x g for 5min.The supernatant was transferred into a new 1.5ml micro-centrifuge tube. One thousand microliter of 100% ethanol was added, then followed by 40µl of 3M sodium acetate and the tube was inverted, stored at -20^{0}C overnight. It was later centrifuged at

12,000 x g for 10 min. The supernatant was discarded and 400μl of 70% ethanol was added and then further centrifuged at 12,000 x g for 5 min. The supernatant was discarded and the remnant in the tube was allowed to cool. Following alcohol precipitations, the purified DNA was suspended in 50μl water.

3.8.2 Primers used

Primer sequences used are listed on Table 3.1, against the expected sizes ofthe amplicons to confirm the presence or absence of each of the region of difference (Warren *et al*., 2006); they were obtained from Bioneer(Inc. USA).

3.8.3 PCR reaction set-up

Each PCR mixture contained 1μl of DNA template, a final concentration of HotStat PCR Premix (1 unit Taq DNA polymerase, 10mM Tris-HCl (pH 9.0), 30mM KCl, 250μM of each dNTPs, 1xPCR buffer and 1.5mM $MgCl_2$), 0.5μl of each of the sequence from the 3-pair primers (summed up to 6μl) and 13μl of sterile distilled water all added to a final reaction volume of 20μl.

3.8.4 RD deletion typing/amplification

The genomic regions of difference targeted were well-characterized (Brosch *et al*., 2002; Parsons *et al*., 2002; Huard *et al*., 2003), and species of *Mycobacteria*were analyzed for the presence or absence of RD1, RD4, RD9 and RD12 using a previously described method of Warren *et al*. (2006). The multiplex PCR was performed using PTC-100^{TM} Programmable Thermal Controller (MJ Research, Inc. USA) at the following set-up: initial denaturation step of 95^0C for 15 min, 45 cycles of denaturation at 94^0C for 1 min, annealing at 62^0C for 1min and extension at 72^0C for 1min; and followed by the final extension step at 72^0C for 10 min (Warren *et al*., 2006). Positive control with DNA template of reference-typed strain (*M. bovis* BCG Pasteur) and negative control mixture (Master mix) without DNA template were included in each amplification run.

3.8.5Electrophoresis

Amplified products (6µl each) and 10µl of DNA-Marker were electrophoresed on 3% agarose gels (Green Bio Research®) stained with 5µl ethidium bromide (Invitrogen®), in 1 x TAE running buffer at 120V for 4 hours, using Horizontal Gel Electrophoresis Apparatus (GIBCOBRL Model H5-Gaithersburg, USA).

3.8.6 Interpretation of typing results

As described by Warren *et al*. (2006), the gel was visualized using UV transilluminator (Gel Doc 2000, BIO RAD) and photographed.The PCR-products were analyzed by comparing sizes between: M: DNA-Marker (100bp Plus DNA ladder) (Bioneer, Inc. USA), Lane 1: Positive control, Lane 2: Negative control, Lanes 3-onwards: samples-PCR products. Presence of bands pattern equivalent to 146bp (for RD1), 268bp (for RD4), 108bp (for RD9) and 306bp (for RD12) were considered positive for *M. bovis*, and bands pattern at 146bp, 172bp, 108bp and 306bp were labeled for *M. caprae*. While bands pattern at 146bp (RD1), 172bp (RD4), 235bp (RD9) and 369bp (RD12) were labeled for *M. tuberculosis*.

3.9 Data Analysis

$$\text{Prevalence} = \frac{\text{Number of samples positive}}{\text{Total samples analyzed}} \times 100\ (\%)$$

Descriptive statistical tables and bar chart were constructed and data was sorted using Microsoft Excel® 2010. The data obtained were also analysed using statistical package for social science (SPSS) version 20.0. Chi-square (χ^2) test was used to determine the possible association of age, sex, and breed with bovine TB infection. The odds ratio

(OR) was calculated to assess the strength of association between variables and bovine TB infection. *p* values of $\leq$ 0.05 were considered significant; and 95% confidence interval (CI) for odds ratio of 0 (indicates no association), <1 (indicates weak association) and >1 was recorded as strong statistically significant association.

For questionnaire survey, the data collated that comprised of the responses for each main area of the questionnaires were classified, frequencies were estimated and data were used to explain variables (risk factors). The data was sorted using Microsoft Excel® 2010 and then analyzed using SPSS 20.0 version. The degree of association between variables was compared by the Chi-square test of significance on the different classes of data. *p* values of $\leq$0.05 were considered significant.

Table 3.1 PCR Primer Sequences and Corresponding Amplification Product Sizes Indicating the Presence or Absence of Genomic Regions of Difference.

Primer Sequence	RD	*M. bovis*	*M. tuberculosis*	*M caprae*
AAGCGGTTGCCGCCGACCGACC	1	RD1 present	RD1 present	RD1 present
CTGGCTATATTCCTGGGCCCGG	1	(146bp)	(146bp)	(146bp)
GAGGCGATCTGGCGGTTTGGGG	1			
ATGTGCGAGCTGAGCGATG	4	RD4 absent	RD4 present	RD4 present
TGTACTATGCTGACCCATGCG	4	(268bp)	(172bp)	(172bp)
AAAGGAGCACCATCGTCCAC	4			
CAAGTTGCCGTTTCGAGCC	9	RD9 absent	RD9 present	RD9 absent
CAATGTTTGTTGCGCTGC	9	(108bp)	(235bp)	(108bp)
GCTACCCTCGACCAAGTGTT	9			
GGGAGCCCAGCATTTACCTC	12	RD12 absent	RD12 present	RD12 absent
GTGTTGCGGGAATTACTCGC	12	(306bp)	(369bp)	(306bp)
AGCAGGAGCGGTTGGATATTC	12			

PCR = polymerase chain reaction; RD = region of difference (Warren *et al*., 2006)

CHAPTER FOUR

RESULTS

4.1 Prevalence of Bovine Tuberculosis

4.1.1 Prevalence of bovine tuberculosis by postmortem findings

Gross lesions typical of bovine tuberculosis (Plates I and II) were detected in 226(6.12%) of the cattle slaughtered during the study period. Most of the encountered organs/tissues with lesions were enlarged, with number of yellowish granulomas or tubercles, some with cheese-like foci on cut-surface (Plates I and II). The tuberculous-like lesions were observed in eleven different tissues/organs from carcasses (Table 4.1). These included lesions in 90 (2.4%) lungs, 76 (2.1%) livers, 16 (0.4%) tracheo-bronchial lymphnodes, 13 (0.4%) mesenteric lymphnodes, 9 (0.2%) prescapular lymphnodes, 8 (0.2%) hearts and 4 (0.1%) spleen samples. Suspected tuberculous lesions were also observed in 3 (0.1%) each of kidneys, mammary glands, pleura, and 1 (0.02%) small intestines. The distribution and occurrence of tuberculous like lesions in different anatomical sites is presented on Table 4.1. Yellowish granulomatous lesions and abscesses (containing yellowish pus) were observed most in 90 (2.4%) lungs compared with the least recorded in 1 (0.02%)small intestines (Table 4.1).

4.1.2 Prevalence of bovine tuberculosis by ziehl-neelsen staining technique

Thirty seven (16.4%) of the 226 suspected tuberculous tissues collected tested positive with Ziehl-Neelsen stain (Table 4.1), which revealed that 15 (6.6%) of the lungs tissues, 3 (1.3%) of the livers, 7 (3.1%) of the tracheo-bronchial lymphnodes, 3 (1.3%) of the mesenteric lymphnodes, 2 (0.9%) of the prescapular lymphnodes, 2 (0.9%) of the heart tissues, all of the 4 (1.8%) spleens and 1 (0.4%) of the pleural sac were positive forAFB

(Plate III) (Table 4.1). None of the kidneys, mammary glands and small intestines was positive for AFB (Plate IV) (Table 4.1).

4.1.3 Prevalence of bovine tuberculosis by RD deletion typing

Out of the 37 AFB tested positive samples, 34 (91.9%) were positive for RD deletion typing (Plates V, VI and VII). Furthermore, of the 34 samples positive for RDdeletion typing and based on the presence of characteristics bands sizes, 30 (88.2%) were identified as *M. bovis*, while 3 (8.8%) were identified as *M. caprae* and 1 (2.9%) was identified as *M. tuberculosis*. The distribution of positive RD deletion typing among different tissues/organs sampled is shown on Table 4.1.

Table 4.1: Distribution and Occurrence of Suspected Bovine Tuberculosis Lesions, AFB and RD Deletion Typing Positives amongst Different Tissues/Organs Sampled from Slaughtered Cattle in Gusau Abattoir, Zamfara State Nigeria.

