

**ISOLATION AND MOLECULAR IDENTIFICATION OF  
*Mycobacterium bovis* IN CATTLE SLAUGHTERED IN MAKURDI  
AND OTUKPO ABATTOIRS, BENUE STATE, NIGERIA**

**BY**

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### **DECLARATION**

I declare that the work in this thesis titled “ISOLATION AND MOLECULAR IDENTIFICATION OF *Mycobacterium bovis* IN CATTLE SLAUGHTERED IN MAKURDI AND OTUKPO ABATTOIR, BENUE STATE, NIGERIA” was carried out by me in the Department of Veterinary Microbiology under the supervision of Dr. M. A Raji, Dr. M. Bello and Prof. A. C. Kudi

The information derived from the literature has been dully acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another Degree or Diploma at this or any other institution.

**Francis Enenche EJEH**

.....

**Signature**

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**Date**

## CERTIFICATION

This thesis entitled ISOLATION AND MOLECULAR IDENTIFICATION OF *Mycobacterium bovis* IN CATTLE SLAUGHTERED IN MAKURDI AND OTUKPO ABATTOIR, BENUE STATE, NIGERIA by Francis Enenche EJEH meet the regulations governing the award of the degree of Master of sciences (MSc) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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### **DEDICATION**

To the glory of the most Holy Trinity, God Almighty (Jehovah Yahweh), the Creator of all that is seen and unseen, His son Jesus Christ, our Redeemer and the Holy Spirit, who proceed from the Father and the Son.

## ABSTRACT

Bovine tuberculosis (BTB) is a disease of high economic relevance within the context of livestock farming as it directly affects animal productivity and also influences international trade in animal products. The aim of this study was to determine the species of *Mycobacterium* isolated from cattle carcasses in Makurdi and Otukpo abattoirs. A total of 63 cattle suspected of BTB were sampled from a total of 587 cattle inspected at the abattoirs. Samples were processed and Ziehl-Neelsen microscopy were carried out based on recommended procedure. Processed tubercle lesions were cultured on Lowenstein-Jensen media with or without pyruvate and incubated for 8 to 12 weeks at 37°C. Region of difference (RD) typing of isolates was carried out as recommended. Out of a total of 587 cattle samples that were examined, 63 (10.7%) had BTB lesions, 47 (74.0%) were positive for acid-fast bacilli. Lymph nodes and lungs were more affected than other organs. Samples from liver, spleen, kidney, heart and pleural surface gave 100% growth on Lowenstein-Jensen slant while lymph nodes and lungs gave 68.2% and 81.3% growth respectively. More growth were seen on media containing pyruvate than glycerol. RD typing of 40 isolates identified the presence of 36 RD1, and absence of 36 RD4, 36 RD9 and 33 RD 12. Therefore, 36 (90.0%) of the isolates were identify as *M. bovis*, while the other isolates were identified as non-tuberculous mycobacteria (NTM). The annual prevalence of bovine tuberculosis in cattle slaughtered in Markudi abattoirs from 2008 to 2012 was 1.9%. A prevalence of 2.9% was recorded in Otukpo abattoir from 2010 to 2012. The overall prevalence of BTB in Makurdi and Otukpo abattoirs was 2.6%. There was statistical difference between prevalence of tubercle lesions and year. A total of 1935 (3046.50Kg), valued at  $N2.91 \times 10^6$  ( $\$1.82 \times 10^4$ ) of edible organs were condemned from 2008 to 2012. There was significant difference between direct economic loss in edible organs condemned in

2008, 2009, 2010 and 2011 ( $P < 0.05$ ). During dry season, 1151 (59.48%) edible organs weighed 1797.50Kg and valued at  $N1.72 \times 10^6$  ( $\$1.07 \times 10^4$ ) while during the raining season, 784 (40.52%), 1249.00Kg valued at  $N1.19 \times 10^6$  ( $\$7483.80$ ) were condemned. There was significant differences in direct economic losses between edible organs condemned during raining season and dry season (Mann - Whitney U statistics =  $7.74 \times 10^3$ ,  $P = 0.034$ ). Organs condemned were lungs, liver, heart, spleen and kidney. Bovine tuberculosis is prevalent in Benue State and accounts for a loss of over 2.9 million Naira from 2008 – 2012 in Makurdi alone. The causative agent of bovine tuberculosis (BTB) in cattle slaughtered in Makurdi and Otukpo was predominantly *M. bovis*, it is zoonotic and is capable of causing clinical diseases in humans in Benue State.

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## ABBREVIATIONS

|                |                                                |
|----------------|------------------------------------------------|
| AD             | Annum Dominium                                 |
| APC            | Antigen Presenting Cell                        |
| AvP/Kg         | Average Price per Kilogram                     |
| BC             | Before Christ Era                              |
| BCG            | Bacillus Calmette-Guerin                       |
| BTB            | Bovine Tuberculosis                            |
| CD             | Cluster of Difference                          |
| CMI            | Cell – Mediated Immunity                       |
| DEL            | Direct Economic Loss                           |
| DNA            | Deoxyribonucleic Acid                          |
| DNR            | Department of Natural Resources                |
| dNTPs          | Dexonucleotide Triphosphates                   |
| DR             | Direct Repeat Region                           |
| ESAT – 6       | Early Secretory Antigenic Target               |
| FAO            | Food and Agricultural Organization             |
| FMH            | Federal Ministry of Health                     |
| HIV            | Human Immunodeficiency Virus                   |
| ICCTT          | Intra Cervical Caudal Fold Tuberculin Test     |
| IgA            | Immunoglobulin – A                             |
| IgE            | Immunoglobulin – E                             |
| IgG            | Immunoglobulin – G                             |
| IgM            | Immunoglobulin – M                             |
| IL             | Interleukins                                   |
| INF – $\alpha$ | Interferon – Alfa                              |
| INF – $\gamma$ | Interferon – Gama                              |
| INH            | Isoniazid                                      |
| IS             | Insertion Sequence                             |
| KDa            | Kilo Dalton                                    |
| LAM            | Lipoarabinomanann                              |
| LJ             | Lowenstein – Jensen Media                      |
| MHC            | Major Histocompatibility Complex               |
| MTC            | Mycobacterium Tuberculosis Complex             |
| MTSA           | Mycobacterium Tuberculosis Secretory Protein A |
| N              | Naira                                          |
| NK             | Natural Killer Cell                            |
| NPC            | National Population Census                     |
| NTM            | Non Tuberculous Mycobacteria                   |
| OFRs           | Open Reading Frames                            |
| OIE            | Office International des Epizooties            |
| PAS            | Para – aminosalicytic Acid                     |
| PCR            | Polymerase Chain Reaction                      |
| PI             | Post Infection                                 |
| PPD            | Purified Protein Derivative                    |
| RNA            | Ribonucleic Acid                               |
| RD             | Region of Difference                           |
| RFLP           | Restriction Fragment Length polymorphism       |
| SCCTT          | Single Cervical Caudal Fold Tuberculin Test    |
| SNPs           | Single Nucleotide Polymorphisms                |

|           |                                                 |
|-----------|-------------------------------------------------|
| TACo      | Tryptophan Aspartate Coat Protein               |
| TB        | Tuberculosis                                    |
| Th – 2    | Thymus Derived Helper Cell 2                    |
| TLR       | Toll – Like Receptor                            |
| TNCo      | Total Number Condemned                          |
| UK        | United Kingdom                                  |
| USA       | United State of America                         |
| V- ATPase | Vesicular Protein – Pump Adenosine Triphosphate |
| VNTR      | Variable Number of Tandem Repeat                |
| WHO       | World Health Organization                       |
| ZN        | Ziehl – Neelsen                                 |

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Study Background

Bovine tuberculosis (BTB) is a chronic bacterial disease of cattle characterized by respiratory disease (O'Reilly and Daborn, 1995) with production of progressive granulomatous lesions affecting organs in the chest and abdominal cavity (Shitaye *et al.*, 2006). The disease is caused by the *Mycobacterium bovis* in cattle (Smith *et al.*, 2006), a member of the Family *Mycobacteriaceae* (Metchock *et al.*, 2005) and belong to the Genus *Mycobacterium* (Smith *et al.*, 2011). The species is a member of the group *Mycobacterium tuberculosis* complex (MTC), these include *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis* (along with the *M. bovis*-derived bacillus Calmette-Guerin (BCG) vaccine strains), *Mycobacterium microti*, *Mycobacterium bovis* subsp. *caprae* (*M. caprae*), *Mycobacterium tuberculosis* subsp. *Canettii* (Brosch *et al.*, 2002; Mostowy *et al.*, 2002) and *Mycobacterium pinnipedii* (Niemann *et al.*, 2005). Bovine tuberculosis caused by other members of the MTC has been reported (Cadmus *et al.*, 2010; Jenkins *et al.*, 2011; Smith *et al.*, 2011; Schrenzel, 2012)

The organism was discovered by Robert Koch in 1882, it an aerobic acid- fast, non motile, non-capsulated, non-spore forming bacillus (Grange, 2006). *Mycobacteria* are obligate intracellular pathogens that can infect several animals species, although *M. bovis* is principally an animal pathogen which is closely related to *M. tuberculosis* and has the broadest host range of any pathogenic Mycobacterial species (Schrenzel, 2012).

Tuberculosis (TB) is humanities greatest killer which is out of control in many parts of the world. The disease is preventable but it has been grossly neglected (Shrestha *et al.*,

2005). It is still a major health concern worldwide and the disease spreads more easily in overcrowded settings and in the conditions of malnutrition and poverty (Mycal *et al.*, 2005).

Epidemiologically, tuberculosis is worldwide in distribution with an estimated incidence rate of 9.4 million in 2009, more than any at other time in history of the disease (WHO, 2010); and bovine tuberculosis accounted for about 5% - 10% of human tuberculosis (Haddad *et al.*, 2001). Tuberculosis in humans due to *M. bovis* is both clinically and pathologically indistinguishable from cases caused by *M. tuberculosis* (Wedluck *et al.*, 2002).

Transmission of tuberculosis from cattle to humans mostly occur through consumption of unpasteurised milk, closed contact with infected animals (Michel *et al.*, 2010) and air borne transmission (Vekemans *et al.*, 1999). The epidemic of HIV infection in developing countries, particularly countries in which *M. bovis* infection is present in animals and the conditions favour zoonotic transmission, could make zoonotic tuberculosis a serious public health threat to persons at risk (Darbon and Grange, 1993; Grange and Yate, 1994; Cosivi *et al.*, 1995; Moda *et al.*, 1996).

The implementation of eradication programme in Europe, USA, Canada and other developed countries contributed immensely to the eradication of zoonotic tuberculosis (Bovine tuberculosis) (Cosivi *et al.*, 1995; 1998; Ayele *et al.*, 2004; Thoen *et al.*, 2004; Amanfu, 2006; Smith *et al.*, 2006). However, the continual presence of the organism in wildlife pose a significant challenge to the control and eradication of bovine tuberculosis in the developed world ( Aranaz *et al.*, 1996; Dalahay *et al.*, 2002; O'Brien *et al.*, 2002).

In Africa, the true extent of the disease has not been evaluated due to economic constraints (Cosivi *et al.*, 1995; Ayele *et al.*, 2004). However, studies carried out by individuals in some African countries show the presence of the disease in all African countries with Nigeria ranking second in Africa and fourth in the world (WHO, 2004).

In South Africa, bovine tuberculosis is thought to have been transmitted from cattle to buffalo in both the Kruger National park and Hluhluwe- Imfolozi park accounting for over 70% prevalence rate (Weyer *et al.*, 1999; Michel *et al.*, 2009). In Ethiopia, a prevalence rate of bovine tuberculosis ranges from 3.4% in small holder production system to 50% in periurban dairy production system (Bogale *et al.*, 2001; Ameni *et al.*, 2007). Prevalence rate of 18% - 30% *M. bovis* of all *M. tuberculosis* complex strains isolated from human patients in rural settings in Tanzania and Uganda were reported by Kazwala *et al.* (2001); Mfinang *et al.* (2004); Cleaveland *et al.*, (2007), while Awah-Ndukum *et al.* (2010) reported a prevalence rate of 31% Ziehl Neelsen, 51% culture and 60% antibodies detection of tested cattle in Cameroon. In Chad a prevalence of 11.5% was reported in 34 transhumant herds (Diguimbaye-Djaibé *et al.* (2006); 3.7% prevalence rate was reported by Boukary *et al.*( 2011) in Niger and 71.7% in cattle sampled at Kumasi metropolitan abattoir in Ghana (Abu- Bobi *et al.*, 2009).

In Nigeria, reporting of bovine tuberculosis is not mandatory and there is no active tuberculosis surveillance program; hence the true of the status of bovine tuberculosis in cattle is unknown (Cadmus *et al.*, 2004). However, few works had been carried out by individuals in Nigeria. Studies dating from the 1970s and 1980s report the proportion of tuberculosis due to *M. bovis* in humans was 10% in northern states and 4% in Lagos (Mawak *et al.*, 2006). In other studies, Cadmus *et al.*(2006) reported 5% in Ibadan and Mawak *et al.* (2006) found an incidence of 15% in the Jos Plateau state.

The diagnosis of tuberculosis in developing countries relies on the acid- fast smear examination and scanty work using bacteriological methods to determine the relative contribution of *M. bovis* and *M. tuberculosis* (Idigbe *et al.*, 1986; Idrisu and Schnurrberger, 1977). However, molecular typing provides a rapid means for discriminating members of the *M. tuberculosis* complex (Haddad *et al.*, 2001).

Recently, molecular epidemiological techniques like spoligotyping, variable number tandem repeat (VNTR) typing, genotyping and deletion typing have helped to characterise members of the *Mycobacterium tuberculosis* complex, these have contributed in revealing sources of infection and on-going transmission of disease in animals and humans (Haddad *et al.*, 2001; Kamerbeek *et al.*, 2007; Rodwell *et al.*, 2010).

## **1. 2. Statement of Research Problem**

Bovine tuberculosis is a disease of high economic relevance within the context of livestock farming as it directly affects animal productivity and also influences international trade in animal products. *M. bovis* infections have also been detected in wildlife and can have severe consequences for the ecosystem. Moreover, bovine tuberculosis bears a zoonotic potential and is therefore of public health concern (Cosivi *et al.*, 1998; Renwick *et al.*, 2007).

Butchers in Benue state face financial losses due to condemnation of organs as result of detection of tuberculous lesions in slaughtered cattle and they are also constantly exposed to infection as result of poor adherence to preventive measures.

The high risk group are individuals with concomitant HIV/AIDS infections (Ayele *et al.*, 2004), and Benue State ranks second in Nigeria with a prevalence rate of HIV/AIDS at 13.5% ( FMH, 2003).

Previous studies in the area (Ofukwu *et al.*, 2008) are based on conventional methods which have limitations in specificity, sensitivity, timing, relative cost and inability to detect latent infection; therefore, further studies using more recent molecular tools which have the advantages of high sensitivity, specificity and rapidity in sample processing are needed to characterise and understand the epidemiology of bovine tuberculosis in Benue State, Nigeria.

*Mycobacterium tuberculosis* complex is multi host, infecting a variety of animal in Nigeria, Jenkins *et al.* (2011) isolated *M. bovis* and *M. tuberculosis* from pigs slaughtered in Ibadan.

Infection with *M. bovis* has also been confirmed in cattle slaughtered in abattoirs in Nigeria (Cadmus *et al.*, 2004; 2008). Infected cattle can transmit *M. bovis* to other species of food animals reared together. 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. Human cases of tuberculosis associated with *M. bovis* have been described in Nigeria (Cadmus *et al.*, 2006).

Risk factors of zoonotic tuberculosis identified in Nigeria and also in Benue State include: consumption of improperly cooked meat and raw milk (Ofukwu *et al.*, 2008; Cadmus *et al.*, 2010; 2011), close association of animals with humans (Awah Ndukum *et al.*, 2010; Abubakar *et al.*, 2011) and implementation of meat inspection policies (Cadmus and Adesokan, 2009).

There is paucity of information on the epidemiology of mycobacterial infection in cattle in Benue state. Molecular typing of bovine tuberculosis has not been carried out in Benue state.

### **1.3 Justification**

Benue State is known as the food basket of the nation, the State is endowed with good natural pasture and large animal resources including cattle (Blench, 1999). The people are known for high meat consumption including beef. Therefore, in the light of increased reports of bovine tuberculosis as a re-emerging zoonoses worldwide and increase incidence of immunosuppressive diseases, such as HIV/AIDS especially in Benue State, Nigeria, (FMH, 2003). There is the need for epidemiological assessments of bovine tuberculosis to provide information on the role of cattle as maintenance host of the agent of bovine tuberculosis (BTb) and as a likely source of human infection. In addition, reporting of BTb is not mandatory and there is no active TB surveillance programme in Nigeria; therefore, the true status of the disease in cattle is unknown. Hence, the outcome of this study will provide information that will be useful to both regulatory agencies and government departments and agencies in policy formulation relating to tuberculosis control in Benue state. Previous studies in the area (Benue State) were based on traditional methods for the diagnosis of tuberculosis which include tuberculin test, culture and smear microscopy. Therefore, there is need to use modern molecular typing technique such as deletion typing to characterize members of *Mycobacterium tuberculosis* complex (MTC) obtained from culture of tuberculous lesions sampled from Otukpo and Makurdi abattoirs in Benue state to understand the current status of bovine tuberculosis in Benue state.

### **1. 4. Aim of the Study**

- Isolation and molecular identification of *Mycobacterium bovis* from tuberculous lesions in cattle slaughtered at the Makurdi and Otukpo abattoirs in Benue state, Nigeria.

### **1. 5. Objectives of the Study**

- Identification of acid-fast bacilli with the use of Ziehl-Neelsen microscopy.
- Isolate *Mycobacterium* from tissues samples suspected of tuberculosis from cattle slaughtered in Makurdi and Otukpo abattoirs in Benue state.
- To identify members of *Mycobacterium* species by using a multiplex PCR based genomic region of difference (RD) typing technique.
- To determine the economic losses due to condemnation of meat suspected of tuberculosis at post mortem inspection for a period five years (2008 – 2012) in Makurdi abattoirs.

### **1. 6. Research Questions:**

- What is the isolation rate of *Mycobacterium* species in cattle slaughtered in Otukpo and Makurdi, Benue state?
- What are the species of *Mycobacterium tuberculosis* complex present in cattle slaughtered in Benue state?
- What are the economic losses due to condemnation of edible organs suspected of tuberculosis at post mortem inspection from 2008 – 2012 in Makurdi, Benue state?

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Historical Perspectives

Tuberculosis (TB) has a long history. It was present before the beginning of recorded history and has left its mark on human creativity, music, art, and literature; and has influenced the advance of biomedical sciences and healthcare (Leão and Portaels, 2007). Its causative agent, *Mycobacterium tuberculosis* complex, may have killed more persons than any other microbial pathogen (Daniel, 2006).

It is presumed that the genus *Mycobacterium* originated more than 150 million years ago (Daniel 2006). *Mycobacterium tuberculosis* was thought to 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).

Identification of genetic material from *M. tuberculosis* in ancient tissues has provided a powerful tool for the investigation of the incidence and spread of human TB in historic periods, it also offers potential new insights into the molecular evolution and global distribution of these microbes (Leão and Portaels, 2007).

