

**ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCE
SCHOOL OF VETERINARY MEDICINE**

**EPIDEMIOLOGY AND PUBLIC HEALTH IMPLICATION OF BOVINE TUBERCULOSIS
IN AND AROUND MEKELLE, NORTHERN ETHIOPIA**

**BY
FIKRE ZERU KFLEWAHD**

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DEBRE ZEIT, ETHIOPIA**

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Board of Examiners:

Signature

1. Dr. Nigussie Deyssa

School of Public Health, College of Health Science

2. Dr. Azage Tegegne

International Livestock Research Institute (ILRI)

3. Professor Getachew Tilahun

Aklilu Lemma Institute of Pathobiology (ALIPB),
College of Health Science

4. Dr. Genene Tefera

Institute of Biodiversity Conservation and Research

Advisors:

Tesfaye Sisay (DVM, MSc, PhD, Assistant Professor)

Gobena Ameni (DVM, PhD, Associate Professor)

Gezahegn Mamo (DVM, MSc, Assistant Professor)

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LIST OF ABBREVIATIONS

A	Adenine
AAU	Addis Ababa University
AFB	Acid fast bacilli
AHA	Animal Health Australia
AIDS	Acquired immunodeficiency syndrome
ALIPB	Aklilu Lemma Institute of Pathobiology
BCG	Bacillus of Calamette and Guerien
BCS	Body condition score
bp	base pair
BTB	Bovine tuberculosis
C	Cytosine
CFP	Culture filtrate protein
CI	Confidence interval
CIDT	Comparative intradermal skin test
CMI	Cell mediated immunity
CO ₂	Carbon dioxide
CSA	Central Statistical Agency
DNA	Deoxyribonucleic acid
DOTS	Direct observed treatment short course
DR	Direct Repeat
DTH	Delayed-type hypersensitivity
DVM	Doctor of Veterinary Medicine
ELISA	Enzyme linked immunosorbent assay
<i>EMbs1</i>	Ethiopian <i>M. bovis</i> strain 1
EPTB	Extra-pulmonary tuberculosis
ESAT	Early secretory target antigen
FAO	Food and Agriculture Organization
G	Guanine
HCL	Hydrochloric acid
HIV	Human immunodeficiency virus

IFN- γ	Interfeeron-gamma
IL	Interleukin
IS	Insertion sequence
IU	International unit
LJ	Löwenstein-Jensen
LN	Lymph node
MAC	<i>Mycobacterium avium</i> complex
MOH	Ministry of Health
m-PCR	Multiplex-polymerase chain reaction
MTBC	<i>Mycobacterium tuberculosis</i> complex
NaOH	Sodium hydroxide
NTM	Non-tuberculous mycobacteria
NVL	Non-visible lesions
OIE	Office International des Epizooties
OR	Odds ratio
PCR	Polymerase chain reaction
PPD	Purified protein derivative of tuberculin
PPD-A	Purified protein derivative-Avian
PPD-B	Purified protein derivative-Bovine
PTB	Pulmonary tuberculosis
RD	Regions of difference
RNA	Ribonucleic acid
SEI	Spoligotype Evolutionary Index
T	Thymine
TAE	Tris-Acetic acid Ethylene Diamine Tetra Acetic acid
TB	Tuberculosis
Th	T-helper cell
UK	United Kingdom
US	united States
WHO	World Health Organization
ZN	Ziehl-Neelsen stain