Tissues/Organs	Postmortem Examination (%)	ZN Positive (%)	RD Deletion Typing Positive (%)
Lungs	90(2.4)	15(6.6)	14 (37.8)
Liver	76 (2.1)	3(1.3)	2 (5.4)
Tracheo-bronchial lymphnodes	16 (0.4)	7(3.1)	7 (18.9)
Mesenteric lymph nodes	13 (0.4)	3(1.3)	3 (8.1)
Prescapular lymph nodes	9 (0.2)	2(0.9)	2 (5.4)
Heart	8 (0.2)	2(0.9)	2 (5.4)
Spleen	4 (0.1)	4(1.8)	4 (10.8)
Kidneys	3 (0.1)	0(0.0)	0 (0.0)
Mammary glands	3 (0.1)	0(0.0)	0 (0.0)
Pleural sac	3 (0.1)	1(0.4)	0 (0.0)
Small Intestines	1 (0.02)	0(0.0)	0 (0.0)
Total	226 (6.1%)	37 (16.4%)	34 (91.9%)

ZN: Ziehl-Neelsen staining and microscopy; RD: regions-of-difference

4.1.3.1 Prevalence of bovine tuberculosis by age and sex using postmortem findings

Among the 226 cattle with the suspected TB like lesions, 77 (34.7%) were young while the remaining 149 (65.9%) were old (Table 4.2). Similarly, of the 226 cattle with the suspected TB like lesions, 79 (3.9%) were males while the remaining 147 (65.0%) were females (Table 4.2).

Table 4.2: Prevalence of Bovine Tuberculosis by Age and Sex using Postmortem Findings

Total no. of animals examined	Total no. of animals positive for TB lesions	Variable	No. positive for TB	Prevalence (%)	OR (95 % C.I.)	χ^2	*p*-value
3690	226	Age					
		Young[a]	77	34.1		4.723	0.000
		Old[b]	149	65.9*	2.614 (1.223 – 3.831)		
		Sex					
		Male	79	35.0		4.457	0.000
		Female	147	65.0*	2.164 (1.223 – 3.831)		

*Reference value; Young[a] = <6years; Old[b] = >6years; C.I: confidence interval; OR: odds ratio

OR (0): no association, OR (<1): weak association, OR (>1): strong association.

4.1.3.2 Prevalence of bovine tuberculosis by age and sex using Ziehl-Neelsen staining technique.

Of the 77 young cattle with suspected tuberculous lesions only 5 (13.5%) were tested AFB positive, and 32 (86.5%) of the tissues/organs from old cattle were positive for AFB (Table 4.3). Furthermore, 8 (21.6%) of the 79 male cattle suspected tissue samples were positive for AFB while 29 (78.4%) of the tissues/organs from the female cattle were positive for AFB (Table 4.3).

Table 4.3: Prevalence of Bovine Tuberculosis by Age and Sex using Ziehl-Neelsen Staining Technique

Total no. of animals positive for TB lesions	Variable	No. of cattle sampled	ZN positive	Prevalence (%)	OR (95 % C.I.)	χ^2	*p*-value
226	Age						
	Young[a]	77	5	13.5		8.324	0.004
	Old[b]	149	32	86.5*	0.254 (0.095-0.681)		
	Sex						
	Male	79	8	21.6		3.460	0.063
	Female	147	29	78.4*	0.458(0.199–1.058)		

*Reference value; Young[a] = <6years; Old[b] = >6years; C.I: confidence interval; OR: odds ratio; ZN: Ziehl-Neelsen staining and microscopy

OR (0): no association, OR (<1): weak association, OR (>1): strong association.

4.1.3.3 Prevalence of bovine tuberculosis by age and sex using RD deletion typing

For RD deletion typing, the prevalence for sex specific rates were 7 (20.5%) for males and 27 (79.4%) for female cattle (Table 4.4), while between age groups the figures were 5 (14.7%) for young and 29 (85.2%) for old cattle (Table 4.4).

Table 4.4: Prevalence of Bovine Tuberculosis by Age and Sex using RD Deletion Typing

Total no. of animals positive for AFB	Variable	No. of cattle positive for ZN	RDT positive	RDT Prevalence (%)	OR (95 % C.I.)	χ^2	*p*-value
37	Age						
	Young[a]	5	5	14.7		6.680	0.010
	Old[b]	32	29	85.3*	0.287 (0.106-0.776)		
	Sex						
	Male	8	7	20.6		3.630	0.057
	Female	29	27	79.4*	0.432(0.179–1.043)		

*Reference value; Young[a] = <6years; Old[b] = >6years; C.I: confidence interval; OR: odds ratio; ZN: Ziehl-Neelsen staining and microscopy; RDT: region-of-difference deletion typing; AFB: Acid-fast bacilli

OR (0): no association, OR (<1): weak association, OR (>1): strong association.

4.2 Comparison between Techniques (Kappa Test)

Results from the two techniques (Ziehl-Neelsen Staining and RD deletion typing) used show varied prevalence rates of 16.4% and 91.9%. The overlapping results are presented on Table 4.8, and it shows that there was an agreement (p =0.000 and k =0.950) between the diagnostic techniques used to detect and identify*Mycobacteria*. There were Specificity (Sp) of 100% and Sensitivity (Se) of 98.4% for RD deletion analysis, while Specificity (Sp) of 91.9% and Sensitivity (Se) of 100% were also recorded for Ziehl-Neelsen staining technique respectively. The agreement between the two methods was very good (k>0.8), indicating a much high specificity. Ziehl-Neelsen staining technique detected the presence of *Mycobacteria* in 37 tuberculous samples, out of the 226 carcasses sampled. The estimated probability of missing *Mycobacteria* present in AFB tested positive samples using RDdeletion typing is 0% (0/34) (Table 4.5).

Table 4.5: Comparison between Ziehl-Neelsen Staining Technique (ZN) and RD Deletion Typing (Kappa test)

ZN		RD Deletion Typing		
		Positive	Negative	Total
	Positive	34	3	37
	Negative	0	189	189
	Total	34	192	226

ZN: Ziehl-Neelsen staining and microscopy; RD: region-of-difference

Kappa value (*k*) = 0.950; *p* = 0.000

k: Kappa signifies degree of agreement between the different methods used to detect bovine tuberculosis; accordingly, 0: means poor agreement, □ 0.5: moderate agreement, □ 0.7: good agreement, □ 0.8: strong or very good agreement.

4.3 Prevalence of Bovine Tuberculosis by Breeds of Cattle

Out of the 226 cattle observed to have bovine TB gross lesions, 129 (57.1%) were identified as White Fulani breed, while 78 (34.5%) were Sokoto Gudali and 19 (8.4%) were Red Bororo. While the prevalence values recorded using Ziehl-Neelsen staining and microscopy (ZN) and RD deletion typing were: 25 (67.6%) and 22 (64.7%) for White Fulani, 8 (21.6%) and 8 (23.5%) for Sokoto Gudali and 4 (8.4%) and 4 (11.8%) for Red Bororo respectively (Table 4.6).

Table 4.6: Prevalence of Bovine Tuberculosis by Breeds of Cattle Sampled at Gusau Abattoir, Zamfara State Nigeria.

Variable	Frequency			Prevalence (%)		
	PM	ZN	RDT	PM	ZN	RDT
Breed						
White Fulani	129	25	22	57.1	67.6	64.7
Sokoto Gudali	78	8	8	34.5	21.6	23.5
Red Bororo	19	4	4	8.4	10.8	11.8
Total	226	37	34	100.0	100.0	100.0

PM: post-mortem examination; ZN: Ziehl-Neelsen staining and microscopy; RDT: region-of-difference deletion typing

4.4 Questionnaire Survey and Responses

In this survey, 101 (67.3%) of 150 questionnaires administered were filled and returned for analysis.Assessment was based on biodata (demographic features), awareness and knowledge(about zoonotic potential of bovine TB and symptoms of TB in humans), attitudes, practices and other risk factors.The significant variables resulting from the Chi-square (χ^2) analysis were: Sex of the respondents (being a male) (χ^2=9.75; p =0.000), educational background (χ^2=22.7; p =0.000), contact of humans with cattle for 1-3yrs (χ^2 = 7.2; p =0.027), length of exposure to carcasses for 1-3yrs (χ^2 =8.0; p= 0.018), knowledge on the transmission of zoonotic TB (χ^2 = 8.0; p = 0.000 and χ^2 = 69.6; p = 0.000) and (χ^2 = 47.1; p = 0.000), poor knowledge on TB screening (χ^2=12.0;p =0.000 and χ^2=78.0; p =0.000),milk consumption (χ^2 = 15.6; p= 0.000) and consumption of meat (χ^2=65.44; p =0.000). All these variables showed a higher risk potential for exposure and transmission of zoonotic TB among persons working in the abattoir, and all of which were statistically significant (Table 4.7-4.13).