Mycobacteria are assumed to be better preserved than other bacteria due to the resistant lipid-rich cell wall and the high proportion of guanine and cytosine in their DNA, which increases its stability. *M. tuberculosis* are found only in the tissues of an infected host, and the characteristic pathology induced by this strictly mammalian pathogen tends to show residual microbial DNA contained in localized lesions (Leão and Portaels, 2007).

These bacteria are, therefore, ideal microorganisms for studying ancient DNA and were the first to be pursued (Leão and Portaels, 2007). These investigations have answered important questions. They proved that TB is an ancient disease with a wide geographical distribution. The disease was widespread in Egypt and Rome (Zink *et al.*, 2003, Donoghue *et al.*, 2004); it existed in America before Columbus (Salo *et al.*, 1994, Konomi *et al.*, 2002; Sotomayor *et al.*, 2004), and in Borneo before any European contact (Donoghue *et al.*, 2004). The earliest DNA-based documentation of the presence of *M. tuberculosis* complex organisms was accomplished in a subchondral articular surface from an extinct long-horned Pleistocene bison from Wyoming, US, which was radiocarbon-dated at 17,870 +/- 230 years before the present (Rothschild *et al.*, 2001).

Another important achievement of the studies on ancient DNA was the confirmation of the TB diagnosis in human remains that showed the typical pathology. Mycobacterial DNA was detected in bone lesions in the spine of a male human skeleton from the Iron Age (400-230 BC), found in Dorset, United Kingdom (Taylor *et al.*, 2005); skin samples from the pelvic region of Andean mummies, carbon-dated from 140 to 1,200 AD (Konomi *et al.*, 2002); and calcified pleura from 1,400 year-old remains, found in a Byzantine basilica in the Negev desert (Donoghue *et al.*, 1998). DNA techniques have also shown the presence of mycobacterial DNA, at a lower frequency, in bones with no pathological changes, suggesting either dissemination of the TB bacilli immediately prior to death or chronic millary TB (Zink *et al.*, 2003).

Spoligotyping was the method used to study the Plesitocene remains of a bison (Rothschild *et al.*, 2001) and was also applied to a subculture of the original tubercle

bacillus isolated by Robert Koch, confirming its species identification as *M. tuberculosis* rather than *Mycobacterium bovis* (Taylor *et al.*, 2003).

Until recently, the search for mycobacterial DNA in human archeological specimens failed to find evidence of the presence of *M. bovis*, a member of the *M. tuberculosis* complex with a remarkably wide spectrum of susceptible mammalian hosts, and once considered a putative ancestor of *M. tuberculosis* (Donoghue *et al.*, 2004). In an up to date publication, the identification of *M. bovis* DNA in South Siberian human remains was confirmed by amplification of *pncA* and *oxyR* genes and analysis of regions of difference (RD). These findings were obtained from remains that showed skeletal evidence of TB. Carbon-dated from 1,761 to 2,199 years ago, they seem to indicate that this population was continuously exposed to wild or domesticated animals infected with *M. bovis*, which could have been reservoirs for human infection (Taylor *et al.*, 2007).

## **2. 2. The Genus *Mycobacterium***

Bacteria of the genus *Mycobacterium* are non-motile and non-sporulated rods. They are grouped in the suprageneric rank of actinomycetes that, unusually, have a high content (61-71 %) of guanine plus cytosine (G+C) in the genomic deoxyribonucleic acid (DNA), and high lipid content in the wall, probably the highest among all bacteria (Barrera, 2007). *Mycobacterium* and other closely related genera (i.e. *Corynebacterium*, *Gordona*, *Tsukamurella*, *Nocardia*, *Rhodococcus* and *Dietzia*) have similar cell wall compounds and structure, and hence show some phenotypic resemblance (Barrera, 2007).

Several mycolic acids in the envelope structure distinguish the mycobacteria. This may act as carbon and energy reserves. They are also involved in the structure and function

of membranes and membranous organelles within the cell. Lipids constitute more than half of the dry weight of the mycobacteria (Barrera, 2007).

However, the lipid composition of the tubercle bacillus may vary during the life cycle in culture, depending on the availability of nutrients. The waxy coat confers the idiosyncratic characteristics of the genus: acid fastness, extreme hydrophobicity, resistance to injury, including that of many antibiotics, and distinctive immunological properties. It probably also contributes to the slow growth rate of some species by restricting the uptake of nutrients (Barrera, 2007)

The species within the genus *Mycobacterium* show great diversity in many aspects. Most of them live and replicate freely in natural ecosystems and seldom, if ever, cause disease. Only a few mycobacteria became successful pathogens of higher vertebrates, preferentially inhabiting the intracellular environment of mononuclear phagocytes. The host-dependent mycobacteria that cannot replicate in the environment are *Mycobacterium leprae*, *Mycobacterium lepraemurium*, *Mycobacterium avium* subsp. *Paratuberculosis*, and the members of the *Mycobacterium tuberculosis* complex. Bacteria within the *M. tuberculosis* complex are able to reproduce *in vitro*, in contrast to *M. leprae* and *M. lepraemurium*, which are uncultivable and require the intracellular milieu for survival and propagation (Barrera, 2007).

Traditionally the genus *Mycobacterium* is divided in two groups; *Mycobacterium tuberculosis* complex (MTC) (*Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium caprae*, *Mycobacterium pinnipedii*, *Mycobacterium africanum*) and nontuberculous mycobacteria (NTM) (*Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium scrofulaceum*, *Mycobacterium ulcerance*) (Metchock *et al.*, 2005).

The *M. tuberculosis* complex are generically called the tubercle bacillus, the various etiologic agents of tuberculosis (TB) have distinct hosts, zoonotic potential and reservoirs. *M. tuberculosis*, and the regional variants or subtypes *Mycobacterium africanum* and “*Mycobacterium canettii*” are primarily pathogenic in humans. *Mycobacterium bovis* and *Mycobacterium microti* are the causative agents of TB in animals, and can be transmitted to humans. Some particular strains isolated from goats and seals have been named *Mycobacterium caprae* and *Mycobacterium pinnipedi*, although sometimes they are identified as *M. bovis* subspecies or variants (Barrera, 2007)

However, the above mentioned agents of TB together with the vaccine bacilli Calmette-Guérin (BCG) strains rank close to each other along a phenotypically continuous taxon (David *et al.*, 1978; Wayne and Lin, 1982; Vincent *et al.*, 1992; van Soolingen *et al.*, 1997; 1998; Niemann *et al.*, 2000; 2002; Mostowy *et al.*, 2005), The rare MTC variants include; the dassie and oryx bacilli (van Soolingen *et al.*, 1994; Mostowy *et al.*, 2004; van Ingen *et al.*, 2012).

### 2.3. TAXONOMY

|         |                        |
|---------|------------------------|
| Phylum  | Actinobacteria         |
| Order   | Actinomycetales        |
| Genus   | <i>Mycobacterium</i>   |
| Species | <i>M. tuberculosis</i> |
|         | <i>M. bovis</i>        |
|         | <i>M. africanum</i>    |
|         | <i>M. microti</i>      |
|         | “ <i>M. canettii</i> ” |

*M. caprae*

*M. pinnipedi*

(Source: Barrera, 2007)

#### **2. 4. Acid Fastness**

Unlike Gram-negative bacteria, mycobacteria do not have an additional membrane in the outer layers of the cell wall. They are structurally more closely related to Gram-positive bacteria. However, mycobacteria do not fit into the Gram-positive category as the molecules attached to the cell wall are distinctively lipids rather than proteins or polysaccharides. Frequently, they do not retain the crystal violet and appear as “ghosts” 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).

Ehrlich discovered the acid fastness of the tubercle bacillus, which has been the prominent characteristic of mycobacteria up until now. The expression “acid-fastness” describes the resistance of certain microorganisms to decolourization with acid-alcohol solutions after staining with arylmethane dyes such as carbol fuchsin (Madison, 2001). This feature is of utmost practical importance in identifying the tubercle bacillus, particularly in pathological specimens (Abe, 2003).

Most of the current knowledge on this phenomenon was disclosed in pioneer experiments. The beading observed inside the cells was interpreted as accumulation of free dye rather than staining of particular structures, which led to the early hypothesis that alkaline stains are retained in the cytoplasm (Yegian, 1947). Later, evidence was provided sustaining the role of lipids in trapping the dyes. Indeed, there is a parallelism between the increasing degree of acid fastness displayed by microorganisms in the

genera *Corynebacterium*, *Nocardia*, and *Mycobacterium*, and the increasing length of mycolic acid chains in their walls. This correspondence suggests that the chemical binding of the dye to these molecules might be a determinant for acid fastness (Metchock *et al.*, 2005).

Bacilli suspended in aqueous solution retain the acid fastness for a long time, even after heating. However, the property is absolutely dependent on the integrity of the bacillus. Unimpaired mycolic acids are required to hinder the penetration of water soluble dyes and bleaching acids (Goren *et al.*, 1978). The acid fastness of the bacillus is obliterated by cell trauma or autolysis (Baisden and Yegian, 1943), infection by specific mycobacteriophages (Gangadharam and Stager, 1976) or treatment with antibiotics targeting cell wall synthesis, such as isoniazid (INH). Acid fastness seems to also be dependent on nutrients and oxygen tension, as suggested by fluctuations in staining observed in different culture conditions (Nyka 1971). Dormant *M. tuberculosis* bacteria bearing cell wall alterations may remain undetected by the classic Ziehl-Neelsen staining (Seiler *et al.*, 2003).

## **2. 5. Microscopic Morphology**

The microscopic appearance does not allow the differentiation of the pathogenic agents of TB, mainly *M. tuberculosis*, from other mycobacteria although some characteristics may be indicative. In smears stained with carbol fuchsin or auramine and examined under light microscope, the tubercle bacilli typically appear as straight or slightly curved rods. According to growth conditions and age of the culture, bacilli may vary in size and shape from short coccobacilli to long rods. A typical curved shape has been described for *M. microti* (van Soolingen *et al.*, 1998). The dimensions of the bacilli have been reported to be 1-10  $\mu\text{m}$  in length (usually 3-5  $\mu\text{m}$ ), and 0.2-0.6  $\mu\text{m}$  in width.

Therefore, the length of the microorganism is comparable to the diameter of the nucleus of a lymphocyte. Unlike some fast growing mycobacteria and other actinomycetales, *M. tuberculosis* is rarely pleomorphic; it does not elongate into filaments, and does not branch in chains when observed in clinical specimens or culture (Abe, 2003)

In the experimental macrophage infection, intracellular bacilli were described as being significantly elongated compared to broth-grown bacilli and, remarkably, to display bud-like structures (Chauhan *et al.*, 2006). When numerous and actively multiplying, the bacilli are strongly acid fast and show an evident and distinctive tendency to form hydrophobic bundles. Free bacilli can also be seen, though, especially at the border of the swarms. In un-lysed host tissue, the bacilli are more numerous within the phagocytic cells (Chauhan *et al.*, 2006).

Once the disease has been controlled, dying bacilli become sparser, often faintly and unevenly coloured, due to partial loss of the internal contents. Of course, irregular staining may also be the consequence of technical defectiveness of dyes or staining procedures (Abe, 2003).

## **2.6. Nutritional and Environmental Requirements for Growth**

The tubercle bacillus is prototrophic (it can build all its components from basic carbon and nitrogen sources) and heterotrophic (it uses already synthesized organic compounds as a source of carbon and energy). The microorganism macromolecular structure and physiological (metabolic) capabilities result in high adaptation to the specific environment. In turn, the nutritional quality of the environment determines the bacillus lifestyle and limitations, either in the natural habitat or in culture media, as do

various physical conditions such as oxygen availability, temperature, pH and salinity (Rastogi and Sola, 2007).

The bacilli have the ability to adapt to environmental change by adapting to different physiological pathways in order to survive even in harsh conditions. This highly adaptive strategy is not only for pathogenicity but also for persistence, *M. tuberculosis* metabolism may shift from an aerobic, carbohydrate-metabolizing mode to one that is more microaerophilic and utilizes lipids (Segal and Bloch, 1956; Neyrolles *et al.*, 2006).

*In vitro*, the members of the *M. tuberculosis* complex are not fastidious unless damaged by some noxious agents. In fact, the medium used by Koch to cultivate *M. tuberculosis* was simply sterile coagulated blood serum. The tubercle bacilli can also grow in salt solutions using glycerol as a carbon source, ammonium ions and asparagine as nitrogen sources, and micronutrients. *M. tuberculosis* is able to metabolize glycerol into pyruvate, whereas *M. bovis* is not. Indeed, the genome sequence analysis confirmed that all the genes required for the formation of pyruvate are non-functional in *M. bovis*. Being defective in this metabolic process, *M. bovis* grows much better in the presence of a pyruvate salt as a source of carbon. Albumin, which is normally provided by adding eggs or bovine serum albumin to the culture media, promotes the growth of these microorganisms (Neyrolles *et al.*, 2006).

Other subsidiary media components may be used, such as Tween 80, a detergent that disperses the bacilli in liquid media. It was postulated that bovine serum albumin may bind the excess of oleate that can be released from the detergent up to toxic amounts. Biotin and catalase have been incorporated to the Middlebrook series media to stimulate the revival of damaged bacilli in clinical specimens (Wayne and Lin, 1982).

Trace elements, inorganic ions, small molecules and macromolecules have a structural or functional roles in microorganisms' cells. Magnesium and iron are essential for life. A deficiency in these elements frequently reduce the virulence of bacteria pathogens (Rastogi and Sola, 2007), including the tubercle bacillus. As iron is usually in the form of insoluble ferric salts in the environment, special iron systems are required to incorporate this element into the cell. Exochelins and mycobactins are the major siderophores used by mycobacteria to perform this function. The former are hydrophilic peptides secreted into the environment for iron gathering. The latter are hydrophobic compounds located within the cell wall to introduce the iron into the cytoplasm. The *mbt* operon is putatively involved in the synthase activities required to produce the mycobactin core (De Voss *et al.*, 2000).

In nature, the bacillus grows most successfully in tissues with high oxygen partial tension, such as the lungs, particularly the well-aerated upper lobes. Carbon dioxide is essential and may be taken from the atmosphere and also from carbonates or bicarbonates. In the laboratory, an atmosphere of 5 to 10 % carbon dioxide favors culture growth, at least during the early stage of incubation. On the other hand, *M. bovis* is microaerophilic, i.e. it grows preferentially at a reduced oxygen tension (Rastogi and Sola, 2007).

*M. tuberculosis* is mesophile and neutrophile as its multiplication is restricted to conditions offered by warm-blooded animals: about 37°C and a neutral pH. The temperature and hydrogen ion concentration ranges, in which the bacillus is able to multiply, are relatively narrow. High saline concentration such as that found in media containing 5 % sodium chloride, inhibits the growth of the microorganism (Rastogi and Sola, 2007)

## 2. 7 Generation Time

Under favourable laboratory conditions, *M. tuberculosis* divides every 12 to 24 hours. This pace is extremely slow compared to that of most cultivable bacteria, which duplicate at regular intervals ranging from about 15 minutes to one hour. The low multiplication rate of the tubercle bacillus was demonstrated by Chauhan *et al.*, (2006). These authors demonstrated the small proportion of cells initiating the separation process prior to division among tubercle bacilli growing either in broth or inside macrophages (Chauhan *et al.*, 2006).

The slow growth rate might be partially determined by the cell wall impermeability that limits nutrient uptake. However, only a minimal stimulus to bacterial multiplication is achieved when the permeability is increased through treatment with some compounds that interact with the cell envelope (Rastogi and Sola, 2007).

Harshey and Ramakrishnan (1977) identified ribonucleic acid (RNA) synthesis to be a major factor associated with the long generation time of the tubercle bacillus. These authors demonstrated that both the ratio of RNA to DNA and the RNA chain elongation rate are ten-fold lower in *M. tuberculosis* compared to *E. coli*. Another unusual feature is the existence of a unique operon commanding RNA synthesis (Verma *et al.*, 1999).

Furthermore, when the tubercle bacillus switches from the stationary to the active multiplying phase, its total RNA content increases only twofold. Consequently, the protein synthesis must be retarded (Verma *et al.*, 1999). The influence of nutrient availability on the ribosome synthesis rate, which is a proxy of metabolic activity, remains controversial (Hampshire *et al.*, 2004).

## **2. 8. The Mycobacterial Region of Differences (RDs)**

The group *Mycobacterium tuberculosis* complex are characterized by 99.9% similarity based on the nucleotide level and identical 16S rRNA 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, insertions, inversions 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 several non tuberculous mycobacteria (NTM) (Parkash *et al.*, 2009), these region of difference (RD) encompasses > 80kb genomic DNA of *M. tuberculosis* and are predicted to have 89 open reading frames (ORFs) capable of encoding an equal number of proteins (Bahr *et al.*, 1999; Mustafa, 2002).

The region of difference contain genes that encode potential virulence factors such as prophage (RD3, RD11), phospholipases C (RD5), invasins (RD7) and an exopolysaccharide biosystem (RD4) (Cole, 2002). RD1 is the only region that appears to be missing from the vaccine strains BCG and *M. microti* but is present in all virulent member 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. 9 *Mycobacterium tuberculosis***

*Mycobacterium tuberculosis* is one of the etiologic agents of tuberculosis in humans (Schrenzel, 2012), Humans are the main reservoir for the bacterium. Other animals such as primate (Schrenzel, 2012) cattle (Cadmus *et al.*, 2006), small ruminants and wild lives also serve as reservoirs of the organism (Schrenzel, 2012).

*M. tuberculosis* bacilli are straight or slightly curved rods occurring singly and in occasional threads rods and ranging in size from  $0.3-0.6 \times 1-4 \mu\text{m}$ . They stain uniformly or irregularly, often showing banded or beaded forms. They are strongly acid-fast and acid-alcohol-fast as demonstrated by Ziehl-Neelsen or fluorochrome procedures. Growth tends to be in serpentine, cordlike masses in which the bacilli show a parallel orientation. Colonies of a virulent form are less compact (Sneath *et al.*, 1986).

On most solid media, *M. tuberculosis* colonies are rough, raised, and thick, with a nodular or wrinkled surface and an irregular thin margin; may become somewhat pigmented (off-white to faint buff or even yellow). Colonies on oleic acid albumin agar are flat, rough, corded, dry and usually non-pigmented. In liquid media lacking a dispersing agent, it forms a pellicle which, with age, becomes thick and wrinkled. In Dubos' Tween albumin medium, growth is diffuse, settling if undisturbed, but readily dispersed (Barrera, 2007)

Generation time for *M. tuberculosis in vitro* under optimal conditions is 14-15 hours. Optimum temperature for growth is  $37^{\circ}\text{C}$ , though some grow at  $30 - 40^{\circ}\text{C}$ . Optimum pH is 6.4-7.0. Its growth is stimulated by incubation in air with 5-10% added  $\text{CO}_2$  and by inclusion of glycerol to 0.5% in the medium. Bacilli grown under highly aerobic conditions die rapidly on abrupt shift to anaerobiosis; when allowed to grow and settle

slowly through a self-generated oxygen gradient, they adapt a tolerance to oxygen deprivation, and exhibit synchronized growth on resuspension (Wayne and Lin, 1982).