ABSTRACT

Bovine tuberculosis (BTB), caused by *Mycobacterium bovis*, is a well-known zoonotic and economically important disease which affects cattle world-wide. A cross-sectional study was conducted on 1248 cattle and 120 cattle owners in Mekelle City and its surroundings from September 2010 to May 2011 to assess the epidemiology and public health implication of BTB using comparative intradermal tuberculin test (CIDT), postmortem and bacteriological examinations, molecular typing and questionnaire. Accordingly, 120 herds comprising 480 cattle were subjected to CIDT, and the herd and individual animal prevalence were 20% (24/120) and 11.25% (54/480), respectively. The herd level prevalence varied significantly among different type of farming ($\chi^2=4.37$; $P=0.036$), herd size ($\chi^2=19.47$; $P<0.001$) and management ($\chi^2=25.04$; $P<0.001$). Cattle herd in intensive type of production (OR=3.67) and under poor management conditions (OR=4.28) had a higher odds for tuberculin reactivity compared to those cattle herd managed under extensive production type and good management conditions, respectively. Cattle kept in herds which had >10 cattle (OR=13.53) and cattle kept under intensive farming system (OR=4.79) had a higher odds for tuberculin reactivity compared to those cattle kept with herds had less than five cattle and cattle managed under extensive type of production, respectively. Of the animal characteristics, sex ($\chi^2=8.1$; $P=0.004$), breed ($\chi^2=15.03$; $P=0.001$) and body condition ($\chi^2=6.65$; $P=0.036$) were significantly associated with the prevalence of BTB. Female (OR=4.81), poor body condition (OR=2.65), exotic (OR=6.13) and cross (OR=6.61) had a higher odds for tuberculin reactivity compared to male, good body condition, and Zebu, respectively. Of the 54 CIDT positive cows, four were slaughtered and TB lesion was detected in all the four cattle and 57 % of the lesions were found in the thoracic cavity. In addition, of the total 768 cattle subjected to postmortem examination, 49 (6.39%) were found to be positive for gross tuberculous lesions. Larger proportion (62.3%) of TB lesion was recorded in thoracic cavity followed by retropharyngeal lymph nodes (22.95%). Culture positivity in primary culture media was confirmed in 44.2% (27/61) in tissue sample and 12.9% (4/31) in milk sample. *Mycobacterium* genus typing of the 29 culture isolates using multiplex PCR analysis indicated that only four isolates positive for the genus *Mycobacterium* of which two were members of the *Mycobacterium tuberculosis* complex. Awareness of cattle owners about BTB was generally poor and thus cattle owners were found to consume raw milk, soured milk product (yoghurt) and shared the same

shelter with their cattle. A total of 1272 TB patients received short course therapy in Mekelle hospital within five years, of which 62.03% (789/1,272) were pulmonary TB and 37.97% (483/1,272) were extra-pulmonary TB patients. In conclusion, this study revealed the importance of BTB in the study area in particular and the region in general. Thus, detection of BTB both in live and slaughtered animals, lower awareness of cattle owners about the disease and the prevailing habit of consumption of animal products and isolation of *M. tuberculosis* complex from animal tissue and milk would suggest the potential role of BTB as a zoonosis. Therefore, practical and sound control methods such as strict meat inspection, boiling of milk and cooking of meat, and public education to raise the awareness on the transmission of the disease are recommended.

Key words: Bovine tuberculosis, Cattle, CIDT test, Epidemiology, Mekelle, *M. tuberculosis* complex, Postmortem examination, Public Health.

1. INTRODUCTION

At the onset of the 21st century, animal production is facing new challenges such as demographic growth, urbanization and economic development contribute considerably to the increasing demand for meat, milk and other animal products (Steinfeld *et al.*, 2006). The concomitant intensification of animal husbandry and the development of peri-urban systems for livestock production have resulted in increased contact between people and livestock and, consequently, increased risk of some zoonotic diseases (Acha and Szyfres, 2003).

Tuberculosis (TB) is an important disease among many zoonoses (Une and Mori, 2007), that affect humans and many vertebrate animals and is characterized by the formation of granulomas in tissues and organs (Amanfu, 2006). Tuberculosis is an infectious disease of worldwide distribution and responsible for the more deaths throughout the world than any other bacterial disease ever today (Omer *et al.*, 1995; Une and Mori, 2007). According to the World Health Organization (WHO), globally, an estimate of 9.4 million incident cases, 14 million prevalent cases and 1.7 million people died of TB in 2009 were reported. An estimated 11-13% of incident cases were HIV-positive; the African region accounted for approximately 80% of these cases (WHO, 2010). In Ethiopia, tuberculosis and leprosy have been recognized as major public health problems since the 1950s. Though there have been efforts to put these diseases under control, they remained to be major disease problems even now (MOH, 2005). Ethiopia ranks seventh among the TB high burden countries globally and third from Africa (WHO, 2010). The estimated new TB cases in Ethiopia during 2009 per 100,000 population was 300 and estimated HIV prevalence in new TB cases was 12% (WHO, 2010).