4.4.1 Demographic features

The survey assessed 101 respondents that comprised of 100 (99.0%) males and 1 (1.0%) female. Amongst occupations, 34 (33.7%) were butchers whomade up of most of the respondents compared to the least proportions recorded on animal health scientists 3 (3.0%) and veterinarians 3 (3.0%).The responses collated showed that 38 (37.6%) of the respondents were between ages of 26 to 30 years, and 35 (34.7%) of them had none-formal educational background (Table 4.7).

Table 4.7: Demographic Information of Respondents in Gusau Abattoir Zamfara State

Variable		No. of responses (%)	Chi-square value	*p*-value
Occupation	Veterinarian	3 (3.0)	83.8	0.000
	Animal health scientist	3 (3.0)		
	Cleaner	5 (5.0)		
	Butcher	34(33.7)*		
	Cattle handler	21 (20.8)		
	Cattle trader	25 (24.8)		
	Meat seller	8 (7.9)		
	Others	2 (2.0)		
	Total	101 (100)		
Age	16-20	10 (9.9)	50.0	0.000
	21-25	21 (20.8)		
	26-30	38 (37.6)*		
	31-35	15 (14.9)		
	36-40	9 (8.9)		
	46 and above	2 (2.0)		
	Missing	6(5.9)		
	Total	101 (100.0)		
Sex	Male	100 (99.0)*	9.75	0.0000
	Female	1(1.0)		
	Total	101 (100.0)		
Educational Background	Primary school	5 (5.0)	22.7	0.000
	Secondary school	28 (27.7)		
	Tertiary school	33 (32.7)		
	None	35 (34.7)*		
	Total	101 (100)		

*Reference value

4.4.2 Risk factors of bovine TB transmission

Evidence of repeated contact between respondents and cattle, as well as years of involvement with cattle carcasses formed the most responses. As found significant, 41 (40.8%) of the respondents have had contact with cattle for 1-3 years (*p* =0.027) and then followed by 47 (46.5%) who had contact with carcasses for1-3 years (*p*= 0.018) (Table 4.8), while 78 (77.2%) of the respondents wear protective clothing while on operations.Although, 25 (24.8%) of the respondents can accurately recognised tissues with typical bTB lesions (*p* =0.000) (Table 4.8), 42 (41.6%) described it to be when there is presence of either abscesses or nodules on/in the lung tissues (*p* =0.000) (Table 4.9). 22 (21.8%) and46 (45.5%) of the respondents reside at crowdy settings (high and medium density areas) (*p* =0.010) and 20 (19.8%) out of all the respondents live more than 3 persons per room (*p* = 0.000) (Table 4.9).

Table 4.8: Risk Factors Assessment

Variables		No. of Responses (%)	Chi-Square	*p*-value
How long have you been in contact with cattle?	< 1 year	21(20.9)	7.2	0.027
	1-3 years	41(40.8)*		
	> 3 years	39 (38.6)		
	Total	101 (100)		
How long have you been exposed to carcasses in the abattoir?				
	< 1 year	26 (25.7)	8.0	0.018
	1-3 years	47 (46.5)*		
	> 3 years	28 (27.7)		
	Total	101(100.0)		
Do you use protective clothing while working in the abattoir?	Yes	78(77.2)*	7.1	0.000
	No	10 (9.9)		
	Missing	13 (12.9)		
	Total	101 (100.0)		
What are the common lesions observed in the lungs of the carcasses you worked on?	Hardened lung tissues with odour	32 (31.7)*	29.9	0.000
	Discoloured lungs but soft	23(22.8)		
	Odourless tissues but with nodules	25(24.8)		
	Hardened and odourless lungs with reduced size	17(16.8)		
	All of the mentioned	4(4.0)		
	Total	101(100.0)		

*Reference value

Table 4.9: Risk Factors assessment

Variable		No. of Responses (%)	Chi-Square	*p*-value
How do you recognise lung tissues with bovine tuberculosis?	Presence of nodules in/on the lung tissues	42(41.6)*	52.9	0.000
	Presence of multiple abscesses in/on the lungs	6(5.9)		
	presence of both abscess and nodules	10(9.9)		
	Discoloured but normal lungs	15(14.9)		
	Hardened and reduced size lungs	10(9.9)		
	All of the mentioned	14(13.9)		
	Missing	4(4.0)		
	Total	101(100.0		
What area of the city do you live?	High density area	22 (21.8)	9.1	0.010
	Medium density area	46 (45.5)*		
	Low density area	30 (29.7)		
	Missing	3 (3.0		
	Total	101		
How many of you on the average live together in a room?	1 person	10 (9.9)	63.6	0.000
	2-3 persons	71 (70.3)*		
	> 3 persons	20(19.8)		
	Total	101(100.0)		

*Reference value

4.4.2.1 Awareness and knowledge on bovine tuberculosis

Moreover, 89 (88.1%) of the respondents claimed to have known that humans can contract diseases from cattle, but only 58 (57.4%) have had the knowledge that bovine TB can be acquired from cattle. However, 69 (68.3%) were also aware of the possible zoonotic transmission of bovine TB through either direct contact with the infected cattle, 17 (16.8%) thought it to be byconsumption of raw/half-cooked meat andor 10 (9.9%) who thought it to be through both, comparing these responses with the 2 (2.0%) of the respondents who believed in none, and thus revealed that on almost all perceptions the four groups differed with the least number exhibiting poor knowledge of whether bTB could be transmitted from cattle to man (p =0.000), and also different modes of transmission (p = 0.000) (Table 4.10). Inaddition, 93 (92.1%) of the respondents have known persons with tuberculosis and they recognised its manifestations when there were cough by 77 (76.2%) and sweat at night by 8 (7.9%). 55 (54.5%) observed any of the obvious symptoms amongst member(s) of their households. But 74 (73.3%) of the respondents believed that TB can be cured and 90 (89.1%) thought it can be treated using modern medicine (Table 4.11).

Table 4.10: Awareness and Knowledge about Zoonotic Tuberculosis

Variable		No. of Responses (%)	Chi-Square	*p*-value
Are you aware that people can contract diseases from cattle?	Yes	89 (88.1)*	8.0	0.000
	No	9 (8.9)		
	Missing	3 (3.0)		
	Total	101 (100.0)		
What type of disease can be contracted?	Tuberculosis	58 (57.4)*	69.6	0.000
	Brucellosis	21 (20.8)		
	Helminthosis	12 (11.9)		
	Others	5 (5.0)		
	Missing	5 (5.0)		
	Total	101		
How do you think man can be infected with TB?	Direct contact with infected cattle	69 (68.3)*	47.1	0.000
	Consumption of raw/half-cooked meat	17 (16.8)		
	All of the above	10 (9.9)		
	None	2 (2.0		
	Missing	3 (3.0)		
	Total	101 (100.0)		

*Reference value

Table 4.11: Awareness and Knowledge about Zoonotic Tuberculosis

Variable		No. Responses (%)	Chi-Square	*p*-value
Do you know of anybody with TB now or before now?	Yes	93 (92.1)*	5.0	0.000
	No	8 (7.9)		
	Total	101 (100.0)		
What are the signs/symptoms of TB you observed in the person you know with TB?	Cough	77 (76.2)*	21.0	0.000
	Wasting	5 (5.0)		
	sweat in the night	8 (7.9)		
	body weakness	4 (4.0)		
	All of the mentioned	4 (4.0)		
	Missing	3 (3.0)		
	Total	101 (100.0)		
Have you observed any member of your household with these symptoms in the past three months?	Yes	55 (54.5)	5.0	0.426
	No	46 (45.5)		
	Total	101 (100.0)		
Do you think TB can be cured?	Yes	74 (73.3)*	81.4	0.000
	No	19 918.9)		
	Do not know	5 (5.0)		
	Missing	3 (3.0)		
	Total	101 (100.0)		
What type of treatment do you think is best for TB?	Modern medicine	90 (89.1)	5.0	0.000
	Traditional medicine	8 (7.9)		
	Missing	3 (3.0)		
	Total	101 (100.0)		

*Reference value

4.4.2.2 Attitudes towards zoonotic TB

The overwhelming 98 (97.0%) of the respondents believed thatit is important to protect oneself while handling diseased cattle/carcass. While 77 (76.2%) of the respondents have had good vaccination history, and so 80(79.2%) have had their children vaccinated against TB (Table 4. 12).