*M. tuberculosis* produces tuberculosis in man, other primates, dogs and some other animals which have contact with man. Experimentally, from inoculum of 0.01 mg, it is highly pathogenic for guinea pigs and hamsters, but relatively non-pathogenic for rabbits, cats, goats, bovine animals or domestic fowls (Mitchison *et al.*, 1963). Inocula of 0.001 mg are used to produce experimental disease in mice. Attenuation of virulence may occur spontaneously upon subculture in artificial media. Virulence can be maintained by selection of appropriate portions of growth on suitable media or by animal passage. Strains of *M. tuberculosis* isolated from patients from southern India may cause localised lesions in guinea pigs and these tend to regress. These strains produce catalase and are susceptible to both hydrogen peroxide and to isoniazid (Mitchison *et al.*, 1963).

Streptomycin, p-aminosalicylic acid, isoniazid, ethambutol, rifampin and some other secondary drugs are used to treat tuberculosis. Spontaneous mutants resistant to one of these drugs may replace the parent strain if treatment is improper. Resistance to isoniazid is frequently accompanied by changes in other properties, such as loss of peroxidase and catalase activity and attenuation of virulence for guinea pigs (Middlebrook and Cohn, 1953; Middlebrook, 1954).

## **2.10 *Mycobacterium bovis***

*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 (Cosivi *et al.*, 1998). *M. bovis* is the agent responsible for bovine tuberculosis, however it can also cause the disease in humans if there is consumption of infected materials. (Cadmus *et al.*, 2006)

Pasteurization of milk has been a major preventive factor in stopping transmission of bovine tuberculosis in humans; however in many underdeveloped countries, where pasteurization is not practiced, there is still a concern with infection by *M. bovis* (Garnier *et al.*, 2003). They are short to moderately long rods. On primary isolation, growth is very poor on glycerol-containing media, although repeated subculture permits adaptation to growth on such media. Furthermore, freshly isolated cultures of *M. bovis* are microaerophilic; inocula dispersed into liquid, semisolid or solid agar media grow in the medium but not on the surface, as distinguished from *M. tuberculosis* which is highly aerobic (Schmiedel and Gerloff, 1965). On repeated subculture, *M. bovis* will adapt to aerobic growth. Dilute inocula on egg media yield small, rounded, white colonies, with irregular edges and a granular surface after 21 days or more of incubation at 37°C. Colonies on transparent oleic acid albumin agar are thin, flat, generally corded; not easily emulsified in absence of a detergent (Schmiedel and Gerloff, 1965).

Strains usually lose catalase on acquiring resistance to isoniazid. Originally isolated from tubercles in cattle; generally more pathogenic for animals than is *M. tuberculosis*. *Mycobacterium bovis* is the primary aetiological agent of bovine tuberculosis. Smith (1898) clearly differentiated it from other types of tubercle bacilli. It has a wider range of pathogenicity for different animal species than any of the other species in the genus (Rich, 1951).

The *M. bovis* genome sequence is 4,345,492 base pairs in length, arranged in a single circular chromosome (Garnier *et al.*, 2003). The genome contains 3,952 genes encoding proteins (Garnier *et al.*, 2003).

The genome is >99.52% identical at the nucleotide level to *Mycobacterium tuberculosis* (Schrenzel, 2012). Comparative sequencing with *M. tuberculosis* revealed 11 deletions from the genome of *M. bovis*, ranging in size from 1-12.7 kb, and have been confirmed by the genome sequence data (Schrenzel, 2012). *M. bovis* contains one unique locus termed TbD1, which is absent from the *M. tuberculosis* strain; therefore, it appears that deletion has been the mechanism in shaping the *M. bovis* genome (Taylor *et al.*, 2007).

*M. bovis* is similar in structure and metabolism to *M. tuberculosis*. *M. bovis* is a Gram-positive, acid-fast, rod-shaped, aerobic bacteria. Unlike *M. tuberculosis*, *M. bovis* lacks pyruvate kinase activity, due to *pykA* containing a point mutation that affects binding of  $Mg^{2+}$  cofactor (Taylor *et al.*, 2007). Pyruvate kinase catalyses the final step of glycolysis, the dephosphorylation of phosphoenolpyruvate to pyruvate (Taylor *et al.*, 2007). Therefore in *M. bovis* glycolytic intermediates are unable to enter into oxidative metabolism (Taylor *et al.*, 2007). Although no specific studies have been performed, it seems that *M. bovis* must rely on amino acids or fatty acids as an alternative carbon source for energy metabolism (Taylor *et al.*, 2007).

The pathology of *M. bovis* is similar to *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 bronchomediastinal lymph nodes (Garnier *et al.*, 2003). Infected cows produce mycobacterial mastitis causing the shedding of the bacteria into milk leading to transmission to humans if the milk is ingested without pasteurization Garnier *et al.*, 2003).

*M. bovis* is the ancestor of the most widely used vaccine against tuberculosis, *M. bovis* bacillus Calmette-Guérin (Garnier *et al.*, 2003) BCG is a strain that was created by growing *M. bovis* on potato slices soaked in ox-bile and glycerol over a period of 13 years (Garnier *et al.*, 2003)

## **2. 11 Other Members of the *Mycobacterium tuberculosis* Complex**

Other members of the MTBC are *M. africanum*, *M. caprie*, *M. microtti* and *M. pinipedi*

### **2.11.1 *Mycobacterium africanum***

*Mycobacterium africanum* is a species from the *M. tuberculosis* complex that include strains which share phenotypic characteristics with *M. bovis* and *M. tuberculosis* (Grange and Yate, 1994). Strains of this species have been isolated from sub Saharan African countries, where it is the cause of human tuberculosis with variable percentages (Niemann *et al.*, 2004; de Jong *et al.*, 2005; 2009).

The species have been classically divided into two groups; the East and West African groups, but recent taxonomic studies have demonstrated that the east African strains are *M. bovis* and the West African strains are the true *M. africanum* species (Niemann *et al.*, 2004). The description of the species includes classical phenotypic characteristics and genotypic markers, including the lack of RD9, the presence of RD12, and a specific *gyrB* gene polymorphism (Niemann *et al.*, 2004)

Disease caused by *M. africanum* is identical to that caused by *M. tuberculosis* (Niemann *et al.*, 2004). *M. africanum* is a human pathogen; however, *M. africanum* has been reported in hyrax (*Procavia capensis*) and milk from domestic cattle (Cadmus *et al.*, 2010; Gudan *et al.*, 2008).

### **2. 11. 2 *Mycobacterium caprae***

In 1999 strains from *Mycobacterium tuberculosis* complex isolated from goats in Spain were described as *Mycobacterium tuberculosis* sub- species *caprae* (Aranaz *et al.*, 1999). The strains share common phenotypic properties with *M. bovis*, although they were susceptible to pyrazinamide. Other properties differentiate these strains from other members of the MTBC. The isolates were subsequently characterised as *Mycobacterium bovis* sub-species *caprae* (Niemann *et al.*, 2002) and finally as a new species, *M. caprae* (Aranaz *et al.*, 1999). Although in the beginning it was described as a cause of tuberculosis in goats, it has been isolated from other mammals in different parts of Europe.

*Mycobacterium caprae* is a pathogen of domestic goats and occurs sporadically in domestic sheep, wild boar, red deer, and humans (Rodríguez *et al.*, 2009; Cunha *et al.*, 2011 and Muñoz *et al.*, 2012). Moreover like *M. bovis*, *M. caprae* has been described as the cause of human tuberculosis.

### **2. 11. 3 *Mycobacterium microti* and *Mycobacterium pinnipedii***

*Mycobacterium microti* is a member of the *Mycobacterium tuberculosis* complex that has been described as the cause of tuberculosis in voles and other animals (van soolingen *et al.*, 1998) and was classically considered non-pathogenic for human. However, recent molecular studies have showed that *M. microiti* can be a cause of disease in humans (van Soolingen., 1998; de Jong *et al.*, 2009), most of them immunosuppressed (van Soolingen., 1998) also immunocompetent patients (van Soolingen *et al.*, 1998; Xavier *et al.*, 2007). Diseases seem to be similar to classical tuberculosis (Schrenzel, 2012).

The natural hosts of *Mycobacterium microti* are voles, wood mice, and shrews, but disease is periodically seen in humans, llamas, camels, badgers, and domestic cats (Zanolari *et al.*, 2009; Xavier *et al.*, 2007).

*Mycobacterium pinnipedii* is the most recently described member of the *Mycobacterium tuberculosis* complex (Cousins *et al.*, 2003). It has been described as a cause of diseases among seals but also among other animals. A report suggests the possibility of human infection (Kiers *et al.*, 2008), infection with *M. pinnipedii* has been recently been identified in a camel and Malayan tapirs. Spoligotyping of the isolates identified the source and probable routes of transmission among the animals at two zoological facilities, allowing measures to be taken to prevent future infections (Kiers *et al.*, 2008; Stetter *et al.*, 1995; Thorel *et al.*, 1998; Smith *et al.*, 2011 and Twomey *et al.*, 2012). Infection has also been found in humans but is very rare (Kiers *et al.*, 2008).

#### **2. 11. 4 *Mycobacterium canettii***

The name “*Mycobacterium canettii*” has been applied to the *Mycobacterium tuberculosis* strains which have glossy and smooth colonies, a rare finding among this species (van Soolingen *et al.*, 1997). Although cases of human tuberculosis caused by these strain have not been described, it is not considered as a separate species or sub-species in the *Mycobacterium tuberculosis* complex (Schrenzel, 2012).

#### **2. 12 Immunology against Tuberculosis**

The re-emergency of tuberculosis worldwide has led to the increasing research efforts directed at examining the host defence and pathological mechanisms operative in *Mycobacterium tuberculosis* complex infections. The immunological response of the host lies mainly in the role of macrophages, T- cells, and the cytokines/ chemokine

network in providing immunity against infections. While so much has been about the immunology of human tuberculosis, Neill *et al.* (2005) believed that not much has been done intense of bovine tuberculosis. However, as in human, bovine tuberculosis appears complex, involving a multiplicity of interactions and infection does not usually lead to active disease. The immune response mounted to the infection is generally successful in containing the infection, although not strong enough to eliminate the pathogen. In most cases of tuberculosis infection, the individual is asymptomatic and non-infectious in animals including humans (Kaplan *et al.*, 2003). These clinical latency often extends for lifetime. However, reactivation of the latent infection can occur in response to perturbation of immune response and active tuberculosis ensues (Kaplan *et al.*, 2003). Infection with human immunodeficiency virus, treatment with corticosteroids, aging, alcohol or drug abuse and malnutrition increase the potential for reactivation of latent tuberculosis (Chan and Flynn, 1999).

## **2. 12. 1 Innate immune response**

### **Neutrophils leucocytes**

Even though macrophages are considered the main targets for infection by *Mycobacterium tuberculosis* complex, it has been proposed that other cell populations can also be infected by mycobacteria and therefore may be important in the development of the disease (Pedrosa *et al.*, 2000).

Characteristically, neutrophils are among the earliest cells recruited into site where any noxious agent enters into the body and or inflammatory signals are triggered (Hernandez- Pando *et al.*, 2007). They also have well characterized microbicidal mechanism such as those dependant on oxygen and the formation of neutrophil extra cellular traps (Urban *et al.*, 2006).

The role of neutrophils in tuberculosis is controversial, the presence of neutrophils at the beginning and several days after infection (Pedrosa *et al.*, 2000; Fulton *et al.*, 2002) were thought to have an important role in the control of mycobacterial growth. The elimination of neutrophils before infection in an experimental study lead to increase growth of mycobacterium in the lungs while treatment of mice with agent that increase neutrophils decrease bacillary growth rate (Appelberg *et al.*, 1995; Fulton *et al.*, 2002). There were reports of neutrophils been able to kill mycobacteria (Jones *et al.*, 1990). It is believed that the function of neutrophils goes beyond their microbiocidal activities. Therefore, neutrophils are thought to contribute to the control of infection through the production of chemokine (Riedel *et al.*, 1997), the induction of granuloma formation (Ehlers *et al.*, 2003) and the transfer of their own microbicidal molecules to infected macrophages (Tan *et al.*, 2006).

Aside the above functions, neutrophils have been reported to responsible for pathology rather than the protection of the host. Animal susceptible to TB were found to have a larger and longer accumulation of neutrophils in TB lesion compare to TB resistant animals (Eruslanove *et al.*, 2005)

### **Mast cells**

Mast cells are effector cells with relevant roles in allergic reactions (Woodbury *et al.*, 1984; Miller, 1996; Galli *et al.*, 1999; Williams and Galli, 2000). Mast cells are important in the development of T-helper (Th2) response (Metcalf *et al.*, 1997; Galli *et al.*, 1999), they are found in the mucosa of the respiratory, gastrointestinal, and urinary tracts and can also be observe in the vicinity of blood and lymph vessels.

Mast cells have receptor with high affinity for IgE, upon the union of the antigen to the active sites bound IgE, mast cell liberate molecules including preformed mediators and mediators synthesised de novo (Metzger, 1992; Turner and Kinet, 1999; Williams Galli, 2000).

Beside the interaction between IgE and antigens, other agents are able to stimulate the activation of mast cells and the liberation of cytokines and other mediators (Ferber *et al.*, 2002; Supajatura *et al.*, 2002; Sabroe *et al.*, 2002; Di Nardo *et al.*, 2003; McCurdy *et al.*, 2003).

Due to their distribution within the lung, mast cells play fundamental role in the immune response of the host against mycobacteria. Ratnam *et al.* (1977) demonstrated an increased number of mast cells and their degranulation in the lungs of animals experimentally infected with *Mycobacterium tuberculosis*.

Munoz *et al* (2012) described the interaction between mast cells and *M. tuberculosis* through the CD48 molecules. The secretory proteins, *Mycobacterium tuberculosis* secretory proteins (MTSA-10) and 6- kilodalton (6-KDa), early secretory antigenic target (ESAT-6) contribute to the activation of mast cells for the liberation of their pro-inflammatory mediators (Trajkovic, 2004; Munoz *et al* ., 2012).

### **Macrophages**

The macrophage are the paradigmatic cells with regards to MTC infection, alveolar macrophages have been shown to play an essential role in the elimination of particles that enters the host through the airways ; and have long been considered the first cell population to interact with tubercle bacilli (Danneberg *et al.*, 1991)

The initial interaction of tubercle bacilli with macrophages takes place through cellular receptor (Schlesinger *et al.*, 1990; 1993; Peterson *et al.*, 1995; Zinnerli *et al.*, 1996; Randhawa *et al.*, 2005). Reaction with Fc receptor increase the production of reactive oxygen intermediates and allows the fusion of the bacteria containing the phagosome with lysosomes (Armstrong *et al.*, 1975), on the other hand interaction with complement receptor -3 (CR-3) prevents the respiratory burst (Le Cabec *et al.*, 2000) and block the maturation of phagosome harbouring the bacteria thus preventing fusion with lysosome (Sturgill-Koszycki *et al.*, 1994).

The interaction of mycobacteria with members of the toll- like receptors are activated by mycobacteria component such as the 19-KDa lipoproteins and lipoarabinomannan (LAM) activate macrophages through TLR-2, promoting the production of IL-12 and inducible nitric oxide synthase (iNos) (Brightbill *et al.*, 1999).

Cellular cholesterol present in the macrophage cell membrane play essential role in the internalization of the bacteria (Gatfield and Pieters, 2000)

Once the bacteria enter the macrophage, they generally locate themselves in the mycobacteria phagosome in contrast to normal phagocytosis, during which the phagosomal content is degraded upon fusion with lysosomes, the mycobacteria block this process. The inhibition of phagocytosis in an active process is induced by viable mycobacteria (Armstrong and Hart, 1971; 1975). Besides having a different morphology, the vacuole in which the bacteria reside present early endosomal component markers instead of the characteristic late endosomes (Clemens, 1996; Hasan *et al.*, 1997). These mycobacteria phagosome also retain early markers such as the Rab5 and Rab14 GTPase and do not acquire the late Rab7 molecule, this is also

observed in blockage of the maturation process from early to late endosome (Via *et al.*, 1997; Kyei *et al.*, 2009).

Another factor which inhibit phagocytosis of mycobacteria in the macrophage is the limited acidification with resultant low concentration or zero concentration of vesicular proton- pump adenosine triphosphatase (V-ATPase) in the mycobacteria phagosome (Sturgill-Koszycki *et al.*, 1994)

Ferari *et al.* (1999) demonstrated the inability of mycobacteria phagosome to mature was due to the retention of a protein present in phagosome known as tryptophan aspartate coat protein (TACO). TACO binds to the plasmatic membrane of macrophages through cholesterol, this shows that both molecules play important role in mycobacterial mechanism for survival (Gatfield and Pieters, 2000)

The inhibition of phagosome maturation may be reverted by cytokines such as interferon- gamma (INF- $\gamma$ ) and tumour necrosis factor-  $\alpha$  (TNF- $\alpha$ ) (Flesch and Kaufman, 1990; Chan *et al.*, 1992). Hydrogen peroxide produced by macrophages activated by cytokines has a mycobacteriocidal activity (Walter *et al.*, 1981).

Also tubercle bacilli present molecules, such as LAM and phenolic glycolipid 1 which work as oxygen radical scavenger molecules (Chan *et al.*, 1991).

### **Dendritic cells**

These cells are found in large number in TB lesion (sturgill-Koszycki *et al.*, 1994; Pedroza-Gonzalez *et al.*, 2004; Garcia- Ramo *et al.*, 2004), hence they may play a vital role in the protection against tuberculosis.

Dendritic cells function as antigen presenting cells (APCs) in the cortex of major histocompatibility complex (MHC) molecules as well as through CD1 (Banchreau and

Steinman, 1998; Gumperz and Brenner, 2001). Dendritic cells bind antigen via C- type lectin receptors and Fc receptors and internalize them by endocytosis (Jiang *et al.*, 1995; Fanger *et al.*, 1996; Engering *et al.*, 1997). *Mycobacterium tuberculosis* endocytosis is carried out via known C- type lectin receptors, such as dendritic cell – specific intercellular adhesion molecule – grabbing non-integrin (DC-SIGN) (Geijtenbeek *et al.*, 2003; Tailleux *et al.*, 2003) this molecule interact with manose capped LAM present in mycobacteria cell wall (Figdor *et al.*, 2002; Geijtenbeek *et al.*, 2003).

Peripheral blood and immature dendritic cells derived from monocytes express TLR-2 and TLR- 4 (Jarrossay *et al.*, 2001; Kadowaki *et al.*, 2001). These interaction seen to induce protective host response.

Internalization of mycobacteria into human and murine dendritic cells have been observed in several in vitro (Inaba *et al.*, 1993; Henderson *et al.*, 1997; Fortsch *et al.*, 2000; Bodnar *et al.*, 2001; Giacomini *et al.*, 2001; Hanekom *et al.*, 2003) and in vivo ((Jiao *et al.*, 2002; García-Romo *et al.*, 2004; Pedroza- González *et al.*, 2004). The ability of infected dendritic cells derived from the monocyte to present lipidic antigen is impaired, thus expression of CD1 decreases (Stenger *et al.*, 1998). Component of mycobacteria cell walls were demonstrated to inhibit maturation of dendritic cells induced by lipopolysaccharides.