Ethiopia, ranked amongst African nations who possess the largest livestock population, with an estimated 50.8 million heads of cattle, 25.9 million sheep and 21.9 million goats and 0.8 million camels (CSA, 2010). While the distribution and the quantity appear diverse according to the type of prevailing animal production systems and agro-ecological zones, but prevailing of various contagious diseases is among the major socioeconomic draw backs of the country's cattle productions (Shitaye *et al.*, 2007). Furthermore, the genetic improvement is becoming a growing

concern being integrated with animal intensification, that there is introduction of diseases of various etiologies (like bovine tuberculosis) in several dairy farms concurrent with importation of exotic breeds (WHO, 1993). Bovine tuberculosis (BTB) becomes a serious problem in cattle when intensive dairying is established, particularly when European breeds are introduced (Bogale *et al.*, 2000; Radostits *et al.*, 2007).

Bovine tuberculosis, caused by *Mycobacterium bovis*, is a well-known zoonotic and economically important disease which affects cattle world-wide (Cosivi *et al.*, 1998; Cousins, 2001). *Mycobacterium bovis* (*M. bovis*) is classified as a risk/hazard group III pathogen for public health (OIE, 2010). The global prevalence of human TB due to *M. bovis* has been estimated at 3.1% of all human TB cases, accounting for 2.1% and 9.4% of pulmonary and extra-pulmonary TB cases, respectively (Cosivi *et al.*, 1998). Bovine tuberculosis aggravates the 'triple trouble' of HIV/AIDS and TB infection and malnutrition (Ayele *et al.*, 2004).

In industrialized countries, human TB due to *M. bovis* is relatively rare as a result of milk pasteurization and animal tuberculosis control programs (Grange, 2001; Cosivi *et al.*, 1998; Amanfu, 2006). In developing countries, however, animal TB is widely distributed, control measures are not applied or are applied sporadically, and pasteurization is rarely practiced (Ayele *et al.*, 2004; Zinsstag *et al.*, 2006). Approximately 85% of cattle and 82% of human populations in Africa live in areas where bovine TB is either partly controlled or not controlled at all (Cosivi *et al.*, 1998). In such countries, where bovine TB is still common and pasteurization of milk is not practiced, an estimated 10 to 15% of human TB cases are caused by *M. bovis* (Ashford *et al.*, 2001). The epidemiology and public health significance of bovine TB in Africa remains largely unknown for politico-economic reasons, including the high cost of a testing programme, social unrest due to political instability, ethnic wars resulting in displacement of large numbers of people and animals, and a lack of veterinary expertise and communication networks (Ayele *et al.*, 2004). In addition, there are few or no laboratories that are capable of isolating and characterizing mycobacteria from animals and human beings (Cosivi *et al.*, 1998).

The available reports in Ethiopia show that the prevalence of bovine TB ranges from 3.4 % in smallholder production systems to 50 % in intensive dairy production systems (Ameni and

Roger, 1998; Kiros, 1998; Bogale *et al.*, 2000; Ameni *et al.*, 2007; Berg *et al.*, 2009). However, recently, Tschopp *et al.* (2010) reported BTB prevalence as low as 0.8% in cattle managed in rural extensive management. Similarly, study conducted in cattle from various region of Ethiopia indicated a prevalence of 3% (Tschopp *et al.*, 2009). Thus, the disease is particularly serious in intensive dairy farms, which serve the public as sources of dairy products, including milk. The situation could be exacerbated by the absence of any control method employed in the country (Ameni *et al.*, 2010).

In Ethiopia, although extent of transmission of *M. bovis* to human beings has not yet been established, the high prevalence of TB in cattle, close contact of cattle and human, habit of the community to drink raw milk and increasing HIV epidemics suggests that the impact of *M. bovis* on human TB could be significant (Ameni *et al.*, 2010). It is estimated that 82% of the milk is supplied unpasteurized by intra and peri-urban producers to consumers, while only 18% is supplied by dairy enterprises in pasteurized form (Ameni *et al.*, 2007). Moreover, substantiating such assumptions, data from the Ministry of Health show a significant rise in the incidence of extrapulmonary TB in Ethiopia, although the extent attributed to *M. bovis* is not known because the routine diagnostic procedures employed by the health personnel could not differentiate between the *Mycobacterium* species of the *Mycobacterium tuberculosis* complex (MTBC) (MOH, 2005).