Table 4.12: Attitudes of Respondents towards Tuberculosis

Variable		No. of Responses (%)	Chi-Square	*p*-value
Do you think that it is important to protect yourself while handling diseased cattle/carcass?	Yes	98 (97.0)*	9.4	0.00
	No	3 (3.0)		
	Total	101 (100.0)		
Have you been given BCG vaccination before?	Yes	77 (76.2)*	5.9	0.000
	No	23 (22.8)		
	Donot know	1 (1.0)		
	Total	101 (100.0)		
Do you give your children BCG vaccination?	Yes	80 (79.2)*	8.2	0.000
	No	20 (19.8)		
	Missing	1 (1.0)		
	Total	101 (100.0)		

*Reference value

4.4.2.3 Practices with regard to risk onbovine tuberculosis

Vaccination had beenchosen by 74 (73.3%)of the respondents to be the most means of protection against infection with zoonotic TB, compared to 23 (22.8%) who only believed in good hygienic practices and 2 (2.0%) who opted for prayers only as a means of prophylaxis.However, because 70 (69.3%) respondents did not fall sick and 22 (21.8%)thought they cannot have TB, 86 (85.1%) of them have not been screened for TB before and hence their TB status is unknown.42 (41.6%) have admitted the consumption of boiled milk, 41 (40.6%) consumed soured milk and 14 (13.9%) consumed fresh un-boiled milk.Meanwhile, the proportion of meat consumption varied according to intake rates amongst respondents, thus on daily basis was 42 (41.6%), while 48 (47.5%) persons only consumed meat weekly and 8 (7.9%) consumed it once a month. More so, 36 (35.6%) of the respondents will treat their cattle when observed to have signs of bovine tuberculosis, while 25 (24.8%) will kill and burry the affected animal and 18 (17.8%) will sell it out; but 4 (4.0%) will eat it and give some to their neighbors (Table 4.13).

Table 4.13: Practices of Respondents towards Tuberculosis

Variable		No. of Responses (%)	Chi-Square	*p*-value
What do you do to protect yourself from diseased cattle/carcass?	immunization/vaccinatio n	74 (73.3)*	210.7	0.000
	Good hygienic practices	23 (22.8)		
	Prayer	2 (2.0)		
	Missing	2 (2.0)		
	Total	101 (100.0)		
Have you ever been tested for TB?	Yes	12 (11.9)	12.0	0.000
	No	86 (85.1)*		
	Missing	3 (3.0)		
	Total	101 (100.0)		
If no, why did you not go for test?	I am not sick	70 (69.3)*	78.0	0.000
	I cannot have TB	22 (21.8)		
	I dont know there is test for it	2 (2.0)		
	Missing	7 (6.9)		
	System	101 (100.0)		
What type of milk do you take?	Fresh un-boiled milk	14 (13.9)	15.6	0.000
	Boiled milk	42 (41.6)*		
	Soured milk	41 (40.6)		
	Missing	4 (4.0)		
	Total	101 (100.0)		
What would you do if you see your cattle having bTB signs?	I will sell it	18 (17.8)	26.0	0.000
	I will kill and bury it	25 (24.8)		
	I will eat and give some to neighbours	4 (4.0)		
	Medication	36 (35.6)*		
	Missing	18 (17.8)		
	Total	101 (100.0)		
How often do you consume meat?	Daily	42 (41.6)	65.44	0.000
	Weekly	48 (47.5)*		
	Once a month	8 (7.9)		
	Not at all	2 (2.0)		
	No response	1(1.0)		
	Total	101 (100)		

*Reference value

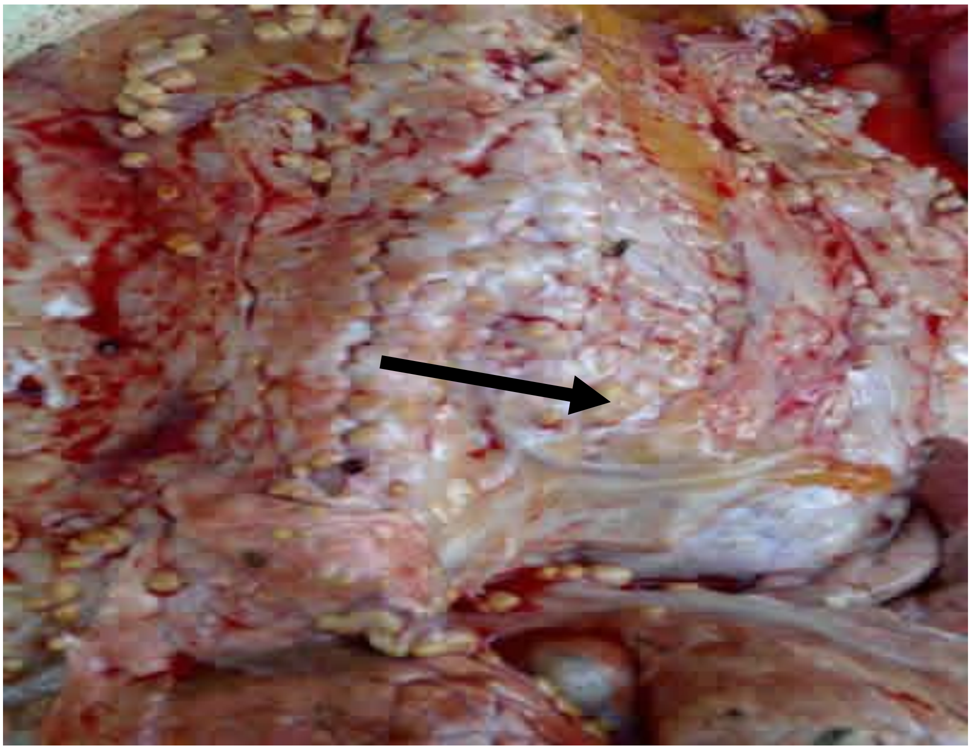

Plate I**:** Showing enlarged tuberculous lung with multiple granulomas on its surface (arrow).

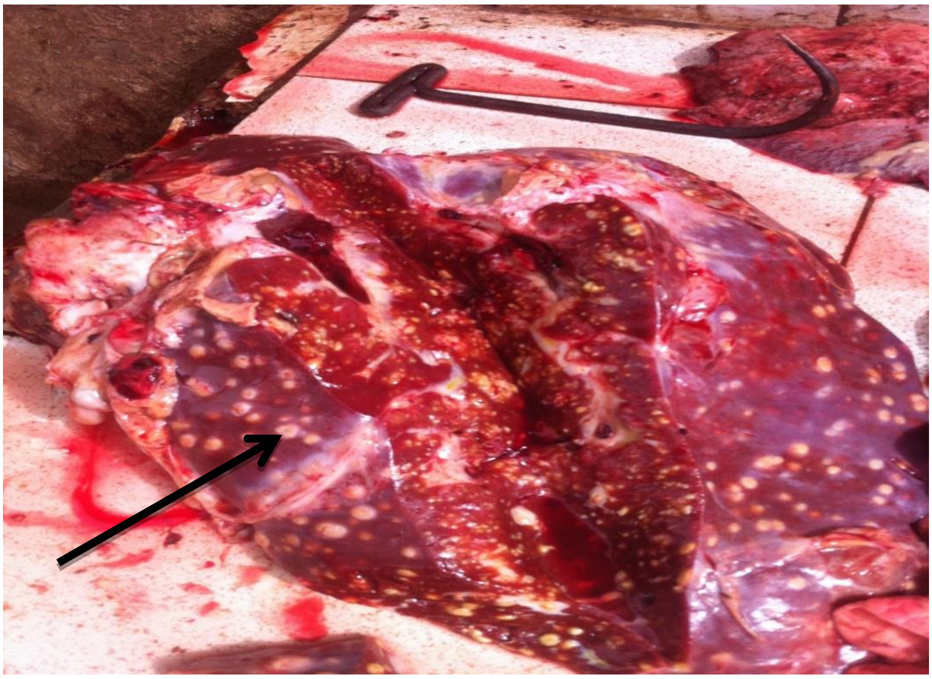

Plate II**:** Showing enlarged tuberculous liver with multiple granulomas on its surface (arrow).