In a protective immune response, dendritic cells induce maturation of T cells towards a T- helper 1 (Th1) profile by secreting cytokines, such as IL-12, IL-18, IL- 23, and probably IFN- $\alpha$  and  $\beta$ , but not IFN- $\gamma$  (Thurnher *et al.*, 1997; Kalinski *et al.*, 1999; Kadowaki *et al.*, 2001; Wozniak *et al.*, 2006). Th1 cells expand in response to the BCG antigens presented by the dendritic cells in the lymphoid nodules and migrate toward

infection sites, such as the lung tissue, where they liberate IFN- $\gamma$ , thus activating local macrophages that control bacilli replication (Humphreys *et al.*, 2006).

### **Natural killer cells (NK)**

Natural killer cells are effector cells of innate immunity, they lyse pathogen directly or indirectly by lysing infected cells monocytes (Raja, 2004). NK produce INF- $\gamma$  and can lyse mycobacterium pulsed target cells (Molloy *et al.*, 1993).

In a study carried out by Nirmala *et al.* (2001), they showed that lowered NK activity in TB infection is probably due to the effect and the cause for the disease. The combine administration of NK and cytokines demonstrated that they can act as adjuvants to TB chemotherapy.

It has been shown that NK are not essential for host resistance, although they are activated during the early response in pulmonary TB (Hernandez-Pando *et al.*, 2007).

### **Epithelia cells**

The member of epithelia cells in the aveoli is 30 times higer than the number of macrophages therefore, it is likely they are the first cell to be exposed to infecting bacilli. Mycobacteria DNA was detected in epithelia cells (endothelia cells) (Hernandez-Pando *et al.*, 2000).

Epithelia cells can host mycobacteria and allow their replication, they are able to establish an initial inflammatory environment by secreting IL-8 (Bermudez and Goodman, 1996) and induce the production of nitric oxide (Roy *et al.*, 2004).

### **2.12.2 Acquired immune response against mycobacteria**

Specific or acquired immunity requires the recognition of specific foreign antigen. Specific immunity can be divided into cell mediated immune response consisting of T-

cell activation and effector mechanism, and humoral immune response consisting of B-cells maturation and antibody production, both mechanisms work together mutually; and T- helper cells are require for antibody maturation, isotype switching and memory. B- Cells also function as antigen presenting cells by T- cells in a specifically driven manner (Collins, 2002).

Mycobacteria are perfect example of intracellular bacteria that persist for a long period within the host, causing latent infection in a chronic asymptomatic infection without pathological changes in the host tissues (Colins, 2002). These bacilli remain within the host in a state of dormancy, although, the host cell mediated immunity mount enough response to control the progression of disease but it is not adequate to exert sterile eradication and hence 90% of animals and persons infected surfer the latent form of TB (Collins, 2002)

Mycobacteria reside inside macrophages and is relatively resistant to microbicidal mechanism that efficiently eliminate other phagocytized bacteria, this is due in part to the ability of the tubercle bacilli to hinder macrophage activation by INF- $\gamma$  and IL-12 (Jouanguy *et al.*, 1999). The role of lung antigen presenting cells has not been adequately address in TB immunity, information on the role of lung antigen presenting cells during pulmonary TB can help better understanding of induction of specific immune response against mycobacteria, and therefore the development of tools that could control the disease effectively (Colins, 2002).

### **Humoral immune response**

Antibody immune response were considered not to be protective against mycobacteria infection because of their residence intracellulaly (Collins, 2002).

The role of antibody immune response against intracellular bacterial infection have been elucidated (Salinas-Carmona and Perez-Rivera, 2004; Williams and Galli 2004; Reljic *et al.*, 2006).

The use of antibodies against TB include, enhance immunity through neutralization of toxin, opsonization, complement activation, promotion of cytokines release, antibody dependent cytotoxicity and enhance antigen presentation (Costello *et al.*, 1992; Teitelbaum *et al.*, 1998; Hoft *et al.*, 1999; Hoft *et al.*, 2002; Williams and Galli, 2004; De Vallière *et al.*, 2005). De Valliere *et al.* (2005) showed that specific antibodies increased the internalization and killing of *M. bovis* BCG by neutrophils and macrophage/monocytes.

Surface antigens such as LAM or protein expressed under stress conditions such as alpha crystalline protein have been shown to be important in the protection of the host against mycobacteria infection (Brown *et al.*, 2003). The use of passive inoculation of IgA antibodies was shown to protect against TB in an experiment using mouse model (Williams and Galli, 2004). The duration of protection was extended by inoculation of INF- $\gamma$  three days before and after infection and further co- inoculation of with IgA at different time point (2h, 2 and 7 days) after aerosol infection with *M. tuberculosis*H37RV (Reljic *et al.*, 2006).

Therefore there is need to further research on the role of antibody response to TB in order to exploit their potential for vaccine development and immunotherapeutic tools.

### **Cell – mediated immunity against TB**

Since the tubercle bacilli reside inside a compartment within the macrophage, their antigens are presented by MHC class II molecules to CD4<sup>+</sup> T lymphocytes. These cells play an important role in the protective response against *M. tuberculosis* and, when

they are absent, growth of the bacilli cannot be controlled (Muller *et al.*, 1987; Caruso *et al.*, 1999). This is the case in patients with an immunodeficiency, such as that caused by HIV infection.

The main function of CD4<sup>+</sup> T cells is the production of cytokines including IFN- $\gamma$ , which activates macrophages and promotes bacilli destruction. Another function has been ascribed to these cells, i.e., helping to develop the CD8<sup>+</sup> T cell mediated response (Scanga *et al.*, 2000). In the same way, CD4<sup>+</sup> T cells may participate in the induction of apoptosis of infected cells and the subsequent reduction of bacterial viability through the CD95 Fas ligand system (Oddo *et al.*, 1998).

The participation of CD8<sup>+</sup> T cells in the control of the infection is well recognized (Flynn *et al.*, 1992). Mice deficient in molecules such as CD8 $\alpha$ , transporter associated with antigen processing (TAP), and perforin, were shown to be more susceptible to *M. tuberculosis* infection than animals which produced these molecules (Flynn *et al.*, 1992, Behar, 1999).

The mechanisms used by these cells for the control of TB seem to be mainly cytokine production and bacterial lysis.

In the lungs of infected mice, CD8<sup>+</sup> T cells showed to be able to secrete IFN- $\gamma$  through activation of the T-cell receptor or by interaction with infected dendritic cells (Serbina and Flynn, 1999). Once again, the function performed by this IFN- $\gamma$  is the activation of the macrophage and promotion of bacterial destruction.

In addition, CD8<sup>+</sup> T cells proved to be efficient in lysing infected cells and in reducing the number of intracellular bacteria (Stenger *et al.*, 1997). The mechanisms of control of the bacterial load seem to be associated with granular exocytosis involving perforin

and granzymes. Still, granulysin, which is found in CD8<sup>+</sup> T granules, is the molecule responsible for killing the bacterium (Stenger *et al.*, 1998).

## **2. 13 Pathology of Tuberculosis**

### **2. 13. 1 Development of lesions**

In an experimental study, Cassidy *et al.* (1998) describe the development of lesions as follows: Microscopic lesions could be observed in cattle infected experimentally as early as seven (7) days post infection (PI), gross lesion was not observed until after 14 days PI. At day 14 post infection WC1<sup>+</sup> and CD2<sup>+</sup> T-cells were found within the granuloma, increasing number of acid fast bacilli were not seen within lesion until 14 days PI. At 21 days PI CD2<sup>+</sup> T- cells could be seen within the necrotic area of the lesions which was surrounded by macrophages. At 21 days PI necrotic area was characterized by the presence of intact and degenerated neutrophils, this area (necrotic) was associated with acid fast bacilli. At 41 days PI mineralization set in characterize by moderate encapsulation with the presence of large number of lymphoid cells. (Cassidy *et al.*, 2001).

Cassidy *et al.* (2001) and Pollock *et al.* (2001) observed the presence of r $\alpha$ t, CD4<sup>+</sup> and CD8<sup>+</sup> T cells in lymphoid cells; the presence of these cells helps in the containment of the bacilli through cytokine release (IL-12 and INF- $\gamma$  for example), which stimulate macrophage activation and chemokine release to recruit T- cell involved in the development of acquired immune response (Cassidy *et al.*, 2001).

A small portion of cattle with tuberculosis lesions have measurable antibody response (McNair *et al.*, 2001), this may be due to the development of anergy (Pollock *et al.*, 2005; Houlihan *et al.*, 2008). However, IgG has been associated with lesions development and may be useful indicator of disease status (Lightbody *et al.*, 2000).

## **2. 13. 2 Gross pathology**

Bovine tuberculosis is characterized by the development of granulomas (tubercles) where bacteria have localized. The granulomas are usually yellowish and either caseous, caseo- calcareous or calcified and often encapsulated (OIE, 2009).

In a study undertaken by Liebana *et al.* (2007) they examined 200 tuberculin reactors for post-mortem lesions, 55.5% showed visible lesion while 44.5% do not show characteristic lesions of TB.

Gross lesions were more in the thoracic cavity than in other regions of the body, the mediastinal lymph nodes are most commonly affected in TB confirmed animals while the tonsils are least affected. In the lungs, the lesions are multiple coalescing foci of caseous necrosis surrounded by thin pale fibrous tissue capsule (tubercles) (Kumar *et al.*, 2008).

The lung parenchyma is almost entirely replaced by variably sized, coalescing, raised, pale nodules. Most of the lymph nodes are replaced by caseonecrotic debris with a laminated appearance reminiscent of caseous lymphadenitis (OIE, 2009), in pigs the center of the lymph nodes is replaced by caseous, mineralized debris (OIE, 2009), while the liver looks pale and slightly raised granulomas are disseminated throughout all liver lobes (OIE, 2009).

In bovine uterus, the endometrium contains numerous raised tubercles (DNR, 2013), the organism is also responsible for endometritis in women (Kumar *et al.*, 2008).

In some species such as deer, the lesions resemble abscesses rather than typical granuloma. Some tubercles are so microscopic that they cannot be seen with the naked eyes except with the aid of microscope in a sectioned tissue (Kumar *et al.*, 2008).

## **2. 14 Transmission of Bovine TB**

*M. bovis* can be transmitted by inhalation of aerosol, by ingestion or through break in the skin. The important of the routes varies between species (OIE, 2009)

Venereal transmission through artificial insemination (AI) had been documented (Wentink *et al.*, 2000). Aerosol transmission occur usually where animals are in closed contact, thus animal density play a major factor in the transmission of *M. bovis* (DNR, 2013).

Bovine tuberculosis is usually maintained in cattle populations (OIE, 2009) and a few others can become reservoir.

Transmission of tuberculosis from cattle to human mostly occur through consumption of unpasteurized milk, closed contact with infected animal (Michel *et al.*, 2010 ) and air borne transmission (Vekemans *et al.*, 1999; WHO, 2009).

## **2. 15 Global Epidemiology of Bovine Tuberculosis**

Bovine tuberculosis is a disease caused mostly by the bacterium *Mycobacterium bovis* (Smith *et al.*, 2006), *Mycobacterium capriae* (Brosch *et al.*, 2002), *Mycobacterium tuberculosis* has been reported as the cause of tuberculosis in cattle (Cadmus *et al.*, 2006) as well as other members of the MTbC (Cadmus *et al.*, 2010; Jenkins *et al.*, 2011).

The disease is chacterized by the formation of granulomas mostly in the thoracic cavity (Shitaye *et al.*, 2006) and other parts of the body including the genitalia.

Tuberculosis in cattle is a disease of economic and zoonotic importance. The disease is distributed worldwide, with Africa and Asian countries ranking highest in terms of disease burden (WHO, 2004). In developed countries, bovine tuberculosis had been

controlled through test and slaughter method (Cosivi *et al.*, 1998; Ayele *et al.*, 2004; Amanfu, 2006; Smith *et al.*, 2006; Theon *et al.*, 2006), among the industrialized countries bovine tuberculosis is still endemic in Australia and the Caribbean Island (Tweddle and Livingstone, 1994).

The increasing incidence of bovine tuberculosis in the UK had been attributed to burgers which act as reservoir of the causative agent in the wild (Gallagher and Clifton-Hadley, 2000). Wherever there is cattle there is bovine tuberculosis (Smith *et al.*, 2006). The disease has been reported in all continents and for most countries were the burden is high neither surveillance nor control programs exist (Theon *et al.*, 2006).

#### **2. 15. 1 Bovine tuberculosis in Africa: The livestock and zoonotic situation**

The situation of bovine tuberculosis is completely different in Africa; with the emergency of HIV/AIDS, poverty and other debilitating diseases in Africa, bovine tuberculosis continue to increase in prevalence in both animals and humans, these situation have potential impact on human health directly and threat to human livelihood by compromising sustainable food supply, income and social status (WHO, 2006). Although studies in recent time have provided information on the important of zoonotic bovine tuberculosis in Africa, the extent of the disease in man and livestock is still largely unknown (Michel *et al.*, 2010).

In Nigeria, most of the studies were based on pathological examination at the abattoir (Igbokwe *et al.*, 2001; Ameen *et al.*, 2008; Aliyu *et al.*, 2009; Kwaghe *et al.*, 2011) and tuberculin skin testing in herd (Yohanna *et al.*, 2008; Danbirni *et al.*, 2012; Ibrahim *et al.*, 2012) with very few works on bacteria isolation and molecular characteristic (Cadmus *et al.*, 2004; 2006; 2009; 2010; Jenkins *et al.*, 2011).

Majority of bacteria isolation and molecular typing were carried in the South-western part of the country, where livestock population is low compared to the Northern part of the country where cattle population are higher (Blench, 1999).

Cadmus *et al.* (2011) shows that strains of *M. bovis* with the spoligotype pattern SBO944 were dominant in cattle slaughtered in Ibadan, they also demonstrated that this molecular type is also identical to *M. bovis* isolated from cattle in neighboring countries. Hence indicates possibility of Trans -border transmission.

Bovine tuberculosis is present in Ethiopia with a prevalence rate ranging from 3.4% in small hold production system to 50% in peri urban dairy production system (Amani *et al.*, 2007; Bogale *et al.*, 2001). *M. bovis* had been isolated from lymph nodes of slaughtered cattle (Kazwala *et al.*, 2001) while the presence of the disease had been reported by several researchers (Markhan, 1952; Jiwa *et al.*, 1997), Ethiopian native zebu cattle were found to be more resistant to infection by *M. bovis* than exotic Holstein- Friesian cattle (Vordermeier *et al.*, 2012). In another study by Tsegaye *et al.* (2010) 53.6% of herd were positive for bovine tuberculosis while individual animal prevalence rate was 34.1%; the high prevalence rate of bovine tuberculosis in Ethiopia, is an indication of the implication of the absence or inadequate control of bovine tuberculosis in poor African countries. Tschopp *et al.* (2009) examined the risk factors of bovine tuberculosis in cattle in rural livestock production system in Ethiopia, in this study they observed that central Ethiopia has the highest prevalence rate while the North had the lowest rate of BTB, 19% of household assessed for potential risk factors of disease transmission between cattle and human were diagnosed with TB or showing signs of pulmonary TB (Tschopp *et al.*, 2009).

In a study in Uganda, Olaya *et al.* (2007) reported a prevalence of 60% of 1522 cattle tested among nomadic transhumance cattle, drinking water from mud hole during dry seasons and introduction of new animal are risk factors that might be targeted to control BTB in transhumance area (Olaya *et al.*, 2007). In another study in Uganda, Faye *et al.* (2005) reported 6% and 74.1% prevalence in individual and herd respectively.

Awah-Ndukum *et al.* (2010) reported a prevalence rate of 31.0% using Ziehl Neelsen stain, 51.0% culture and 60.0% antibody detection of tested cattle in Cameroon. Bovine tuberculosis is high in exotic breed than in gudali and white Fulani ; and that the rate of BTB is higher in meat (beef) production than in others while large herd are less affected than smaller herd (Awah-Ndukum *et al.*, 2012).

In Chad, Ngandolo *et al.* (2009) reported a prevalence rate of 7.7% while in Ghana, 71.7% was reported in cattle slaughtered in Kumasi in metropolitan abattoir (Adu- bobi *et al.*, 2009) and 3.7% was reported in Niger (Boukaery *et al.*, 2011).

In Mali, Muller *et al.* (2008) reported the molecular characteristic of *M. bovis* from slaughtered cattle at the Bamako abattoir, they observed that 13 of the strain lack spacer 30, a characteristic common among strains of *M. bovis* found in West Africa and 6 had spoligotype pattern identical to strains commonly isolated in France and Spain.

Like in Nigeria most of the studies carried out in Africa relied on tuberculin testing and gross pathological examinations of tissue samples from abattoir.

### **2. 15. 2 Host rang**

The genetic evolution of the *Mycobacterium bovis* and its transition from a common ancestor with *Mycobacterium tuberculosis*, the major cause of tuberculosis in humans through gene loss (Brosch *et al.*, 2002) resulted to its ability to infect cattle. The switch

in host from human to cattle appears to have broaden *M. bovis*'s infective capacity (Nugent, 2011a, b). *Mycobacterium bovis* has the broadest host range of known zoonotic pathogen (O'Reilly and Daborn, 1995; Schrenzel, 2012). The IS6110 transpositions in *M. bovis* was suggested as the driving force in the adaption of *M. bovis* from animal to human host (De Kantor *et al.*, 2008). The organism can infect a wide range of host, ranging from wildlife to domestic animal (livestock and pets) and man.

In the UK, the maintainance of *M. bovis* by wildlife species had been incriminated as the obstacle militating against control of zoonotic bovine tuberculosis in both cattle and man (Corner, 2006; Smith *et al.*, 2006)

### **2. 15. 3 Bovine tuberculosis in man**

*M. bovis* had been isolated from mummy dated back to the Stone Age (Taylor *et al.*, 2007). In a research by de Kantor *et al.* (2008), they observed that *M. bovis* was present in human in Latin America, they also observed that the non-inclusion of pyruvate containing media appropriate to isolate *M. bovis* contributed to an understanding of the problem. They reported a prevalence of 0.34% - 1.0% of *M. bovis* among tuberculosis patients.

In another study in the United States of America from 1995 – 2005, 1.4% of 11860 cases of tuberculosis in human were identified as *M. bovis* (Hlavsa *et al.*, 2008) the isolates used in this study were obtained from retrospectively submitted isolate of selected cases, and the initial interest may not be targeted towards *M. bovis* isolation, hence, the low prevalence rate.

Rodwell *et al.* (2010) implicated cattle in Mexico as the origin of *M. bovis* infection in humans in the USA. With the help of spoligotyping technique, they discovered that over

91% of human *M. bovis* infections had spoligotype pattern that were identical to those isolated from cattle in Mexico. *M. bovis* accounted for 34.9% among tuberculosis-HIV/AIDS co – infection, they demonstrated that abdominal diseases were strongly associated with *M. bovis* disease (Park *et al.*, 2010). This finding seen to substantiated the theory that zoonotic tuberculosis is mostly transmitted through oral route and *M. bovis* is largely responsible for mesenteric disease (Leao and Portaels, 2007; Pollock and Neill, 2000; Biet *et al.*, 2005).

In Nigeria and other African countries *M. bovis* had been reported as the cause of tuberculosis in man.