Despite the introduction of Directly Observed Treatment, Short Course (DOTS) strategy in 1995 in Tigray region, northern Ethiopia, the burden of tuberculosis has not been reduced substantially. Increasing incidence of HIV, low access to health care and other factors contributing to patient delay in seeking medical treatment are thought to be contributing to the persistent burden of tuberculosis and counteracting the expected improvement due to DOTS (Mesfin *et al.*, 2005). However, the contribution of zoonotic tuberculosis in human tuberculosis is not considering in this regional state of Ethiopia. Furthermore, a number of extra-pulmonary tuberculosis commonly cutaneous tuberculosis TB patients are admitted to public and private hospital each year (personal communication) which depicts that tuberculosis is a major human health problem in the study area.

Currently, as livestock extension packages programme, the Ministry of Agriculture is promoting milk production by encouraging the expansion of smallholder dairy farms in different regions of the country. Mekelle which is one of the places in Tigray region where dairying is commonly practiced using small herd size. People in the area were observed with a great tendency to own cross-breed and exotic animals and implement intensive cattle production system via owning few productive animals. A number of small-scale and modern/intensive dairy farms serve as a source of milk to the community. Any of the dairy production or other sector does not practice pasteurization, rather they sell raw milk. People residing in the area consume raw and locally soured milk, and raw meat. Moreover, cattle are kept in close proximity to farmers' homes (personal observation). However, the intensification in cattle rearing, keeping exotic and cross breeds, and mixing up of cattle herds without any appropriate BTB control strategy may favors the spread of BTB between cattle. Though various types of cattle husbandry methods and cultural practices that could facilitate transmission of BTB between cattle and humans exist, the extent of bovine TB in Tigray regional state particularly in and around Mekelle is not known. Hence, the degree of zoonotic transmission of tuberculosis from animals to humans is unknown. In order to embark a future national BTB control program, the epidemiology of the disease has to be assessed in terms of prevalence in animals, and in terms of risk factors for disease transmission. Elaboration of a transmission model is therefore a prerequisite for any further economical assessment of the most profitable intervention of bovine tuberculosis in Ethiopia.

Therefore, the objectives of this study were:

- To determine the extent of BTB in cattle residing in and around Mekelle,
- To identify the risk factors associated with bovine tuberculosis infection in cattle,
- To investigate the possible risk factors predisposing cattle owners to zoonotic tuberculosis and to assess the public awareness about zoonotic importance of bovine tuberculosis,
- To isolate and identify the agent responsible for bovine tuberculosis in the area.

2. LITERATURE REVIEW

2.1. The mycobacteria

2.1.1. Taxonomy

The genus mycobacterium belongs to the Kingdom of Bacteria; Phylum of Actinobacteria; Order of Actinomycetales; Family of Mycobacteriaceae (Seifert, 1996; Quinn *et al.*, 2004). 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 a high lipid content in the wall, probably the highest among all bacteria (Palomino *et al.*, 2007). The mycobacteria comprise more than 80 species, within a complex of related and poorly studied organisms (Rainey *et al.*, 1995). 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 *M. leprae*, *M. lepraemurium*, *M. avium* subsp. *Paratuberculosis*, and the members of the *M. tuberculosis* complex (Palomino *et al.*, 2007).

Comprised within the *M. tuberculosis* complex and generically called the tubercle bacilli, the various etiologic agents of tuberculosis have distinct hosts, zoonotic potential and reservoirs (Vincent *et al.*, 1992; Palomino *et al.*, 2007). The *M. tuberculosis* complex which includes *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, *M. canettii*, *M. bovis* subsp. *caprae* and *M. pinnipedii* (Tauro *et al.*, 1996; Haddad *et al.*, 2004). *M. tuberculosis*, and the regional variants or subtypes *M. africanum* and *M. canettii* are primarily pathogenic in humans (Palomino *et al.*, 2007). *M. bovis* and *M. 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 *M. caprae* and *M. pinnipedi*, although sometimes they are identified as *M. bovis* subspecies or variants. It could be expected that the major evolutive shifts involved in adaptation to different hosts would have entailed significant microbiological differentiation (Niemann *et al.*, 2000; Mostowy *et al.*, 2005).