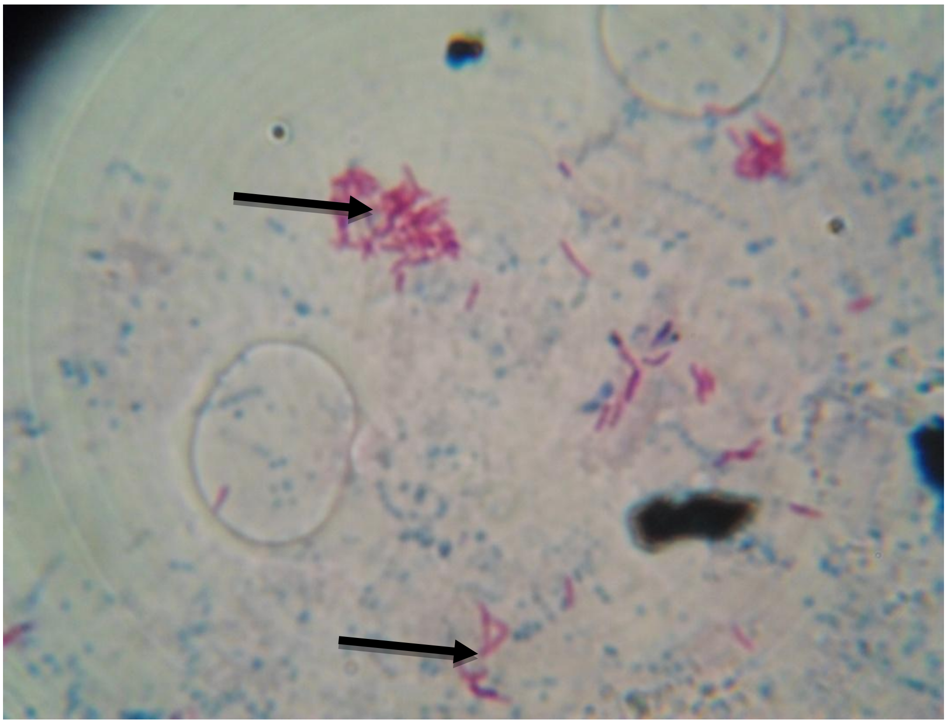

Plate III: Photomicrograph of a field showing Acid-Fast Bacilli (AFB) appeared pinkish (arrow) in a bluish background (x100 oil immersion).

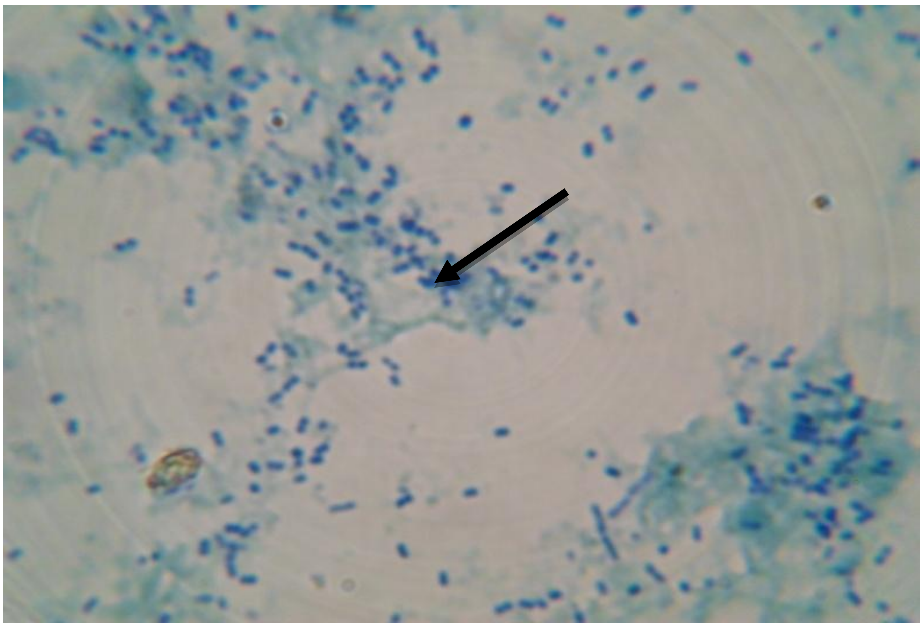

Plate IV: Photomicrograph of a fieldshowing Acid-fast negative that appeared blue (arrow) in bluish background (x100 oil immersion).

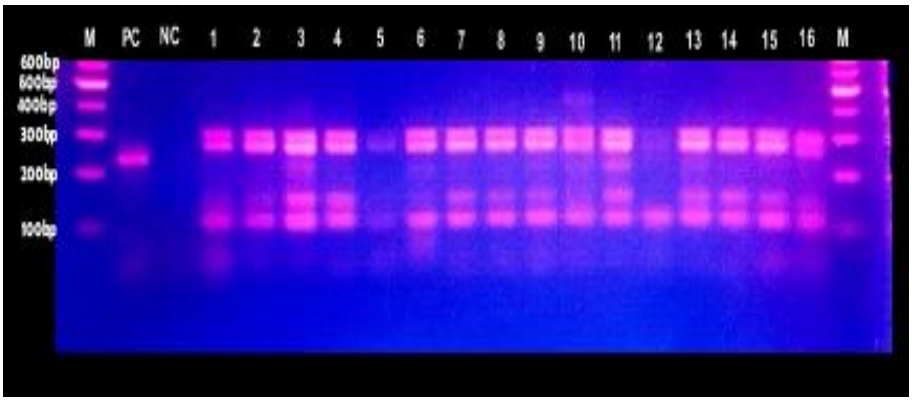

Plate V: Electrophoretic separation of PCR products on 3% agarose gel by RD1, RD4, RD9 and RD12 deletion typing of mycobacterial DNA extracted from slaughtered cattle tissues.

Legend: Lane M = 100bp plus DNA ladder

Lane PC = Positive Control (Master mix + reference-typed strain DNA template)

Lane NC =Negative Control (Master mix + distilled water)

Lanes 1-4, 6-11 and 13-16 were identified as*M. bovis* [RD9 absent (108bp); RD1 present (146bp); RD4 absent (268bp); RD12 absent (306bp)].

Lanes 5 and 12 were identified negative.

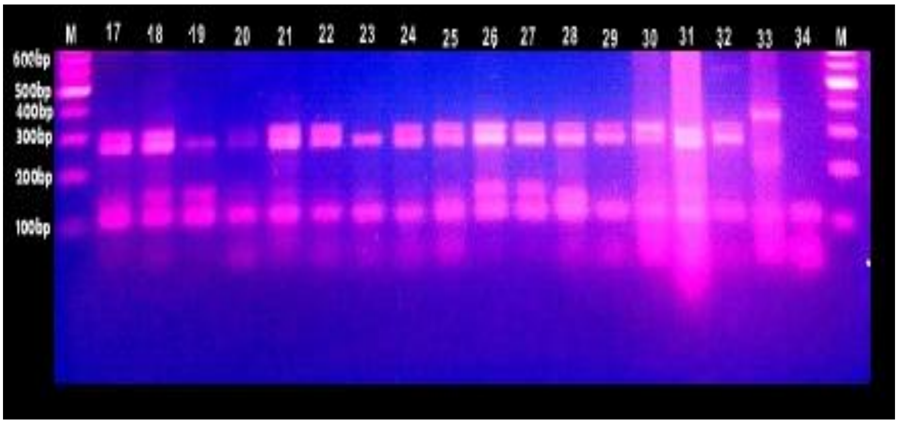

Plate VI:Electrophoretic separation of PCR products on 3% agarose gel by RD1, RD4, RD9 and RD12 deletion typing of mycobacterial DNA extracted from slaughtered cattle tissues.

Legend: Lane M = 100bp plus DNA ladder

Lane PC= Positive Control

Lane NC= Negative Control (Master mix + distilled water)

Lanes 17, 18, 21, 22, 24 to 32 were identified as *M. bovis*[RD9absent (108bp); RD1 present (146bp); RD4 absent (268bp); RD12 absent (306bp)].

Lanes 19, 20 and 23 were identified as*M. caprae*[RD9 absent (108bp); RD1 present (146bp); RD4 present (172bp); RD12 absent (306bp)]

Lane33 was identified as*M. tuberculosis* [RD1 present (146bp); RD4 present (172bp); RD9 present (235bp); RD12 present (369bp)]

Lane 34 was identified negative.

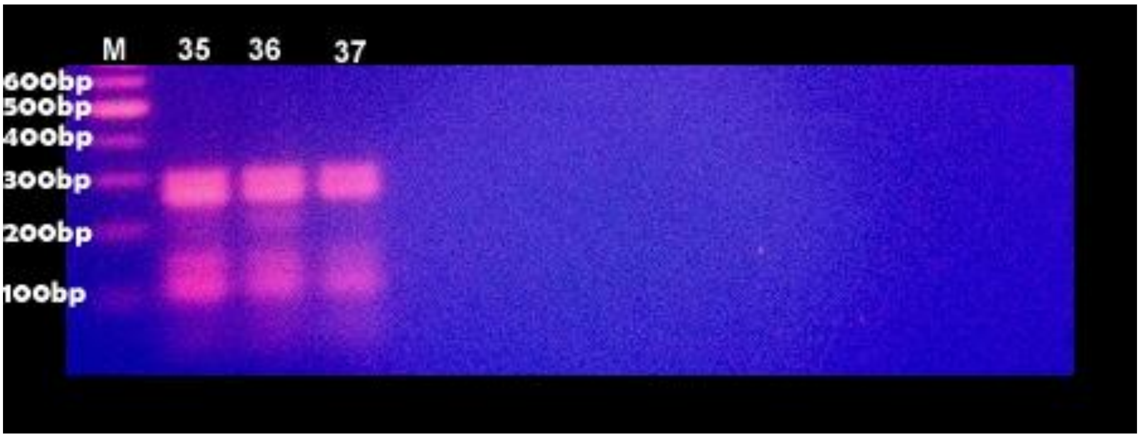

Plate VII: Electrophoretic separation of PCR products on 3% agarose gel by RD1, RD4, RD9 and RD12 deletion typing of mycobacterial DNA extracted from slaughtered cattle tissues.