In a study by Jenkins *et al.* (2011) *M. bovis* were isolated from two patients with pulmonary tuberculosis, suggesting aerosol transmission of *M. bovis* to man, one of the *M. bovis* isolated from human in this study had spoligotype pattern (SB0944) identical to the *M. bovis* strains isolated in this area (Cadmus *et al.*, 2011) which also share identical spoligotype pattern to 4 other *M. bovis* strains the study (Jenkins *et al.*, 2011). With the help of deletion typing, Cadmus *et al.* (2005) showed that 13% of tuberculosis disease in human were caused by *M. africanum* and *M. bovis* rather than tuberculosis, they further demonstrated a closed similarity of *M. bovis* isolated in Nigeria to those reported in Cameroon (Cadmus *et al.*, 2005). *M. bovis* had been isolated from unpasteurized milk samples from Northern Nigeria (Cadmus *et al.*, 2005).

Cattle in china were identified as maintenance host of *M. bovis* and *M. tuberculosis*, hence pose challenge to the stop TB plan for human in china (Chen *et al.*, 2009), their finding revealed that cattle harbor Beijing family strains of *M. tuberculosis* and that 0.34% of tuberculosis patients were diagnosed of *M. bovis* infection. The authors concluded that TB in high burden countries like China were bovine and human

tuberculosis coexist, the fact that cattle maintain both *M. bovis* and *M. tuberculosis* would be a potential challenge to both stop TB plan of human and bovine tuberculosis eradication scheme.

## **2. 16 Diagnosis of Tuberculosis in Cattle and Man**

On-farm *in vivo* skin testing of cattle, with subsequent abattoir inspection and laboratory monitoring for disease has been pivotal in all national programs for control and eradication of bovine tuberculosis. Diagnostic accuracy (i.e. test sensitivity and specificity) is therefore of paramount importance, particularly where there is potential for immune exposure to environmental, non-tuberculosis causing mycobacteria, or where there is the possibility of mycobacterial vaccines being used (Pollock *et al.*, 2001).

Delayed type hypersensitivity reactions, demonstrated using intradermal skin testing with tuberculin purified protein derivatives, have proved effective for diagnosis in most instances, as this highlights CMI responses, the primary reactions following *M. bovis* infection in cattle (Pollock *et al.* 2001). Where cattle trade is important, tuberculin skin testing remains the definitive diagnostic assay for tuberculosis. However, eradication of tuberculosis from cattle in some countries has been unexpectedly protracted and this has raised questions about the effectiveness of skin testing, particularly when the incidence of disease in the population is low or where there is potential of contact with environmental mycobacteria. Therefore recent research has focused on developing alternative and complementary testing procedures and searching for antigens that are more specific than the relatively crude but complex antigen mixture of constituents in tuberculin (Pollock *et al.*, 2001; Wood and Jones, 2001).

There has been renewed interest in applying the latest technologies to serological diagnosis of bovine TB (Lyashchenko *et al.*, 2004), but the sensitivity and specificity of most diagnostic tests developed in recent years have proved inadequate for general acceptance. However, an assay, based on the detection of antigen-specific bovine interferon gamma responses has proved most promising in field trials in many countries and is now becoming widely acceptable as a reliable diagnostic test for bovine tuberculosis (Wood and Jones, 2001). Similarly, there was significant interest in low molecular weight proteins secreted from tubercle complex mycobacteria, some of which have proved to be dominant antigen targets in tuberculous cattle (Pollock and Anderson, 1997). Application of these antigens in assays when testing cattle for tuberculosis in Northern Ireland and New Zealand, has yielded promising results (van Pinxteren *et al.* 2000; Pollock *et al.*, 2001). From sequencing studies, it is apparent that a large amount of *M. tuberculosis* genome coding capacity is devoted to lipid metabolism. A surprise finding was the identification of two families of unusual glycine-rich acidic proteins, the PE and PPE families. These proteins are over-represented in analyses aimed at defining genes essential for viability (Lamichlane *et al.*, 2003), they have a potential role in host-pathogen interaction or immune evasion and are targets of the host immune system (Plotkin *et al.*, 2004).

A family of more than 20 proteins, secreted by some members of the *M. tuberculosis* complex, was also identified. These included the small proteins, ESAT-6, CFP-7 and CFP-10, and are already known to be potent T-cell antigens in humans and cattle and candidates for improved diagnostic tests, with potential to differentiate infected and vaccinated individuals. Exploiting the sequence information more fully will undoubtedly identify combinations of components, with appropriate immunological

recognition in cattle infected with *M. bovis*, to better assist diagnosis (Pollock *et al.*, 2001).

A requirement for future sensitive tests should be differential ability, for example, capable of: detecting cattle that have been exposed to *M. bovis*, without developing disease; identifying cattle that pose the threat of spreading infection; and distinguishing vaccinated cattle from those infected with *M. bovis*. Genomic approaches have begun to be applied to the characterization of useful *M. bovis* antigens, and novel candidates are being identified (Brosch *et al.*, 2002; Cockle *et al.*, 2002; Aagaard *et al.*, 2003).

PCR has been the most studied of the different molecular techniques that exist for the species identification of Mycobacterium (Anon, 2009). It is widely used in all wildlife species for differentiation of mycobacteria of the *M. tuberculosis* complex from non-tuberculous mycobacteria, as well as for more specific differentiation of *M. bovis* from other members of the *M. tuberculosis* complex (Miller *et al.*, 1997; Harmsen *et al.*, 2003; Cleaveland *et al.*, 2005; van Helden *et al.*, 2008). It can be performed after culture or directly in the suspect samples (Angkawanish *et al.*, 2010), but the latter approach demands a sufficiently high bacterial load, as obviously it is influenced by irregular shedding of the bacteria (Clifton – Hadley *et al.*, 1993).

## **2. 17 Control of Tuberculosis**

Control programs for tuberculosis in animals are primarily focused on control of infection with *M bovis*. These programs can be considered as having 4 components: prevention, treatment, eradication, and surveillance (Kaneene and Theon, 2004)

Bovine tuberculosis can be controlled by test-and-slaughter or test-and-segregation methods. Affected herds are re-tested periodically to eliminate cattle that may shed the

organism; the tuberculin test is generally used. Infected herds are usually quarantined, and animals that have been in contact with reactors are traced. Only test-and-slaughter techniques are guaranteed to eradicate tuberculosis from domesticated animals (Anon, 2009).

Once eradication is nearly complete, slaughter surveillance, with tracing of infected animals, may be a more efficient use of resources (Anon, 2009). This includes antemortem testing and slaughter surveillance of livestock and captive animal species (Kaneene and Theon, 2004). The policy has numerous constraints in developing countries. Alternative strategies (e.g. programs based on slaughter house surveillance or trace back of tuberculosis (TB) animals to herds of origin) may be technically and economically more appropriate in these countries (Cosivi *et al.*, 1998).

## **2. 17. 1 Challenges (problems) of tuberculosis control**

### **Wildlife reservoir hosts**

The reemergence of *M bovis* infection in captive and free-ranging wild animals, with subsequent transmission of infection to domestic animals, is of concern to livestock producers and regulatory officials in the United States and in several other countries of the world (Wahlstrom *et al.*, 1998; Wyss *et al.*, 2001; Schmitt *et al.*, 2002).

Significant wildlife reservoir hosts currently exist for *M. bovis* infection of cattle including: Eurasian badgers in Great Britain and Ireland (Clifton- Hadley *et al.*, 1993; Griffin *et al.*, 2005), white-tailed deer in the United States (O'Brien *et al.*, 2002; Schmitt *et al.*, 2002), brushtail possums in New Zealand (Karlson and Carr, 1970; Coleman *et al.*, 2006; Porphyre *et al.*, 2007), wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) in Spain (Naranjo *et al.*, 2006;), and African buffalo (*Syncerus caffer*), lechwe (*Kobus lechwe*), warthog (*Phacocoerus africanus*) and kudu

(*Tragelaphus strepsiceros*) in Africa (Bengis *et al.*, 2004; Michel *et al.*, 2006). Eradication of *M. bovis* in these regions has been impossible, in part, due to continued spillover of infection from the respective reservoir hosts. Without control of *M. bovis* in the reservoir host, it will be very difficult if not impossible, to eradicate *M. bovis* infection in cattle in Africa.

### **Transboundary transmission**

*M. bovis* infection in man in the US had been traced to strain isolated from cattle in Mexico (Rodwell *et al.*, 2010), spoligotype pattern of *M. bovis* isolated from cattle in Nigeria were found to be identical to those isolated in Cameroon (Cadmus *et al.*, 2006; Jenkins *et al.*, 2011), suggesting trans-border transmission of bovine tuberculosis. Therefore, for effective eradication of zoonotic and bovine tuberculosis there is need for regional collaboration and involvement of global organization such as WHO, FAO and OIE (WHO, 2009)

### **Political willigness**

Countries where the government are willing to eradicate tuberculosis instituted the eradication program, in such countries bovine and zoonotic tuberculosis had been reduced to minimal rate.

Moda *et al.* (1996) made the following observations:

1. The political and health authorities of all nations must be kept up-dated on the prevalence of bovine tuberculosis and the means available for its control.
2. Surveillance systems and co-operation between the medical and veterinary professions must be reinforced.

3. Public health programs must be planned and financially supported through international co-operation.
4. Countries lacking compulsory elimination programs based on tuberculin testing and slaughter of reactors should institute control programs based on restrictive action whenever resources permit.

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3. 1 Study Area**

Benue State lies between latitudes 6°25'N and 8°8'N and longitudes 7°47'E and 10°E' (Figure: 3.1). It is surrounded by five states, namely Nassarawa to the north, Taraba to the northeast, Cross River to the south, Anambra to the southwest and Kogi to the west. There is also a short international boundary between the state and the Republic of Cameroun along Nigeria's southeast border (NPC,2006)

Benue State is located in the Guinea Savanna zone, it has abundant pasture for livestock support. The indigenous cattle breed reared in Benue State is the Muturu, other breeds of cattle that are reared in the State include: Bunaji and Adamawa Gudali (Blench, 1999)

The major abattoirs in Makurdi and Otukpo are Wurukum abattoir, modern market abattoir and Otukpo abattoir. Cattle slaughtered in abattoirs in Benue State are sourced from within the State and from cattle markets in neighbouring States such as Nasarawa, Niger, Plateau, Bauchi, Adamawa, Taraba and Cross River State (Personal Communication).

The above abattoirs were chosen for the study because more cattle are slaughtered on a regular basis at these abattoirs and for convenience of sampling.

The climatic condition of Benue State is influenced by two air masses: the warm, moist south westerly air mass and the the dry northeasterly air mass. Raining season begins in the month of May and end in October.  
(NPC, 2006).



### **3. 2 Prospective Study of Bovine Tuberculosis**

#### **3. 2.1 Samples collection from suspected tuberculous lesions**

Sample were collected during participatory post-mortem examination of cattle carcasses from two abattoirs in Makurdi, Wurukum and Modern market abattoirs. At Wurukum and morden market abattoirs cattle carcasses were examined on daily basis for gross lesion typical of bovine tuberculosis from 24th July, to 12th August, 2012. While suspected of bovine tuberculosis lesions were collected from cattle carcasses inspected at Otukpo abattoir on daily basis from November, 2012 to December, 2012.

Briefly, tissue samples from the lungs, lymph nodes, liver, kidney, pleural and spleen were collected from the animals suspected of tuberculosis. Samples were obtained by careful visual palpation and incision procedure for nodules and granulomatous lesions as described by OIE (2008). Tissue bearing lesions from suspected cases were collected and placed in a labeled sterile screw-capped, leak-proof specimen container containing 0.9% normal saline, frozen and transported to Tuberculosis laboratory, University of Ibadan for processing.

#### **3. 2. 2 Processing tissue samples, acid-fast staining and culture**

The processing of lesions was based on the Becton Dickinson digestion and decontamination procedures. In order to prepare tissue samples for culture, the tissues were first homogenized by using a pestle and mortar as described by OIE (2009), it was then followed by decontamination in a 15ml centrifuge tube containing equal amount of specimen and NALC (N- acetyl- l –cysteine) NaOH (about 2ml each). The tube containing the mixture was allowed to stand until the specimen was liquefied and then shaken for 15 minutes at room temperature followed by neutralization using 6ml phosphate buffer. The mixture was then centrifuged at 3000×g for 15min. The supernatant was carefully decanted; 2ml of distilled water was added to suspend the

sediment. The suspension was inoculated onto Lowenstein Jensen slope with pyruvate or glycerol and incubated at 37°C between 8 and 12 weeks.

Two smears of the homogenates of each specimen were made and stained by the Ziehl-Neelson (Z-N) method as described by Elmer (1992). Presence of acid-fast bacilli was a suggestive of Bovine tuberculosis infection.

The culture positive samples were further subjected to smear microscopy using Ziehl-Neelsen (ZN) stain as described above.

### **3. 2. 3 Crude Mycobacteria DNA extraction**

Two loops full of Mycobacteria cells was resuspended in 250µl 1 × TE in an ependorf tube and incubated at 80<sup>0</sup> C for one hour. The tube containing the heat killed cells was centrifuge at 1300 × g for two minutes, the supernatant was discarded and the pellet was resuspended in 500µl of mM NaCl. This procedure was repeated twice. Finally the supernatant was discarded and the residue was resuspended in 25 µl of distilled water (Jenkins *et al.*, 2011).

### **3. 2. 4 Genomic deletion typing of mycobacteria isolates**

Heat killed acid-fast bacilli isolated in this study were subjected to a multiplex Polymerase Chain Reaction (PCR) deletion typing method (Warren *et al.* 2006). The presence or absence of RD1, RD4, RD9 and RD12 were used as criteria for identification of the isolate. Primer that were used and expected results are represented in table 3.1.

### **3. 2. 5 Primers used**

Primers were obtained from Inqaba Biotec (South Africa) (Table 3.1). Primers were directed against RD1, RD4, RD9 and RD12 according to previously described protocol

(Brosch *et al.*, 2002 and Warren *et al.*, 2006). The HotStarTaq master mix system from QIAGEN (Hilden, Germany) was used for the PCR.

**Table 3.1: Primer sequences used for deletion analysis and expected results**

| Primer sequences        | RD            | <i>M. tuberculosis</i> | <i>M. bovis</i> | <i>M. africanum</i> |
|-------------------------|---------------|------------------------|-----------------|---------------------|
| AAGCGGTTGCCGCCGACCGACC  | 1 (forward)   | Present                | Present         | Present             |
| CTGGCTATATTCCTGGGCCCCGG | 1 (internal)  | (146bp)                | (146bp)         | (146bp)             |
| GAGGCGATCTGGCGGTTTGGGG  | 1 (reverse)   |                        |                 |                     |
| ATGTGCGAGCTGAGCGATG     | 4 (forward)   | Present                | Absent          | Present             |
| TGTACTATGCTGACCATGCG    | 4 (internal)  | (172bp)                | (268bp)         |                     |
| AAAGGAGCACCATCGTCCAC    | 4 (reverse)   |                        |                 |                     |
| CAAGTTGCCGTTTCGAGCC     | 9 (forward)   | Present                | Absent          | Absent              |
| CAATGTTTGTTGCGCTGC      | 9 (internal)  | (235bp)                | (108bp)         |                     |
| GCTACCCTCGACCAAGTGTT    | 9 (reverse)   |                        |                 |                     |
| GGGAGCCCAGCATTTACCTC    | 12 (forward)  | Present                | Absent          | Present             |
| GTGTTGCGGGAATTACTCGG    | 12 (internal) | (369bp)                | (306bp)         | (369bp)             |
| AGCAGGAGCGGTTGGATATTC   | 12 (reverse)  |                        |                 |                     |

### **3. 2. 6 PCR amplification**

Each PCR reaction contained 1 µl DNA template, 5 µl Q-buffer, 2.5 µl  $\times 10$  buffer, 2 µl 25 mM MgCl<sub>2</sub>, 4 µl  $\times 10$  mM dNTPs, 0.5 µl of each primer (50 pmol/ µl), 0.125 µl HotStarTaq plus DNA polymerase (Qiagen, Hilden, Germany) and was made up to 25 µl with DEPC treated H<sub>2</sub>O. Amplification was initiated by initial denaturation at 95°C for 15 min, followed by final denaturation at 94°C for 1 min, Annealing at 62°C for 1 min, extension at 72°C for 1 minute for 45 cycles and final extension at 72°C for 10 minutes on a Perkin-Elmer GeneAmp machine. PCR amplification products were electrophoretically separated in 3.0% agarose in 1X TBE pH 8.3 at 6V/cm for 4 h, and visualized by staining with ethidium bromide (Warren *et al.*, 2006).

DNA templates from previously characterised *M. avium* and *M. tuberculosis* (H37Rv) were included as positive controls.

## **3. 3 Retrospective study of the Prevalence and Direct Economic Losses from Bovine**

### **Tuberculosis.**

#### **3. 3. 1 Collection of data**

Abattoir records for a period of five years (2008-2012) were collated from the Ministry of Agriculture and Natural Resources, Makurdi, Benue State. From abattoir records, data on tuberculosis cases per month were extracted. These include: number of cattle examined before slaughter, number and types of whole edible organs condemned as a result of the presence of tuberculosis lesions. Partially condemned edible organs were not included in the study because their actual or estimated quantity were not recorded. There was no record for whole carcass condemnation from detection of tuberculosis

lesions. It was not possible to get the correct data on age, breed, and sex for each slaughtered cattle during the study period due to poor abattoir recording system at the Ministry of Agriculture and Natural Resources (MANR) Makurdi. Veterinarians who are staff of the Ministry of Agriculture and Natural Resources (MANR) Makurdi carried out post-mortem examination at the abattoirs.

### **3. 3. 2 Estimation of Financial Losses due to Condemnation of tuberculous organs in Makurdi abattoirs.**

The average cost per kilogram of edible organs was obtained through oral interviews with the butchers and meat traders at the abattoirs. The average costs of organs like lungs and spleen, which are sold without weighing, were also obtained through oral interviews with butchers and meat traders. Total number of liver, lungs, heart, spleen, and others condemned as unfit for human consumption during meat inspection were noted for cattle slaughtered in Makurdi abattoirs for a period of five years.

Financial losses in Naira and Dollar was subsequently calculated based on the basis of a previous pilot study (Mbaya *et al.*, 2010) and the formula:  $Del = nW \times Av.P/kg$  was used to determine financial losses.

Where: Del = direct economic losses due to total meat condemned

n = total number of condemned organs for the period

W = Total weight of condemned organ

Av.P/Kg = average price of whole normal or passed organ/ kilogram

## **CHAPTER FOUR**

### **RESULTS**

#### **4. 1 Prospective Survey**

##### **4. 1. 1 Gross pathological lesions and acid-fast test of tubercule lesions from cattle slaughtered at Makurdi and Otukpo abattoirs.**

A total of 926 cattle were slaughtered at Wurukum and Modern Market abattoir in Makurdi from July to August, 2012, 625 (67.5%) cattle were slaughtered at Wurukum abattoir, while 301 (32.5%) cattle were slaughtered at modern market abattoir. Out the 926 cattle slaughtered, 338(37%) carcasses were examined for lesions typical of tuberculosis (TB). Forty three (12.7%) of the 338 cattle carcasses examined showed signs typical of tuberculosis (TB) out of which 32 (74.4%) were positive for Acid-fast bacilli (Table 4.1).