Mycobacterium species other than the MTBC that cause TB like diseases in man and animals are commonly called 'atypical mycobacterium' (Quinn *et al.*, 2004). Atypical mycobacterium are not pathogenic to man and animals except in certain situations such as direct inoculation into wound or introduction into immunocompromised hosts due to immune suppressive therapy or due to HIV infection (Thoen *et al.*, 2006); however, they are important during diagnosis as they sensitize man/animals to tuberculin test (Carter and Chengappa, 1991).

2.1.2. Morphology and staining

Mycobacteria are thin rods of varying length (0.2-0.6 by 1.0-10.0 μm) and sometimes branching filamentous, non-motile, non-spore forming, aerobic and oxidative (Tauro *et al.*, 1996; Quinn *et al.*, 2004). *M. tuberculosis* is straight or slightly curved rod, whereas *M. bovis* is usually straighter, stouter and shorter (Gupte, 2006). All mycobacteria are acid fast and share a characteristic cell wall, thicker than in many other bacteria, which is hydrophobic, waxy and rich in mycolic acid/mycolates (Palomino *et al.*, 2007). The cell wall of mycobacteria has high lipid content (60%) (Sharma and Adalakha, 1996). 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 (Palomino *et al.*, 2007). It probably also contributes to the slow growth rate of some species by restricting the uptake of nutrients (Palomino *et al.*, 2007).

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 (Palomino *et al.*, 2007). The high lipid content renders mycobacteria hydrophobic and makes them difficult to stain with commonly used aniline dyes at room temperature. The bacteria, therefore, take up the stain with dyes only by prolonged application or by heating (Sharma and Adlakha, 1996). Usually the Ziehl-Neelsen technique of staining is employed for identification of acid-fast bacteria including mycobacteria. Moreover, mycobacteria can also be stained with fluorescent dyes like auramine rhodamine (Biberstein and Hirsh, 1999).

2.1.3. Growth and cultural characteristics

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 (Palomino *et al.*, 2007). According to WHO (1998) tubercle bacilli can be cultivated on many different media like egg-based media, agar-based media and liquid media but, the only media which allow abundant growth of tubercle bacilli are egg-enriched media containing glycerol/pyruvate and asparagines, and agar or liquid medium supplemented with serum or bovine albumin. In addition, tubercle bacilli may also be grown on chick embryos and in tissue culture (Gupte, 2006). To date, the most frequently used media for isolation of *M. bovis* are Löwenstein-Jensen (LJ) and Ogawa-medium (both containing eggs phosphate, magnesium) and the former contains asparagine (Seifert, 1996).

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. *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 (Palomino *et al.*, 2007). All the members of the mycobacteria complex are slow growers (Seifert, 1996). Therefore, the inoculated media may have to be incubated at 37°C up to 8 to 12 weeks (Quinn *et al.*, 2004).

The growth requirements and cultural characteristics of mycobacteria are summarized in Table 1. With regard to the colony characteristics, on solid media *M. tuberculosis* appears as dry, rough, raised and irregular colony. It is creamy white first and becomes yellowish or buff coloured later on and is not emulsified easily. On the other hand, the colony of *M. bovis* is flat, smooth, white and breaking up easily when touched (Gupte, 2006).

Table 1: The growth requirements and cultural characteristics of pathogenic mycobacteria

Growth characteristics and requirements	<i>M. tuberculosis</i>	<i>M. bovis</i>	<i>M. avium</i> complex	<i>M. avium</i> <i>para-tuberculosis</i>
Growth rate	Slow (3-8 weeks)	Slow (3-8weeks)	Slow (2-6weeks)	Very slow (up to 16 weeks)
Atmospheric requirements	Aerobic	Aerobic	Aerobic	Aerobic
Colonial features	Rough, buff, difficult to break apart	Cream colored raised with central roughness, break apart easily	Sticky, off white, break apart easily	Small, hemispherical, some pigmented
Essential growth supplement	None	None	None	Mycobactin
Effect of added glycerol	Enhanced growth (Eugonic)	Growth inhibited (Dysgonic)	Enhanced growth (Eugonic)	-

Source: Quinn *et al.* (2004).

2.1.4. Virulence factor

Mycobacteria are intra-cellular organisms in which the ability to produce diseases in animals depends on their virulence factor, appear to be related to the ability to survive and multiply within microphages (Palomino *et al.*, 2007). The mechanism for such survival is multifactorial phenomenon, requiring the participation and cumulative effect of several components, and may vary from species to species (Thoen and Chiodin, 1993).