Legend: Lane M = 100bp plus DNA ladder

Lane PC= Positive Control

Lane NC = Negative Control (Master mix + distilled water)

Lanes 35 to 37 were identified as*M. bovis* [RD9 absent (108bp); RD1 present (146bp); RD4 absent (268bp); RD12 absent (306bp)].

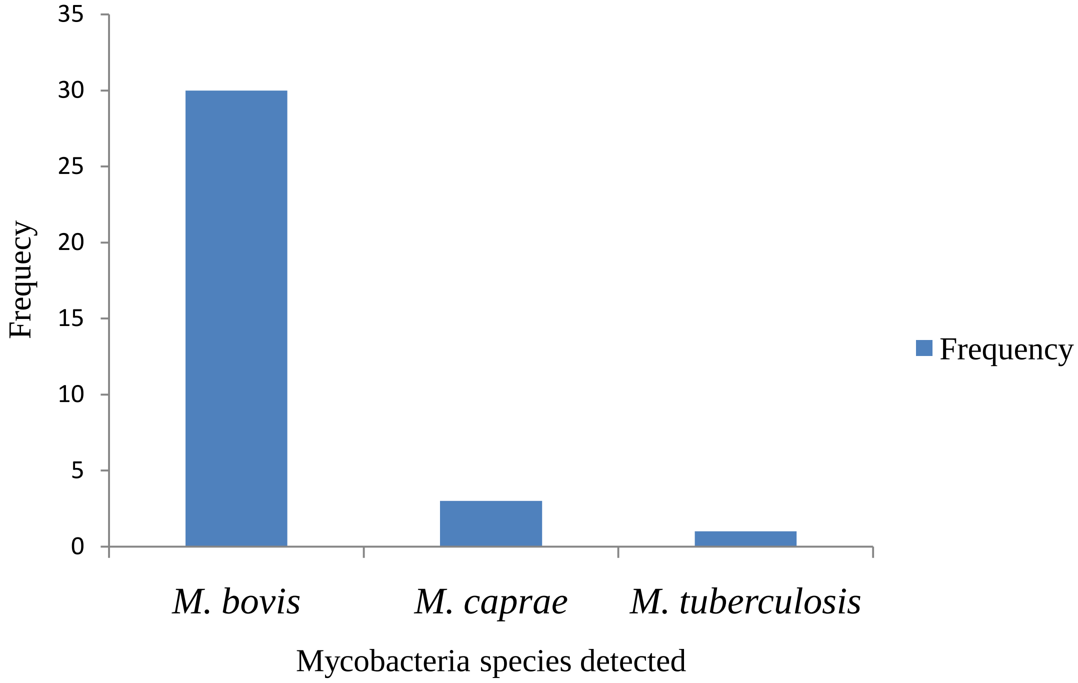

Figure 4.1: frequency of different mycobcaterial species detected by RD deletion typing

CHAPTER FIVE

DISCUSSION

The prevalence of 6.1% was recorded based on detection of gross lesions in tissues and organs of slaughtered cattle by participatory meat inspection. It was similar to the slaughter-slabs prevalence of 4.7% reported by Hena *et al*. (2012) in Bakura Local Government Area of the same Zamfara State (Northwestern Nigeria). Moreover, it was similar to 4.5% prevalence reported by Cadmus *et al*. (2009) from Bodija Municipal Abattoir in Ibadan (Southwestern Nigeria) and 4.4% prevalence published by Damina *et al*. (2011) from Jos abattoir (Northcentral Nigeria). However, compared to the data obtained in other region of Nigeria, this finding was lower than the previous report of a retrospective study by Aliyu *et al*. (2009) that documented a relatively high prevalence rate in Gombe State at 12.3% (Northeastern Nigeria). But it was higher than the following abattoir-based studies recorded in other States, it includes: 1.1% prevalence from Maiduguri Borno State documented by Raufu and Ameh (2010) (Northeastern Nigeria) and 0.54% prevalence from Ogbomosho, Oyo State (Ameen *et al*., 2008) (Southwestern Nigeria). Also when compared with the data recorded elsewhere in Africa, the abattoir bovine TB prevalence recorded in this study was higher than 3.6% prevalence rate reported in Niger Republic (Boukary *et al*., 2011), 1.0% prevalence reported in Cameroon by Koro *et al*. (2013) and 2.5% reported in Ghana (Asante-Poku *et al*., 2014), but it was similar to the 7.3% prevalence of bovine tuberculosis based on the detection of suggestive macroscopic lesions in Chad Repulic (Diguimbaye-Djaibé *et al*., 2006). As asserted by Koro *et al*. (2013), these differences observed in rates could be intriguing because there is frequent cattle movement between these countries, which can lead to the strain dissemination and transmission of bovine tuberculosis. In addition, this result is consistent with the finding of Aylate *et al*. (2013) who recorded 6.1% prevalence in an abattoir-based study in Ethiopia.

Furthermore, as suggested by Awah-Ndukum *et al*. (2012), the differences observed between prevalence in various reports above could be explained by many factors including differences in the disease status in animal population, sources of these animals, and it could also be associated with an increase in the number of slaughtered cattle (due to an increasing public demands for meat). It could also be coincided with the enhanced efficiency of meat inspection. More so, the increased in prevalence of suspected bTB lesions recorded in this study compared to the published report of Hena *et al*. (2012) in the same Zamfara State but different study area, could be associated with the slaughter figure at Gusau abattoir that was highest in the State (due to the increasing meat demands consequence of the socioeconomic status of Gusau town, being the State capital and that it records the highest human population).Thus, the increasing trend recorded may have been an actual increase in the disease state but is probably due to improved diagnostic awareness of the disease and intensification of slaughter/meat inspection procedure.However, the possibility of under-recording and under-detection remains.

The 16.4% prevalence recorded following Ziehl-Neelsen staining technique was lower than 30% prevalence rate reported by Chukwu *et al*. (2013) from suspected tuberculous tissues, but it was higher than 3.7% prevalence published by Damina *et al*. (2011) all from Jos Plateu State, Northcentral Nigeria. Also when compared with the data recorded elsewhere in Africa, a prevalence rate by direct microscopy of the suspected tissues after decontamination recorded in this study was lower than 31% of acid-fast bacilli detected from suspected tuberculous tissues without culture reported by Awah-Ndukum *et al*. (2010), and 58% reported in an abattoir-based study by Koro *et al*.

(2013) all from Cameroon abattoirs. While it was similar to an abattoir-based 11.8% direct microscopy prevalence reported by Asante-Poku *et al*. (2014) in Ghana. However, the probable reasons for these differences could had been because, 5% acid-alcohol was used in this study instead of the regular 3% acid-alcohol solution, and also in this study while decolorizing, during staining technique the timing was extended to 10 min decolourization instead of the usual 5 min, all of which this might had helped to rule out other weak acid-fast non-tuberculous agents; and hence, might had improved the specificity of the technique.

The prevalence recorded using RD deletion typing was 91.9% for all the AFB tested positive samples. For overall prevalence rate (91.9%), it was higher than 79.6% reported by Damina *et al*. (2011) in Jos Plateu State, Northcentral Nigeria, while it was consistent with 92.6% reported by Ameni *et al*. (2010) in Ehiopia; all were abattoir-based studies and detection was made from suspected tissue samples. However, the species specific prevalence rates of: 88.2% for *Mycobacterium bovis* was higher than 75.6% for *Mycobacterium bovis* recorded by Damina *et al*. (2011), while 2.9% *Mycobacterium tuberculosis*detected was lower than 7.4% reported by Ameni *et al*. (2010) and 4.2% reported by Damina *et al*. (2011). The differences observed could had been that in this study, cocktail of primers (that replicate regions RD1, RD4, RD9 and RD12) were used which might had improved specificity of the technique. In addition, Damina *et al*. (2011) confirmed dual infection of *Mycobacterium bovis* and *Mycobacterium tuberculosis* from tuberculous tissues collected from cattle in Jos, Nigeria. While in Ethiopia Ameni *et al*. (2011) detected *M. tuberculosis* in tuberculous cattle tissue and Aylate *et al*. (2013) confirmed *Mycobacterium bovis* (75%) and *Mycobacterium tuberculosis* (25%) from tuberculous tissues collected from slaughtered cattle. This indicates that zoonotic bovine TB is a real public health problem that has been underestimated and not being investigated.