From Otukpo abattoir, 314 cattle were slaughtered and 249 (79%) were examined for signs typical of tuberculosis. Twenty (8.0%) showed lesions typical of tuberculosis, while 15 (75.0) of those that showed lesions infected cattle were positive for Acid-fast bacilli (Table 4.1).

Overall total of 1240 cattle were slaughtered during the course of the study, 587 (47%) were examined for tuberculous lesions; 63 (10.7%) exhibited characteristic lesion of tuberculosis and 47 (74%) that presented lesions typical of tuberculosis (TB) were positive for acid-fast bacilli (Table 4.1).

**Table 4. 1: Gross pathology and Ziehl-Neelsen microscopy of tissues sample collected from cattle slaughtered in Makurdi and Otukpo abattoirs**

| <b>Location</b>   | <b>N<sub>o</sub> slaughtered</b> | <b>N<sub>o</sub> examined (%)</b> | <b>N<sub>o</sub> infected (%)</b> | <b>ZN Positive (%)</b> |
|-------------------|----------------------------------|-----------------------------------|-----------------------------------|------------------------|
| <b>Makurdi</b>    |                                  |                                   |                                   |                        |
| Wurukum           | 625                              | 224 (36)                          | 36 (16.1)                         | 27 (75.0)              |
| M. market         | 301                              | 114 (38)                          | 07 (6.1)                          | 05 (71.4)              |
| <b>Sub- total</b> | <b>926</b>                       | <b>338 (37)</b>                   | <b>43 (12.7)</b>                  | <b>32 (74.4)</b>       |
| <b>Otukpo</b>     |                                  |                                   |                                   |                        |
|                   | 314                              | 249 (79)                          | 20 (8.0)                          | 15 (75.0)              |
| <b>Total</b>      | <b>1240</b>                      | <b>587 (47)</b>                   | <b>63 (10.7)</b>                  | <b>47 (74.6)</b>       |

M. market = morden market

#### **4. 1. 2 Distribution of Lesion in Different Organs/Tissues**

Table 4.2 shows the distribution of lesions and acid-fast bacilli in different organs /tissues. The lymph nodes and lungs were more commonly affected by TB than other organs/tissues examined. Forty four (40%) of lymph nodes and 39 (55.45%) of lungs (Table 4. 4) showed lesions characteristic of TB, Tuberculosis lesions were observed predominately in the lungs, the pleura 2 (1.82%) and heart 3 (2.73%) were least affected. More lymph nodes were collected (Table 4. 2) considering the overall number of cattle sampled (Table 4. 1). The total number of organs/tissue collected (110) (Table 4. 2) indicates that some animals had multiple organ infection.

Gross lesions encountered are shown in Plate I hypertrophied lymph node with yellowish granulomatous appearance and Plate II lung tissue with yellowish tubercle lesions. Other photograph of tubercle lesions are presented in appendix I.



Plate I: hypertrophied lymph node with yellowish granulomatous appearance



Plate II: Lung tissue with yellowish tubercle lesions

**Table 4. 2: Distribution of lesions in organs/tissues and Ziehl-Neelson microscopy**

| <b>Tissues sampled</b> | <b>N<sub>o</sub> sampled (%)</b> | <b>ZN-Positive (%)</b> | <b>ZN-Negative (%)</b> |
|------------------------|----------------------------------|------------------------|------------------------|
| Lung                   | 39 (35.5)                        | 26 (66.7)              | 13 (33.3)              |
| Lymph nodes            | 44 (40.0)                        | 23 (52.3)              | 21 (47.7)              |
| Liver                  | 9 (8.12)                         | 5 (55.6)               | 4 (44.4)               |
| Spleen                 | 7 (6.4)                          | 5 (71.4)               | 2 (28.6)               |
| Kidney                 | 2 (1.8)                          | 2 (100.0)              | 0 (0.0)                |
| Heart                  | 3 (2.7)                          | 3 (100.0)              | 0 (0.0)                |
| Diaphragm              | 4 (3.6)                          | 2 (50.0)               | 2 (50.0)               |
| Pleural                | 2 (1.8)                          | 0 (0.0)                | 2 (100.0)              |
| <b>TOTAL</b>           | <b>110</b>                       | <b>66 (60.0)</b>       | <b>44 (40.0)</b>       |

#### **4. 1. 3 Mycobacteria Culture from Tissues Specimen in Makurdi and Otukpo**

##### **Abattoirs**

Table 4.3 shows mycobacterial isolates from organs/tissue specimens collected from Markudi and Otukpo abattoirs. Growth rate from tuberculous lesions was generally high (80%). Samples from liver, spleen, kidney, heart and pleura gave 100% growth on Lowenstein-Jensen media, while the lymph nodes and lungs gave 68.2% and 81.3% respectively. More growth were seen on media containing pyruvate than glycerol (Plate: III).

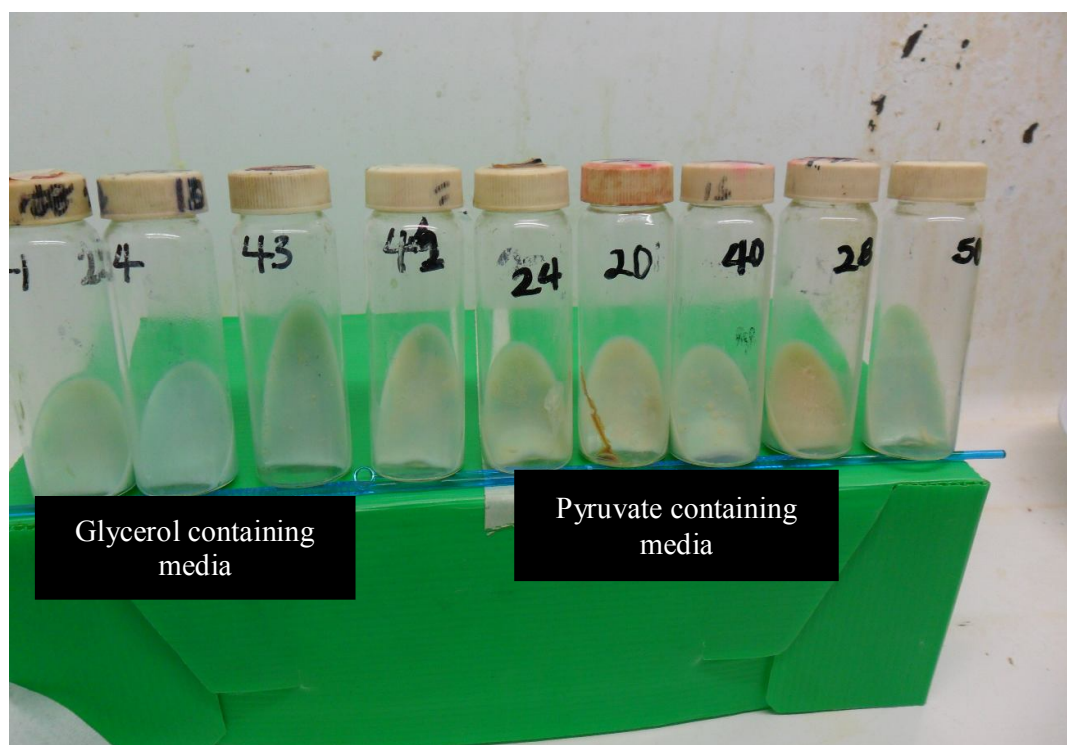


Plate III: Comparison of the growth of mycobacteria on Lowenstein-Jensen containing Media glycerol and pyruvate

**Table 4. 3: Mycobacteria culture from cattle specim in Makurdi and Otukpo abattoirs**

| Type of specimen | No cultured | <u>Growth on Lowenstein-Jensen</u> |                  |
|------------------|-------------|------------------------------------|------------------|
|                  |             | LJ Positive                        | LJ Negative      |
| Lungs            | 16          | 13 (81.3)                          | 3 (18.8)         |
| Liver            | 4           | 4 (100.0)                          | 0 (0.0)          |
| Lymph nodes      | 22          | 15 (68.2)                          | 7 (31.8)         |
| Spleen           | 4           | 4 (100.0)                          | 0 (0.0)          |
| Kidney           | 1           | 1 (100.0)                          | 0 (0.0)          |
| Heart            | 1           | 1 (100.0)                          | 0 (0.0)          |
| Pleural          | 2           | 2 (100.0)                          | 0 (0.0)          |
| Diaphragm        | 0           | 0 (0.0)                            | 0 (0.0)          |
| <b>Total</b>     | <b>50</b>   | <b>40 (80.0)</b>                   | <b>10 (20.0)</b> |

#### **4. 1. 4 Deletion Typing of *Mycobacterium bovis* Isolated from Different Organs**

Plates I and II show the products generated after PCR amplification of DNA extracted from 40 isolates cultured from tissue samples with lesions typical of tuberculosis, using primers RD1A, RD1B, RD1C, RD4A, RD4B, RD4C, RD9A, RD9B, RD9C, RD12A, RD12B, and RD12C. The size of each product corresponds to the in silico predictions, thereby indicating whether regions RD1, RD4, RD9, and RD12 were absent or present in the respective mycobacterial isolates (Table 3. 1).

Of the 40 mycobacterial isolates, the presence of RD1 was successfully amplified in 36, absence of RD4 was amplified from 36 out of 40, in the same way absence of RD9 was amplified from 36 out of 40 isolates that were tested. Out of the 36 isolates that successfully amplified the presence of RD1 (146bp) and absence of RD4 (268bp) and RD9 (108bp), three (n = 3) failed to amplify the presence or absence of RD12 (Table 4.6). Therefore, 36 (90.00%) generated bands typical of *M. bovis* and 4 (10.0%) failed to generate appropriate bands typical of MTC and were tentatively identified as NTM (appendix 8). Isolate number 9, 23 and 40 failed to amplify RD 12 (Plate IV, V and appendix 8)

Co infection of *Mycobacterium bovis* and non tuberculous mycobacteria were identified among three cattle (isolate no 9 and no 4, no 34 and no 22, and 36 and 28 are from the same animals)

All isolates (100%) from spleen, kidney, heart, and pleurae were identified as *M. bovis*, and 76.9%, 50%, 86.7% of isolates from lungs, liver and lymph nodes respectively were identified also as *M. bovis*. (Table 4.4). Animals from which kidney, spleen, heart

and pleural sample were collected showed generalized bovine tuberculosis (BTB) at the time of PM examination.

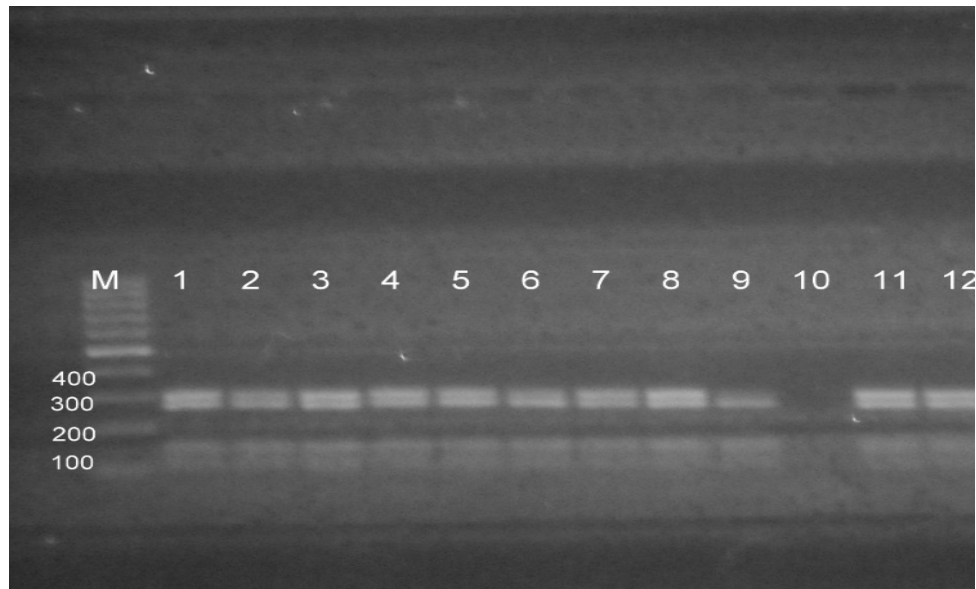


Plate: IV: Electrophoretic fractionation of PCR products in 3% agarose. M. 100bp molecular ladder (1Kbp molecular weight marker), sample 1 – 9 (*M. bovis*), 10 Negative control, 11 – 12 (*M. bovis*).

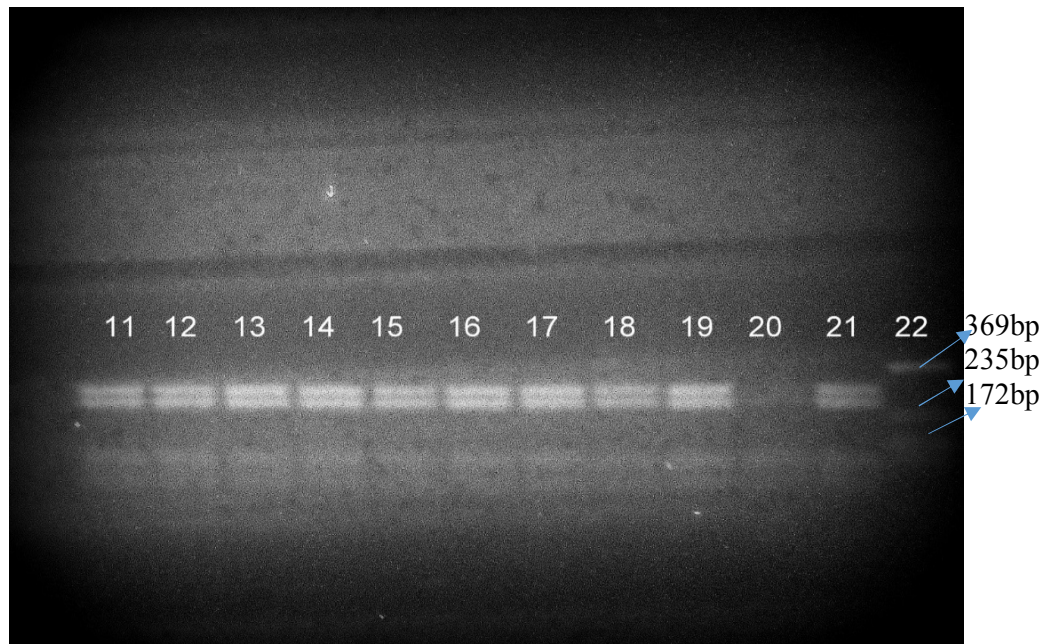


Plate: V (continuation of plate IV above) Electrophoretic fractionation of PCR products in 3% agarose. Sample 20 (No amplification), 21 (*M. bovis*), 22 (positive control mycobacterium tuberculosis H37Rv).

**Table 4. 4 Molecular typing of isolates from different organs/tissues**

| Organs       | Isolates  | <i>M. bovis</i>   | NTM              |
|--------------|-----------|-------------------|------------------|
| Lungs        | 13        | 12 (76.9)         | 1 (7.8)          |
| Liver        | 4         | 3 (50.0)          | 1 (25.0)         |
| Lymph nodes  | 15        | 13 (86.7)         | 2 (13.3)         |
| Spleen       | 4         | 4 (100.0)         | 0 (0.0)          |
| Kidney       | 1         | 1 (100.0)         | 0 (0.0)          |
| Heart        | 1         | 1 (100.0)         | 0 (0.0)          |
| Pleural      | 2         | 2 (100.0)         | 0 (0.0)          |
| Diaphragm    | 0         | 0 (0.00)          | 0 (0.0)          |
| <b>Total</b> | <b>40</b> | <b>36 (90.00)</b> | <b>4 (10.00)</b> |

#### **4. 1.5 Sex Specific Prevalence of Bovine Tuberculosis**

Sex distribution shows that more female cattle were affected than male cattle. There was no statistical difference between sex and *Mycobacterium bovis* infection (Table 4.5)

**Table 4. 5 Sex specific prevalence of bovine tuberculosis**

| Sex          | Total cultured | LJ positive (%)  | Deletion typing     |                |
|--------------|----------------|------------------|---------------------|----------------|
|              |                |                  | <i>M. bovis</i> (%) | NTM (%)        |
| Male         | 6              | 3 (50.0)         | 3 (50.0)            | 0 (0.0)        |
| Female       | 17             | 12 (70.5)        | 12 (70.5)           | 0 (0.0)        |
| <b>Total</b> | <b>23</b>      | <b>15 (65.2)</b> | <b>15 (65.2)</b>    | <b>0 (0.0)</b> |

$\chi^2 = 0.8248$ ;  $p = 0.38248$ ;  $p > 0.05$

## **4. 2     Retrospective Study of the Prevalence and Economic Implication of Bovine Tuberculosis.**

### **4. 2. 1 Annual prevalence of bovine tubercle lesions in Makurdi and Otukpo abattoirs**

Table 4. 6 shows the annual and seasonal distribution of the prevalence of bovine tuberculosis (BTB) from 2008 to 2012. A total of 1942 (37.51%) cattle were slaughtered in 2008 with a lower prevalence of 0.90% (95% CI:0.65 – 1.18%) while in 2012, data were collected for a period of six (6) months, showed a higher prevalence of 4.04% (95% CI:-3.17 – 13.13). Annual prevalence rate of bovine tuberculosis ranges from 0.90% in 2008 to 2.30% in 2011 and a prevalence of 4.04% in 2012 for which data were collected for six months. There was significant difference between the prevalence of bovine tuberculosis recorded in 2012 and 2008, 2009 ( $P < 0.05$ ). A Prevalence rate of 1.90% (95% CI: 1.45 – 3.05) was recorded for a period of five years in Makurdi abattoirs.

The annual prevalence of bovine tuberculosis (BTB) in Otukpo abattoir was recorded from 2010 to 2012 because as at the time data were collated from the Ministry of Agriculture and Natural Resources (MANR) Makurdi, Benue State, previous records for Otukpo were not available. In 2010, a sum of 15360 slaughtered cattle were recorded in Otukpo abattoir, 1.9% (95% CI: 1.4 – 2.5) exhibited lesions typical of BTB, while the highest prevalence was recorded in 2011, 3.9% (95% CI: 3.3 – 4.5).

A prevalence of 2.9% of tuberculous lesion were recorded in Otukpo abattoirs. There was significant difference between the prevalence of BTB in 2010 and 2011 in cattle slaughtered in Otukpo abattoir ( $P < 0.05$ ).

There was no statistical significant difference between the prevalence of bovine tuberculosis (BTB) during the raining season and dry season in both Makurdi and Otukpo abattoirs. (Mann-Whitney U statistics = 267.50,  $p = 0.12$ ).

The overall retrospective prevalence of BTB in cattle slaughtered in Makurdi from 2008 to 2012 and Otukpo abattoirs from 2010 – 2012 was 2.6%.