In an infected animal, *Mycobacterium bovis* can be excreted in all bodily fluids, meaning transmission is possible directly through close contact (aerosol transmission, meat or milk ingestion) or indirectly via contact with fluids or excreta in the environment (Neill *et al*., 2001; Radostits *et al*., 2007). Knowledge of the excretion routes is crucial to improve the control strategies to reduce bTB in animals (Santos *et al*., 2015). However, determining which route(s) is(are) more prevalent is fundamental to define the likelihood of interspecies transmission as fecal shedding tends to promote indirect transmission through contamination of the environment, while oronasal shedding, in addition to environmental contamination, allows also an easier direct transmission by aerosols (Morris *et al*., 1994; Corner, 2006). Regarding the locations of tuberculous-like lesions and confirmed infections in carcasses studied, 126 (3.3%) of the lesions compatible with bovine tuberculosis and established infections observed in this study were more restricted to the organs/tissues of thoracic cavity and its associated lymph nodes, while 100 (2.8%) lesions were seen in the organs/tissues of abdominal cavity and posterior quarters of the carcasses.Granulomatous lesions or abscesses (containing yellowish pus) detected during postmortem examination were more in lungs 90 (2.4%), tracheo-bronchial lymph nodes 16 (0.4%), pre-scapular lymph nodes 9 (0.2%), heart 8 (0.2%) and pleural sac 3 (0.1%). Thus, the frequency of the lesions in the lungs and lymph nodes associated with the thoracic and fore-quarters of most animals recorded in this study, agrees with previous reports (Hojle, 1990; Herenda *et al*., 1994; Whipple *et al*., 1996; Headley, 2002) and it suggests that the respiratory route is the main mode of animal to animal transmission of bovine TB as reported by researchers (Francis, 1971; Pollock and Neill, 2002; Pollock *et al*.,2006), which it serves as primary foci for the excretion of infective *M. bovis* as concluded byCorner (1994) and Neill *et al.* (1994a).While the prevalence values recorded for mesenteric lymph nodes and associated organs suggest oral transmission of *M. bovis*infection (Palmer *et al*., 2000; Gavier-Widen *et al*., 2001; Phillips *et al*., 2003), these findings of gross lesions and identified infections in the organs/tissues of abdominal cavity and posterior quarters of the carcasses agree with the previous studies in Ethiopia, which demonstrated a localization of lesions and established infection in digestive tract and associated lymph nodes mainly mesenteric lymph nodes in cattle kept in extensive production system (Ameni *et al*., 2006; 2007; 2011).

Herd breed and management systems **(**Alvarez *et al*., 2012; Menzies and Neill, 2000), are considered among factors to have been associated with the persistence of bTB in a given environment (Alvarez *et al*., 2014), also included is the disease endemicity in the surrounding areas (Olea-Popelka *et al*., 2004). Results from this study revealed that age and sex had effect on bovine tuberculosis status of the animal; they were found to be related to the occurrence across the two groups with high prevalence values been recorded in old and female category, and low values in young and male cattle (ZN >6 years: OR = 0.254, 95% CI 0.095-0.681; RD deletion typing >6 years: OR = 0.287, 95% CI 0.106-0776). Most of the affected animals were female and old with an average age of ≥6 years. Hence, old cattle have been reported to be most affected and at higher risk of bovine TB infection (Philips *et al*., 2003; Ameni *et al*., 2007; Cleaveland *et al*., 2007; Tschopp *et al*., 2009). These findings are similar to the report of Awah-Ndukum (2012), they also agree with the published observations by other studies (Cook *et al*., 1996; Asseged *et al*., 2000; Ameni *et al*., 2003; Cadmus *et al*., 2004). Cattle of all ages are susceptible to bTB, but the probability of becoming infected generally increases with age due to the proportionate increase in exposure time, with older animals being said to have a greater risk on account of their longer life spans (O'Reilly and Daborn, 1995). However, Pavlik *et al*. (2002b) suggested that, since most of the female cattle might had calved several times, they may not be immunologically as active as males because of the stress of pregnancy; which would had made them more vulnerable and easily succumb to infection; those explained the higher prevalence recorded in female than male cattle in this study.

In this study, it was noted also that bovine tuberculosis was more prevalent in White Fulani and Sokoto Gudali than in Red Bororo cattle breed, which could had been related to the ratio of White Fulani and Sokoto Gudali to Red Bororo in the region and their availability for slaughter at the abattoir. This finding disagrees with the established observation of Nfi and Ndi (1997) that reported a higher proportion of Mbororo cattle with bovine TB infection in Cameroon, Diguimbaye-Djaibé *et al*. (2006) also confirmed more *M. bovis* infection in Red Bororo than other zebu cattle in Chad Republic; they concluded that this finding may indicate that Mbororo are more susceptible to *M. bovis* in the two African countries and high ongoing cross-border transmission of *M. bovis* in cattle is suspected. More so, Awah-Ndukum *et al*. (2012) in Cameroon recorded lower prevalence values amongst White Fulani and Gudali compared to the Red Bororo cattle; thus, asserted that the reasons underlying the differences between breeds in their study remain unclear. However, elsewhere in Africa (Ethiopia and Uganda), a low infectiousness of the local zebu cattle (Ameni *et al*., 2006; 2007; Inangolet *et al*., 2008) was found to be a notable feature in their study environments.

The multiplex PCR-based RD deletion typing used in this study showed evidence of*Mycobacterium tuberculosis* from tuberculous lesions of cattle, which suggests a possible interaction and transmission of TB between humans and cattle, as Cadmus *et al*. (2009) detected *M. bovis* and *M. tuberculosis* in goats suggesting transmission of bovine and human tuberculosis from the primary hosts to goats in Nigeria. Likewise, transmission of *Mycobacterium tuberculosis* from human TB patients to cattle could also be possible (Ocepek *et al*., 2005). *Mycobacterium tuberculosis* has been isolated from cattle (Cadmus *et al*., 2006; Fatene *et al*., 2011); cattle may serve as a source of *Mycobacterium tuberculosis* infections to humans, indicating the possibility of reverse zoonosis (Mengistu *et al*., 2015), or either of them (cattle and humans) could be a source of infection for the other creating a sort of vicious cycle (Cosivi *et al*., 1998). However, inter-species transmission of disease may occur through direct or indirect interactions; direct interaction refers to direct physical contact or very close proximity with the source of the infection like by inhalation of aerosol particles from the exhalation of an infected individual, while indirect interaction refers to shared space use where individuals visit the same location at different times (Cowie *et al*., 2015). The two interactions are likely to be driven by intake behavior such as feeding or drinking (Hutchings and Harris, 1997), particularly if food and water resources are not evenly distributed, causing aggregation of multiple species (Barasona *et al*., 2014). In Nigeria, these interactions (inter- and intra-species) maybe aggravated further due to low socioeconomic development, where animals are not housed in segregated holdings and are kept next to man's living rooms sharing both space and resources (water and food), which narrows the gaps even more further between-animal species-and-man and it enhances repeated contacts. In this study, the evidence of *M. tuberculosis* from tuberculous cattle spleen samples suggests a possible human to animal transmission; which might had resulted when cattle grazed on grass contaminated with sputum coughed out by human TB patients, and or when they fed on feeds or garbage contaminated by the activities of human TB patients; though this needs to be further investigated.Although the anatomical location of lesions *per se*provides some information on the potential routes of transmission, this indirect association needs to be interpreted carefully as abdominal lesions can be caused by swallowing of infected pulmonary secretions or hematogenous spread of infection (Morris *et al.*, 1994; Gallagher and Clifton-Hadley, 2000; Corner, 2006).

Furthermore, varying rates of 16.4% and 91.9% recorded from the detection techniques used in this study. Most of the tuberculous-like lesions detected during post-mortem examination had turned out to be negative for Ziehl-Neelsen staining technique and RD deletion typing. This shows that, other granuloma causing agents might have been among the probable causes of tubercle-like lesions often observed in tissues and organs during participatory meat inspection. However, both the two techniques have showed high specificity and sensitivity interms of detection capability.