**Table 4. 6: Annual prevalence of tuberculous lesions in cattle slaughtered in Makurdi and Otukpo abattoirs from 2008 to 2012**

| <b>Parameter</b> | <b>No Slaughtered (%)</b> | <b>No TB Lesions</b> | <b>Prevalence (%)</b> | <b>95% CI</b>     |
|------------------|---------------------------|----------------------|-----------------------|-------------------|
| <b>MAKURDI</b>   |                           |                      |                       |                   |
| <b>Year</b>      |                           |                      |                       |                   |
| 2008             | 19429(31.51)              | 175                  | 0.9 <sup>a</sup>      | 0.7 – 1.2         |
| 2009             | 17011(27.60)              | 341                  | 2.0 <sup>a</sup>      | 1.2 – 2.6         |
| 2010             | 10988(17.82)              | 265                  | 2.4                   | 1.7 – 3.3         |
| 2011             | 10104(16.39)              | 230                  | 2.3                   | 0.9 – 3.7         |
| 2012             | 4131(6.70)                | 167                  | 4.0 <sup>b</sup>      | -3.2 – 13.1       |
| <b>Season</b>    |                           |                      |                       |                   |
| Raining          | 37262(60.44)              | 631                  | 1.7                   | -2.6 – 0.6        |
| Dry              | 24392(39.56)              | 547                  | 2.2                   | -2.9 – 0.8        |
| <b>Sub total</b> | <b>61654 (62.23)</b>      | <b>1172</b>          | <b>1.9</b>            | <b>1.5 – 3.1</b>  |
| <b>OTUKPO</b>    |                           |                      |                       |                   |
| <b>Year</b>      |                           |                      |                       |                   |
| 2010             | 15360 (41.11)             | 290                  | 1.9 <sup>c</sup>      | 1.4 – 2.5         |
| 2011             | 14340 (38.32)             | 570                  | 3.9 <sup>d</sup>      | 3.3 – 4.9         |
| 2012             | 7722 (20.64)              | 210                  | 2.7                   | 0.8 – 4.6         |
| <b>Season</b>    |                           |                      |                       |                   |
| Raining          | 23419 (62.59)             | 631                  | 2.7                   | 2.1 – 3.4         |
| Dry              | 13999 (37.41)             | 440                  | 3.1                   | 2.1 – 4.6         |
| <b>Sub total</b> | <b>37419 (37.79)</b>      | <b>1071</b>          | <b>2.9</b>            | <b>2.38 – 3.6</b> |
| <b>TOTAL</b>     | <b>99073</b>              | <b>2243</b>          | <b>2.6</b>            | <b>1.9 – 3.1</b>  |

Mean percentages with different letter(s) in the same column were different significantly ( $P > 0.05$ )

#### 4. 2. 2 Annual economic losses

Table 4. 7 shows the financial (direct economic) losses, numbers and weight of edible cattle organs condemned from 2008 to 2012 in Makurdi abattoirs alone. This is because abattoir record of number of whole edible organ condemnation were not available in Otukpo. A total of 1935 (3046.50Kg) edible organs valued at  $\square 2.91 \times 10^6$  ( $\$1.82 \times 10^4$ ) were condemned.

In 2009 a total of 675 (34.89%) organs which weigh 1070Kg, amounting to  $\square 1.00 \times 10^6$  ( $\$6293.14$ ). These figures were about two times higher than the amount and number of edible organs condemned in 2010 and 2011 all together, while in 2012 a total of 236 (12.20%) weighing 363.00Kg and worth  $\square 3.56 \times 10^5$  ( $\$2231.26$ ) edible organs were condemned.

There was no significant difference between direct economic loss in edible organs condemned in 2009 and 2012 ( $P > 0.05$ ). However, there was significant difference between the direct economic losses in 2008, 2009, 2010 and 2011 ( $P < 0.05$ ).

There was statistical significant between edible organs condemned during the dry season and raining season (Mann Withney U statistics =  $7.74 \times 10^3$ ,  $P = 0.034$ ).

During the study period a total of 912 (47.13%) lungs were condemned, this figure was valued at  $\square 9.12 \times 10^5$  ( $\$5700.00$ ), number of spleen condemned were 219 (11.32%), valued at  $\square 8.30 \times 10^4$  ( $\$547.50$ ). lungs and spleen were sold at  $\square 1000$  ( $\$6.25$ ) and  $\square 350$  ( $\$2.50$ ) per organ respectively, without weighing.

A total of 523 (27.03%), weighed 1569.00Kg and valued at  $\square 1.57 \times 10^6$  ( $\$9806.25$ ) liver were condemned, while 176 (9.06%) weighed 262.50Kg valued at  $\square 1.62 \times 10^5$  ( $\$1640.73$ ) and 105 (5.43%), weighed 84.00Kg and valued at  $\square 8.47 \times 10^4$  ( $\$525.00$ ) kidneys were condemned.

There was significant difference between the direct economic loss among edible organs condemned as result of tuberculous lesions.

Statistical analysis showed that there was significant difference between direct economic loss (Del) from condemnation of edible organs due to bovine tuberculosis during the raining season and dry season (Mann-Whitney Ustatistics =  $7.745 \times 10^3$ ,  $p = 0.034$ ).

**Table 4.7 Direct Economic Losses from Condemnation of Edible Organs as a Result of Detection of Tubercle Lesion in Cattle Slaughtred in Makurdi Abattoirs**

| Parameters     | No Condemned (n) (%) | Weight(W) (Kg) | **Del                                |                                      |
|----------------|----------------------|----------------|--------------------------------------|--------------------------------------|
|                |                      |                | (□)                                  | (\$)                                 |
| <b>Year</b>    |                      |                |                                      |                                      |
| 2008           | 322(16.64)           | 517.30         | $5.01 \times 10^5$                   | 3101.90                              |
| 2009           | 675(34.89)           | 1070.50        | $1.00 \times 10^6$                   | 6293.14                              |
| 2010           | 358(18.50)           | 554.50         | $5.34 \times 10^5$                   | 3345.65                              |
| 2011           | 344(17.78)           | 541.20         | $5.18 \times 10^5$                   | 3247.53                              |
| 2012           | 236(12.20)           | 363.00         | $3.56 \times 10^5$                   | 2231.26                              |
| <b>Organs</b>  |                      |                |                                      |                                      |
| Lungs*         | 912(47.13)           | 912.00*        | $9.12 \times 10^{5a}$                | 5700.00                              |
| Liver          | 523(27.03)           | 1569.00        | $1.57 \times 10^{6b}$                | 9806.25                              |
| Heart          | 176(9.20)            | 262.50         | $1.62 \times 10^{5c}$                | 1640.73                              |
| Spleen*        | 219(11.32)           | 219.00*        | $8.30 \times 10^{4c}$                | 547.50                               |
| Kidney         | 105(5.43)            | 84.00          | $8.47 \times 10^{4c}$                | 525.00                               |
| <b>Seasons</b> |                      |                |                                      |                                      |
| Raining        | 784(40.52)           | 1249.00        | $1.19 \times 10^{6d}$                | 7483.80                              |
| Dry            | 1151(59.48)          | 1797.50        | $1.72 \times 10^{6e}$                | $1.07 \times 10^4$                   |
| <b>Total</b>   | <b>1935(100)</b>     | <b>3046.50</b> | <b><math>2.91 \times 10^6</math></b> | <b><math>1.82 \times 10^4</math></b> |

\*Quantity in number not in Kg, \*\* Del =  $nW \times Av.P/Kg$ , □ = Naira, \$ = US Dollar, Amount with the different letter(s) in the same column were different significantly (P > 0.05)

## CHAPTER FIVE

### DISCUSSION

During the prospective study of bovine tuberculosis in Makurdi and Otukpo local Government areas of Benue State, a prevalence of 10.7% based on identification of tubercle lesions and 8.0% based on Z-N microscopy were observed, respectively. The prevalence was higher in Makurdi than in Otukpo although not statistically significant, the reason might be due to the fact that cattle slaughtered in Makurdi and Otukpo were sourced from different cattle markets. The prevalence was higher than the prevalence of BTb recorded in the retrospective study of BTb in both local Government areas. Abattoir meat inspection was observed to be less thorough and may account for the differences observed. Other factors include difference in duration and due to the fact that non-veterinarians, who are not “professionals” were involved in meat inspection.

In this study, mycobacterial culture and RD deletion typing were used to identify causative agents of bovine tuberculosis in Otukpo and Makurdi, Benue State. The high isolation rate (80.0%) of mycobacterial colonies from 40 out of 50 tuberculous organs may be due to the efficiency of mycoprep(R) (N-acetyl L- cysteine NaOH) used as decontaminating agents compared to reports of Awah-Ndukum *et al.* (2010) who used 4% NaOH as decontaminating agent.

The successful culture of mycobacteria from affected tissue demonstrates the presence of generalized or miliary tuberculosis in slaughtered cattle in Makurdi and Otukpo, Benue State. It further demonstrates that all lesions were not detected during routine post-mortem meat inspection by at the abattoirs for BTB in carcasses. About 74.6% cattle sampled were positive acid fast bacilli upon staining of tuberculous lesions. This

result agree with the report of Adu-bobi *et al.* (2009), who reported an overall prevalence of 73.1% in abattoir samples in Ghana, but higher than that reported by Awah-Ndukum *et al.* (2010) in Cameroon.

This study has for the first time demonstrated the importance of tuberculosis in slaughtered cattle in Benue state by the use of molecular typing technique. Differentiation of *Mycobacterium tuberculosis* complex by PCR amplification of genomic region of difference (RD) was applied to all 40 isolates obtained from LJ culture. This PCR technique enables the rapid and accurate identification of members of MTC (Warren *et al.*, 2006). Out of the 40 isolates obtained from organs cultured on LJ slopes, 36 (90.0%) were identified as *M. bovis*.

All 36 isolates carried the RD1 region; this proved that cattle slaughtered in Benue state were not vaccinated or that vaccine strains were not present in cattle slaughtered in Benue state. This finding is of significant important because no history of vaccination of cattle against tuberculosis had been reported in Benue state.

Although there was no amplification of RD12 from 3 isolates, these isolate were however identify as *M. bovis* because of the presence of bands (268bp) indicating the absence of RD4. RD4 is a genotypic marker for *M. bovis* (strict sense) and absence of RD4 can be used to differentiate *M. bovis* and *M. bovis* BCG from the other MTC subspecies (Huard *et al.*, 2006)

The absence of RD9 and RD12 indicates that none of these isolates was *M. tuberculosis*. However, Cadmus *et al.* (2010) and Jenkins *et al.* (2011) reported *M. tuberculosis* in cattle in the Southwestern region of Nigeria.

Coinfection of *M. bovis* and nontuberculous mycobacteria was detected in three cattle, although further diagnostic procedure to determine the species of the nontuberculous mycobacteria was not carried out, however, nontuberculous mycobacteria that might cause granulomatous lesions in cattle is *Mycobacterium avium* sub-species *paratuberculosis* (Shrenzel, 2012).

The isolation of *M. bovis* from slaughtered cattle in Benue state confirms the presence of bovine tuberculosis and hence the risk of transmission of zoonotic tuberculosis from animals to humans.

In developed countries, control/eradication of zoonotic tuberculosis was achieved through thorough meat inspection, pasteurization of milk and test and slaughter method (Thoen *et al.*, 2006). In sub-Saharan Africa including Nigeria, these methods are rarely practice. In Nigeria, herdsman still drink milk directly from the udder of cattle and most cattle rarer do not pasteurize their milk before consumption (Onoja *et al.*, 2010).

Meat inspection are rarely carried out in most part of Nigeria and where it is practice, the act of trimming grossly affected parts and passing the other parts as we observed in Otukpo and Makurdi abattoirs exposes the public, including immunoconpromised individuals, such as HIV/AIDS patients to the danger of consuming infected meat. This is because the act of trimming affected parts cannot completely remove all infected parts and since some lesions are microscopic and cannot be detected grossly Adu-bobi *et al.* (2009). Although Adu-bobi *et al.* (2009) suggested that total rejection cannot be implemented fully when the abattoir management is not in the position to bear the cost of a condemned carcass. Government, farmers and cooperate organization should take up these responsibility by compensating the butchers of condemned carcass to help

spread the burden of economic losses due to tuberculosis in our society and this will contribute immensely to the “Stop TB programme.”

Tuberculosis in human due to *M. bovis* had been reported in Nigeria (Cadmus and Adesokan, 2009; Jenkins *et al.*, 2011; Cadmus *et al.*, 2011) and other parts of Africa (Cosivi *et al.*, 1998; Niobe-Eyangoh *et al.*, 2003; Zinsstag *et al.*, 2006). Individual most at risk of contracting *M. bovis* are animal handlers (Awah-Ndukum *et al.*, 2010) and immunocopromised patients including HIV/AIDS patients (Cadmus *et al.*, 2010).

Detection rate of gross pathological lesions of bovine tuberculosis (BTB) were high (4.0%) in 2012 in Makurdi, while in Otukpo abattoir the highest prevalence was 3.9%. There was a gradual increase from 2008 to 2012 in Makudi, This pattern seem to disagree with the results of Opara (2005) who observed that there was a decrease in the prevalence of bovine tuberculosis along three years (1999 to 2002) in Akwa Ibom State. He further explained that the decrease could result from recent public awareness campaign about tuberculosis and better meat inspection. This explanations might be different from the situation in Makurdi, where information on bovine tuberculosis is scares and meat inspection at abattoirs was less thorough. Although, in Otukpo abattoir no particular pattern was observed, this might result from the limited available records.

Similar pattern of increament was reported in Maiduguri (Igbokwe *et al.*, 2001). Other studies in Nigeria do not follow a particular pattern (Aliyu *et al.*, 2009). In Cameron, Awah-Ndukum *et al.* (2010) reported a fluctuation in annual prevalence of BTB, the authors further explained that the reason for the fluctuation was not clear and emphasized that inadequacies in capacity and lack of thoroughness of veterinary staff carrying out meat inspection could have played a major role.

Detection of BTB lesions in both Makurdi and Otukpo has no relationship with season. This agreed with the results of Awah-Ndukum *et al.* (2010) who further observed that, BTB detection rate was high during stressful periods such as inter-season and peak season periods and also when slaughter was elevated during religious feasts and sociocultural ceremonies.

Ameen *et al.* (2008) also made similar findings, while Opara (2005) reported differences in seasonal prevalence, he explained that, Fulani herdsmen brought their cattle to the southern part of Nigeria to graze, and emigrate when the rains begins in the North, and that possibly, these cattle might had acquired the infection up North before embarking on the South ward migration for pasture.

The overall prevalence of bovine tuberculosis from 2008 to 2012 in Makurdi and from 2010 to 2012 in Otukpo was 2.6%. This result was lower than previous prevalence of BTB in neighboring Nasarawa state where a prevalence of 15.08% was reported among cattle population (Yohana *et al.*, 2008), in Taraba state, 2.8% (Danbirni *et al.*, 2013) and in other parts of the country such as in abattoirs in Oyo state, 4.47% (Cadmus *et al.*, 2006), lower prevalence of 0.54% was reported in Ogbomosho (Ameen *et al.*, 2008).

Between 2008 and 2012 a total number of 1935 edible organs, weighed 3046.50Kg, valued at two million nine hundred and ten thousand naira (₦2910000) [eighteen thousand two hundred US Dollar (\$18200)] were condemned as a result of detection of tuberculosis lesions in cattle at meat inspection in Makurdi abattoirs.

Condemnation of edible organs valued at a huge sums of money as reported here might explain the aggressive behavior of butchers toward meat inspector at abattoirs in Nigeria (Cadmus and Adesokan, 2009) and other parts of Africa (Shitaye *et al.*, 2006),

also butchers are not compensated for partial, whole organs or carcass condemnation, hence they bear the financial burden alone (Ibironke and Fasina, 2010). This finding might be an under estimation of the true direct economic losses from condemnation of edible organs due to tuberculosis in Makurdi abattoirs as meat inspection was observed to be less thorough.

This disease condition may contribute to the economic suffering of the people in the study areas, because some farmers and traders depends entirely on the proceeds from sales of cattle offal as their source of livelihood (Ibironke and Fasina, 2010). Condemnation of edible organs without compensation deprive these group of people their source of livelihood.

Edible organs include lung, liver heart, spleen and kidney, these organs are sometimes prescribe by health officials for children, pregnant mothers, immunocompromised individuals and people suffering from other health conditions as these are excellent sources of minerals, vitamins, amino acids and other nutrients (Huang, *et al.*, 2005).

Condemnation of large quantity of organs without compensation might led to increase in their cost price, thus depriving the economic poor in the society access to such source of vital nutrients (Huang, *et al.*, 2005).

More lung were condemned than other organs, this agrees with the result of Rohnoczy *et al.* (1996) who observed that gross lesions of tuberculosis were most often in the lung, mycobacterium are obligatory, aerobic, intracellular pathogens which have a predilection for the lung tissues rich in oxygen supply (Raja, 2004).

There was significant difference in economic losses from condemned liver and other edible organs. This is because the liver is heavier than other organs condemned, it is

also very expensive as its demand is very high due to its high nutrient contents (FAO, 1990)

## CHAPTER SIX

### CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 Conclusions

Bovine tuberculosis is prevalent in Benue State and accounts for a loss of over 2.9 million Naira from 2008 – 2012 in Makurdi alone. The causative agent of bovine tuberculosis (BTB) in Benue State is predominantly *M. bovis*.

This study has for the first time to the best of my knowledge, reported the prevalence of BTB in Otukpo abattoir, determined the economic implication of condemnation of edible organs at postmortem as a result of tuberculosis in cattle slaughtered in Makurdi abattoirs, successfully cultured mycobacterial from tissue samples from Otukpo and Makurdi abattoirs and used modern molecular technique (deletion typing of mycobacterial genomic region of difference, RD typing) to characterize the mycobacterial isolates.

The isolation of *M. bovis* from slaughtered cattle in Benue state confirms the presence of bovine tuberculosis and hence the risk of transmission of zoonotic tuberculosis from animals to humans, especially animal handlers.

#### 6. 2 Recommendations

This study is an indicative that bovine tuberculosis is prevalence in Benue State. In view the important of tuberculosis, most especially zoonotic tuberculosis in immunodeficient patients and the public health and economic implication of tuberculosis in animals and humans, the following recommendations are made:

1. A good trace-back system should be introduced to monitor the source of tuberculosis infection among cattle slaughtered in abattoirs and to control infection at source.