The questionnaire survey has assessed and established the potential risk factors amongst abattoir cleaners, animal health scientists, butchers, cattle handlers and traders, meat sellers and veterinarians operating in Gusau abattoir Zamfara State, Nigeria. However, 100 (99.0%) of the respondents were males that it was similar to 78.2% reported by Hambolu *et al.* (2013), out of the demographic factors, sex was found to be an important risk factor; that is, in terms of repeated contact with cattle/carcasses potentially infected and also exposure to other risks. Moreover, other risk factors include: none formal education in 35 (34.7%), contact with cattle 41 (40.8%), contact with carcasses 47 (46.5%), low knowledge levels about bTB 43 (42.7%), poor TB screening history 86 (85.1% not screened), consumption of fresh un-boiled milk 14 (13.9%) and soured milk 41 (40.6%), and daily consumption of meat by 42 (41.6%) persons. Although, 93 (92.1%) persons claimed that they were aware of tuberculosis in humans, the level of awareness at 58 (57.4%) amongst respondents about bovine TB was low considering prolong repeated contact with cattle and carcasses as high risk factors for exposure and transmission of zoonotic TB amongst assessed occupations.

The findings in questionnaire survey were consistent with the report of Mairo (2014) which revealed that 53.2% of the respondents had a good knowledge about bTB, and concluded that butchers and meat handlers have inadequate knowledge of bTB and by their practices they are prone to exposure and its transmission. They were also similar to the published report of Hambolu *et al*. (2013) that assessed predictors of bovine TB risk behaviors amongst meat handlers in an abattoir setting in Lagos Nigeria, which recorded low knowledge levels and high prevalence of risk behaviours amongst meat handlers. More so, these findings were similar to the study that assessed cattle owners' on awareness about bovine TB and its public health implication in Ethiopia: Zeru *et al*. (2014) documented the lack of awareness regarding bTB and its routes of transmission, which they concluded that this may allow circulation of the agent between communities and animals.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study has for the first time in almost two decades since creation of Zamfara State in Northwestern Nigeria, used laboratory-based diagnostic techniques to investigate the prevalence of bovine TB infection; other than the subjective visual postmortem examination of suspected TB lesions. Results obtained have confirmed and established the prevalence of *Mycobacterium bovis* and other members of MTC group (*Mycobacterium caprae* and *Mycobacterium tuberculosis*) infections amongst cattle slaughtered in the study area.*Mycobacteria* were identifiedto the species level and that species prevalence rates were 88.2% for *Mycobacterium bovis*, 8.8% *Mycobacterium caprae* and 2.9% as *Mycobacterium tuberculosis*. This provides information on the species of *Mycobacteria* that could had been implicated in the often observed tuberculous-like lesions among cattle slaughtered in Gusau abattoir and elsewhere in Nigeria; which these findings would be useful for modifying and implementing TB control measures in the State and country at large.

However, evidence of *Mycobacterium tuberculosis*infectionfrom cattle tissues suggests a possible interaction and transmission of the pathogen from humans to cattle; hence, the potential for reverse zoonotic transmission. Therefore, an unknown interface between bovine TB and human TB could be implied given that there are many opportunities for close and repeated human-livestock interactions in the study area; consequence of predominant livestock production system that includes cattle feedlots kept within man's niche sharing same environmental space, water and food sources, and or bulk of cattle rearing serving as means of livelihood for most environs in the State.

This study has further revealed that most of the diseased cattle were old and female, it can therefore be inferred that, there are many infected old and aging female cattle maintaining the disease in the area and have been acting as source ofinfection in various production systems across the region and may transmit the infectious agentsto: their offspring, other domestic animals (sheep and goats) being housed/herded together, and susceptible in-contact humans; especially those whose occupations bring them close to cattle, also immune-compromised persons (such as human HIV patients) and consumers of poorly processed cattle products (e.g.:milk and meat). Therefore, there exists an eminent risk of zoonotic TB transmission from abattoir to the general public. However, a high prevalence values recorded in White Fulani and Sokoto Gudali zebu cattle in this study, suggested their apparent higher susceptibility to bovine tuberculosis than the Red Bororo breed; but this is open to further investigation in the follow-up epidemiological studies in the area.

In addition, *M*ycobacterium*tuberculosis* isa pathogen of humans but it was detected in spleen specimens from tuberculous cattle in this study. Its presence in cattle may had been caused by direct transmission from infected human TB patients, particularly infected herdsmen as they have been known to have intimate and close repeated contacts with their cattle. This might also had been due to gastro-intestinal infection through oral route exposure; either by ingestion of pasture/water contaminated by the coughed out sputum from human TB patients or from the cough-swallow of sputum from the same infected cattle; since *Mycobacterium tuberculosis*induced pathology is often limited to the head region and hence its most means of release of the infective foci from the infected cattle. To that end, this study presents a preliminary base-line information on the effectiveness of using combined diagnostic methods(microscopic examination and RD deletion typing) for an improved efficiency in detection and identification of *M. bovis*, its subtypes and other members of*Mycobacterium tuberculosis* complex group that might had been the cause of bovine tuberculosis in cattle in Zamfara State.

This study contributes to knowledge on the potential dangers of TB-infected/contaminated meat being sold at meat shops in and around the study area, and to those neighboring areas (Bungudu, Maru and Tsafe Local Government areas) where beef processed in Gusau abattoir is being distributed to. However, this study has identified the potential source of Mycobacterial infections to animals other than cattle (sheep, goats and camels) and humans in the area; though it is ought to be further investigated. It also focuses on bringing to light the dangers that could be faced having diseased animal-derived products in food chain via consumption of contaminated meat, milk or other products; hence, this trend is deemed to be of public health problem. In addition, with confirmed infections in carcasses that showed suspected gross bTB lesions, none would have been condemned to be unfit for human consumption if it were in home/backyard slaughters or at slaughter slabs located in other areas of the State; where similar occurrences might have been going-on un-noticed due to the none deployment of skilled inspecting authorities (veterinarians).

The results obtained on questionnaire surveyhaveprovided preliminary information about potential risk factors for exposure and transmission of zoonotic bovine TB, amongst abattoir cleaners, animal health scientists, butchers, cattle handlers and traders, meat sellers and veterinarians operating in Gusau abattoir Zamfara State, Nigeria. However, by virtue of their gender and routines such as close and repeated contact with cattle and carcasses, as well as inadequate knowledge about bovine TB and its modes of transmission, feeding habits and food preference (consumption of unpasteurized milk and meat), they are prone to dangers of exposure and transmission of Zoonotic TB.

6.2 Recommendations

Accordingly, the following are hereby suggested to be the recommendations:

(a) There is the need for more study to determine the prevalence of bovine TB in other animal species in the State. Detailed epidemiological studies are needed to investigate the modes of transmission, risk factors of the disease, distribution of tuberculousMycobacterialspecies andstrains, and differential susceptibility to tuberculous*Mycobacteria* amongst the indigenous cattle breeds and other domestic animals tended together as it is for most herdsin the State, as well as in humans.

(b) There is the need to increase effort in thorough meat inspection with a view to reduce the possible incidence of the disease in humans. However, intensification and standardization of abattoir inspection protocols specially detailed meat inspection, establishment of enabling environment for an added confirmatory tests for bovine TB (e.g.: genomic analysis), enhanced training among staff, reviewing and enforcement of enacted laws governing meat inspection; such methods are recommended as essential and cost-effective interventions to improve bovine TB surveillance in Nigeria, with subsequent protection of animal and man health.

(c) Moreover, in order to put this to check, restrictions in random movements of cattle herds should be imposed to minimize transmission of infection from diseased and in-contact to clean susceptible animals. Government should impose it as a policy: to register any sold-out animal along with its detailed records of all previous ownership(s) with address(es); this will make control at the abattoir easy to trace carcass(es) with bovine TB lesions back to their origins, and also impose regular herd screening of cattle using tuberculin skin test for maximum and accurate detection withthe consequent elimination of tuberculin positive reactors by segregation and phasing out of the infected animals. Quarantine measures should be regularly observed, especially for the trade cattle since Zamfara State shares border with the Niger Republic.

(d) There is the need for public awareness on the role of bTB in animals and also TB amongst humans in the State. Although risk factors of bovine TB infection have been scarcely studied in the country.

(e) Further studies on the risk factors of zoonotic TB transmission in humans require case-control studies on those persons with already established TB infection. However, before it is embark on the national bTB control program, an in depth knowledge of possible risk factors is an essential prerequisite and should, therefore, also include risk factors associated with the dominant animals management systems in the country, food hygiene, food preference and feeding habits amongst humans, the influence of the environment, cultures and common norms, and nutritional challenges.

(f) Cattle professionals such as butchers, cattle handlers, cattle traders and meat sellers should be cautioned and enlightened on the dangers of infection, and should be enforced to adhere to the appropriate preventive measures. However, it should be made mandatory through Government-backed legislature the routine testing of butchers and other abattoir personnel for TB, and when tested positive; personnel should be banned from handling animals or being involved in animal related operations till his/her full recovery is assured.

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