2. Abattoir meat inspection should be left in the hands of professionals (veterinarians) only for efficient meat inspection.
3. Further study to determine to extent of zoonotic tuberculosis in both humans and animals in Benue State should be conducted.
4. Initiation of awareness programme through the department of veterinary services on the importance of zoonotic diseases especially tuberculosis.
5. Compensation should be reintroduced and enforced in slaughter houses and abattoirs to act as incentive for butchers.
6. There is need for attitudinal change toward meat inspection by veterinarians

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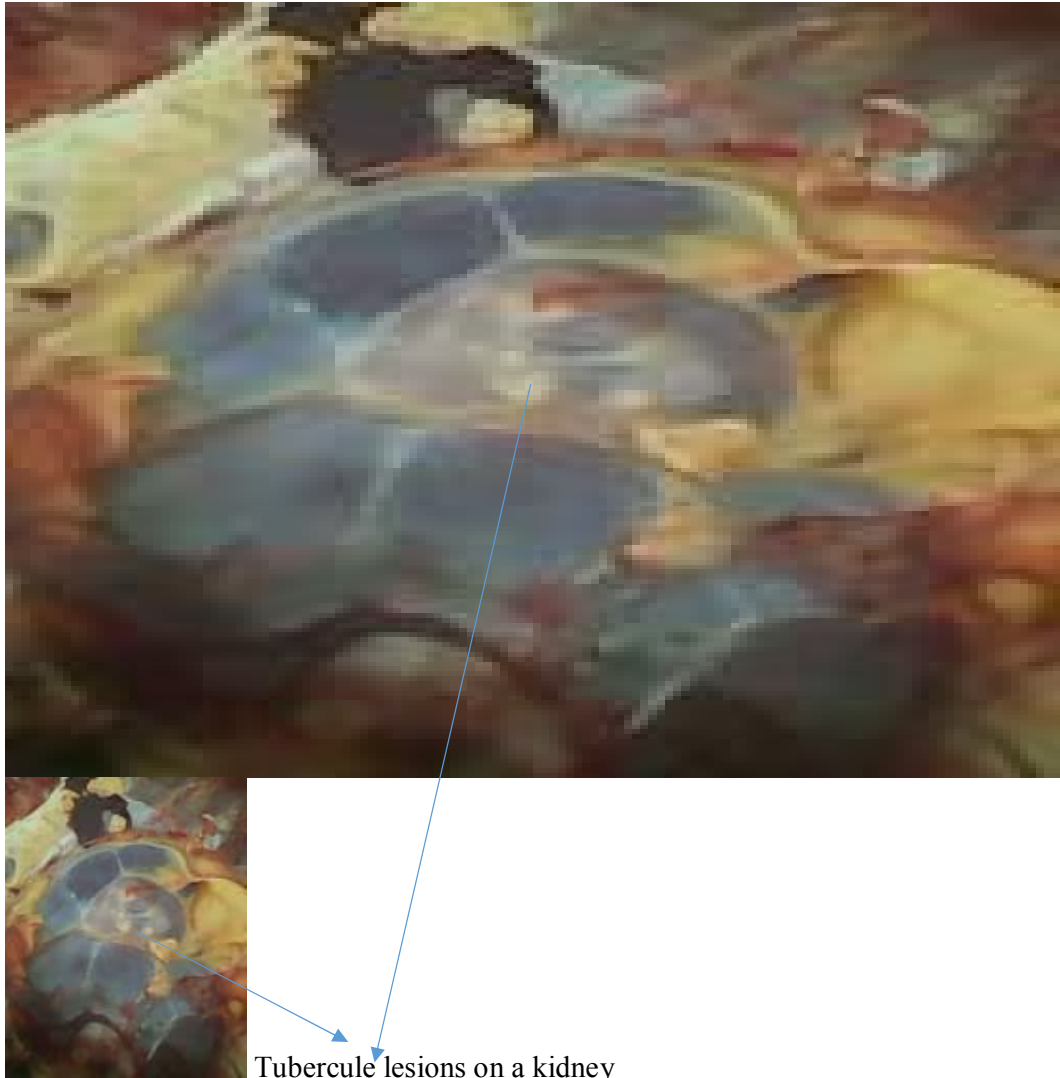
Egyptian mummies by spoligotyping. *Journal of Clinical Microbiology*, **41**, 359–367.

## APPENDICES



Appendix 1: lymph node showing TB lesion

Caecious granulomatous tubercle lesion in a lymph node

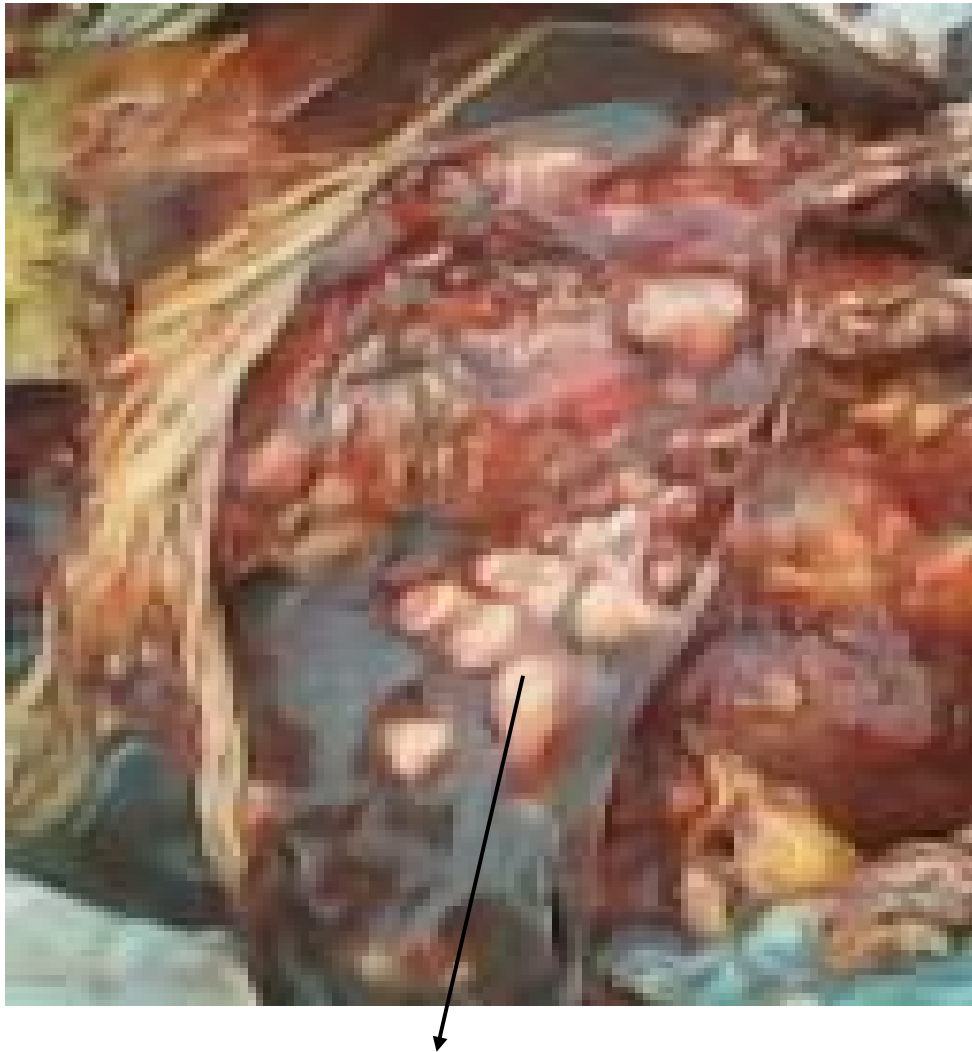


Tubercle lesions on a kidney

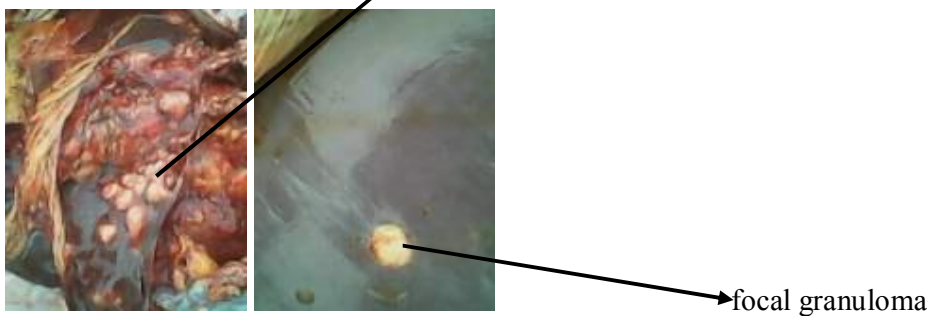
Appendix 2: kidney showing TB lesions



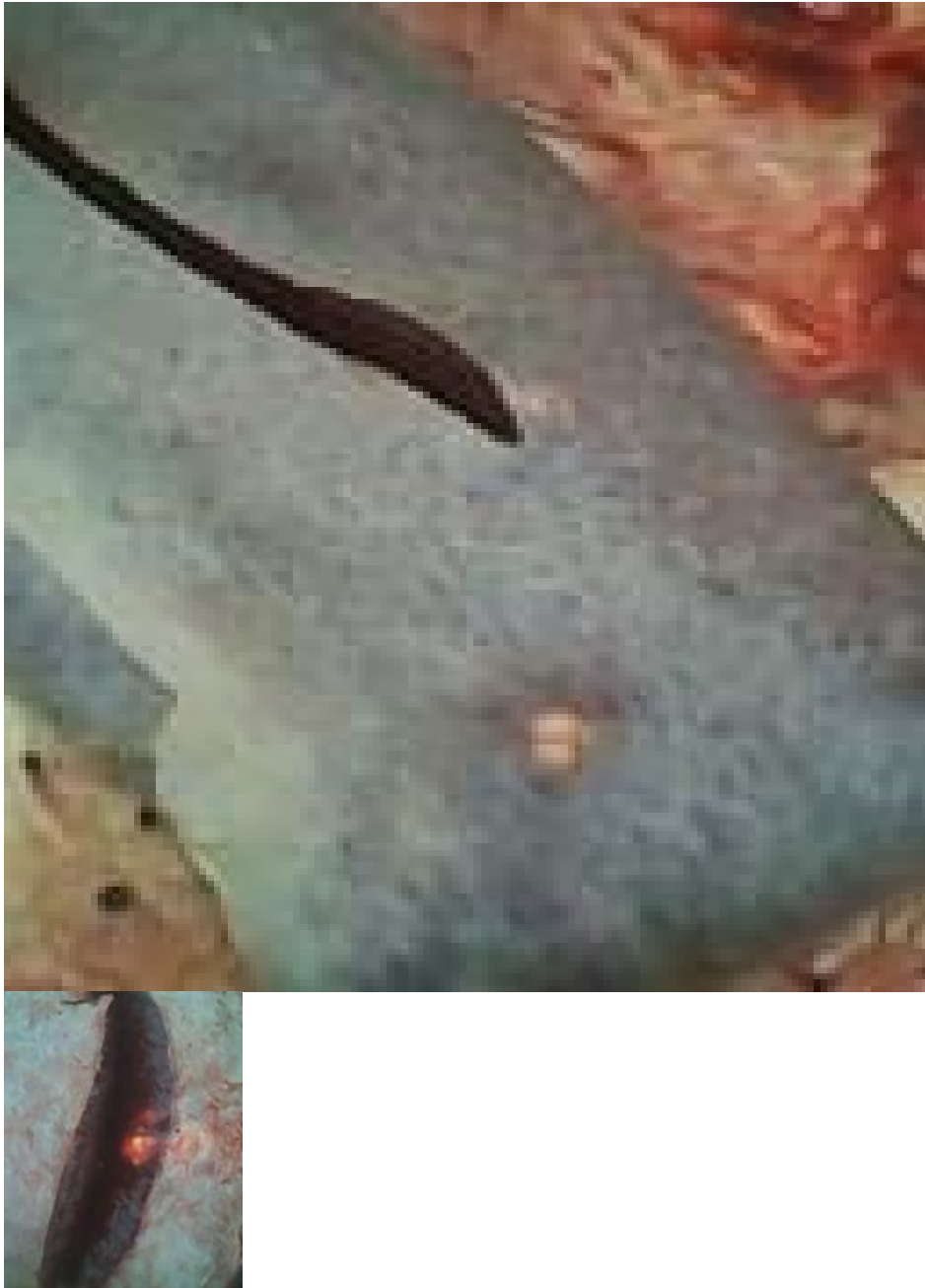
Appendix 3: lung showing tubercle lesions



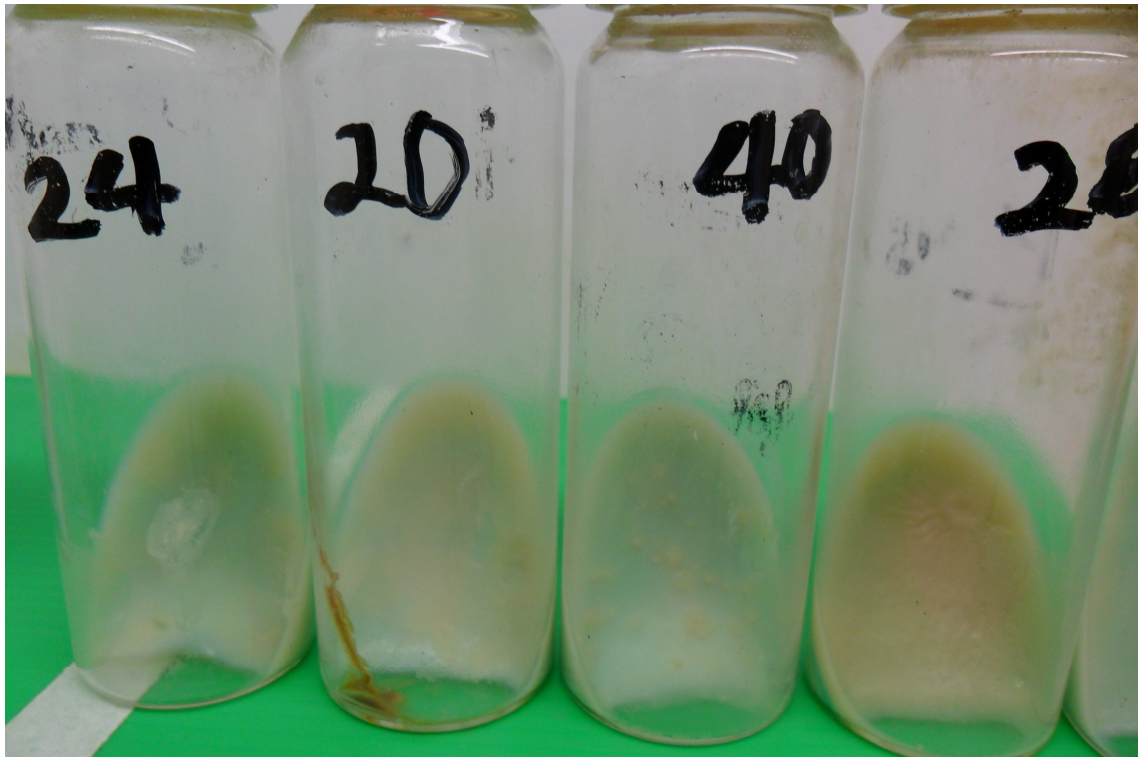
Granulomas



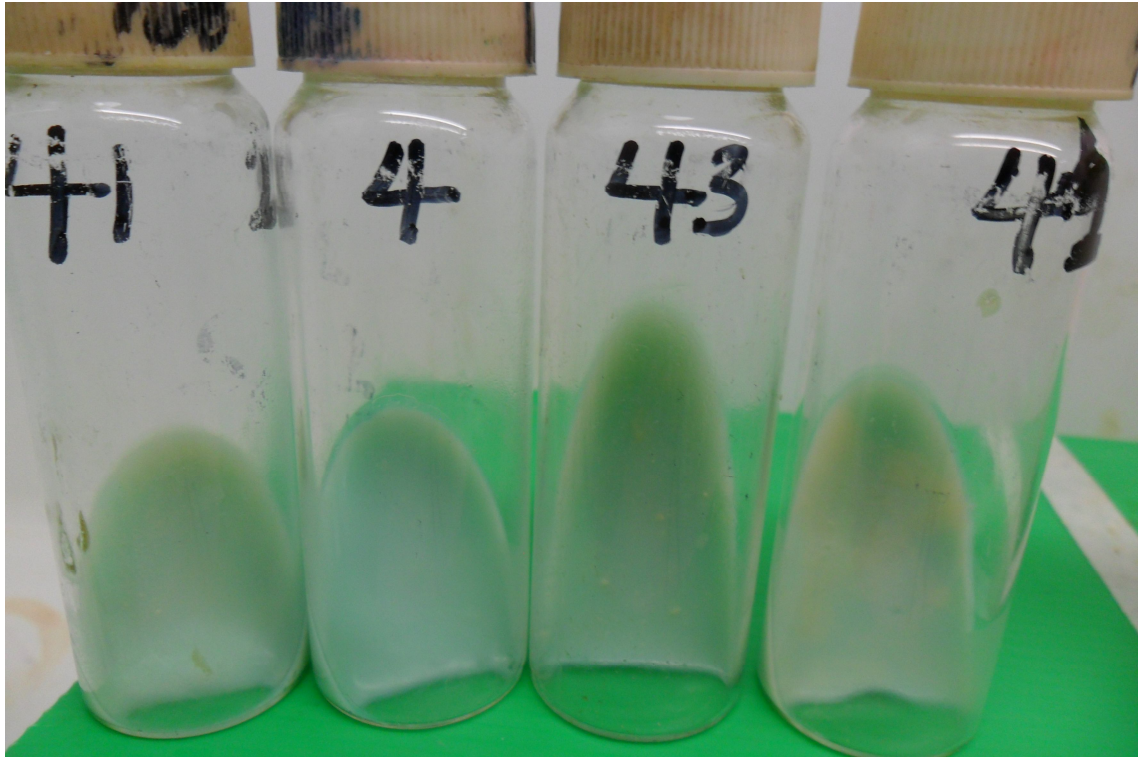
Appendix 4: liver showing numerous and a focal calcified granulomas



Appendix 5: A focal granuloma in a spleen infected with TB



Appendix 6: Growth of mycobacteria on LJ containing pyruvate



Appendix 7: Growth of mycobacteria on LJ containing glycerol

## Appendix 8 Result of deletion typing of region of differences (RDs)

| Sample/No | RD1          | RD4          | RD9          | RD12            | Remark                                       |
|-----------|--------------|--------------|--------------|-----------------|----------------------------------------------|
| 1         | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 2         | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 3**       | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 4         | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 5         | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 6         | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 7         | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 8         | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 9         | —            | —            | —            | <i>M. bovis</i> |                                              |
| 10        | Neg. control | Neg. control | Neg. control | No Amp          | <i>M. bovis</i> *                            |
| 11        | +            | —            | —            | Neg. control    | Neg. CTL\$                                   |
| 12        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 13        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 14        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 15        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 16        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 17        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 18        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 19        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 20#       | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 21        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 22        | +            | +            | +            | +               | <i>M. tuberculosis</i><br>(Positive control) |
| 23        | +            | —            | —            | No Amp          | <i>M. bovis</i> *                            |
| 24        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 25        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 26        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 27        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 28        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 29        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 30        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 31        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 32        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 33        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 34        | No Ampl      | No Ampl      | No Ampl      | No Ampl         | NTM                                          |
| 35        | No Ampl      | No Ampl      | No Ampl      | No Ampl         | NTM                                          |
| 36        | No Ampl      | No Ampl      | No Ampl      | No Ampl         | NTM                                          |
| 37**      | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 38        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 39        | Neg. control | Neg. control | Neg. control | Neg. control    | Neg. CTL\$                                   |
| 40        | +            | —            | —            | No Amp          | <i>M. bovis</i> *                            |
| 41        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 42        | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 43**      | +            | —            | —            | —               | <i>M. bovis</i>                              |
| 44        | No Ampl      | No Ampl      | No Ampl      | No Ampl         | NTM                                          |
| 45        | +            | +            | +            | +               | <i>M. tuberculosis</i><br>(Positive control) |

\*\* Repetition of sample from the same organ, \* No amplification of RD12, # Few DNA, therefore bands were very faint, \$ Negative control.