Virulence appears to reside in the lipids of the cell wall. Mycosides, phospholipids and sulpholipids are thought to protect the tubercle bacilli against phagocytosis. Glycolipids cause granulomatous response and enhance the survival of phagocytosed mycobacteria. Wax D and

various tuberculo proteins induce a delayed hypersensitivity reaction detected in the tuberculin test (Quinn *et al.*, 2004). According to Palomino *et al.* (2007) the distinctive characteristics of the virulent bacilli have been attributed to the trehalose 6,6'-dimycolate. This compound, also known as cord factor, was described as an extractable glycolipid consisting of two mycolic acid molecules loosely bound in the outer layer of the cell wall. A myriad of biological activities related to pathogenicity, toxicity and protection against the host response have been attributed to this molecule. However, it does not seem to be essential for bacterial multiplication *in vitro* (Indrigo *et al.*, 2002).

By microscopic observation, Robert Koch first described the arrangement of bacilli in braided bunches and associated this phenomenon with virulent strains of *M. tuberculosis*. In general, fresh virulent *M. tuberculosis* bacilli produce rough textured colonies on solid media, expanded gummy veils on the surface of liquid media and serpentine on microscopic smears. In contrast, non-virulent mycobacteria and tubercle bacilli attenuated by prolonged cultures usually develop smooth colonies on solid media, form discrete mats in liquid media and distribute randomly in loose aggregates when smeared. The recognition of these two peculiarities, cording and crumbly colony formation, provides a reliable and timely clue to the experienced microbiologist for the presumptive distinction of *M. tuberculosis* from other mycobacteria in cultured specimens and even in sputum smears (Palomino *et al.*, 2007).

2.1.5. Evolution of *Mycobacterium tuberculosis* complex

Mycobacteria are likely to represent a very ancient genus of bacteria. Probably, the *Mycobacterium* genus originates from a common ancestor whose offspring specialized in the process of colonizing very different ecological niches (Palomino *et al.*, 2007). The evolutionary relationships between organisms of the genus *Mycobacterium* have been investigated on the basis of the analysis of derived similarities ("shared derived traits", synapomorphies). The discovery in 1993 of the polymorphic nature of the Direct Repeat (DR) locus, and the subsequent development of the spoligotyping method based on DR locus variability, introduced more modern concepts and tools for *M. tuberculosis* complex genotyping (Groenen *et al.*, 1993; Palomino *et al.*, 2007).

Genetic systems have developed to demonstrate the complete genetic blueprint of *M. tuberculosis* and *M. bovis* which in turn provides major insight in to evolutionary relationship and virulence factor (Brosch *et al.*, 2002; Garnier *et al.*, 2003). Even though *M. tuberculosis* complex shows host specificity, they are 99.9% similar in regard of their DNA, with identical 16S rRNA sequences (Brosch *et al.*, 2002).

The genome of mycobacteria has a high GC (GC% ~65%) content, and its polymorphism is very limited compared to its genome size (4.4 Mb). But some regions are highly polymorphic, either by a variation in number and/or position or by a variation in primary structure (Haddad *et al.*, 2004). Strikingly, the genome sequence of *M. bovis* is greater than 99.95% identical to that of *M. tuberculosis*, but deletion of genetic information has led to a reduced genome size (Garnier *et al.*, 2003). Moreover, *M. bovis* does not have any new genetic material when compared with genome of *M. tuberculosis* (Thoen and Barletta, 2006). Thus, the genome difference between *M. tuberculosis* and *M. bovis* is attributed to DNA deletion in *M. bovis* and hence deletion more than 2000 single nucleotide polymorphism have been found (Thoen and Barletta, 2006).

Different genomic analysis indicated that *M. canettii* is a potential ancestral species of *M. tuberculosis* complex (Brosch *et al.*, 2002; Palomino *et al.*, 2007). Successive DNA deletion from ancestral species resulted in creation of other members of *M. tuberculosis* complex including *M. africanum*, *M. microti* and *M. bovis*. Moreover, *M. bovis* BCG experienced further deletion during in vitro adaptation, and the loss of region RD1 has been implicated as the mechanism of virulence attenuation (Figure 1) (Brosch *et al.*, 2002).

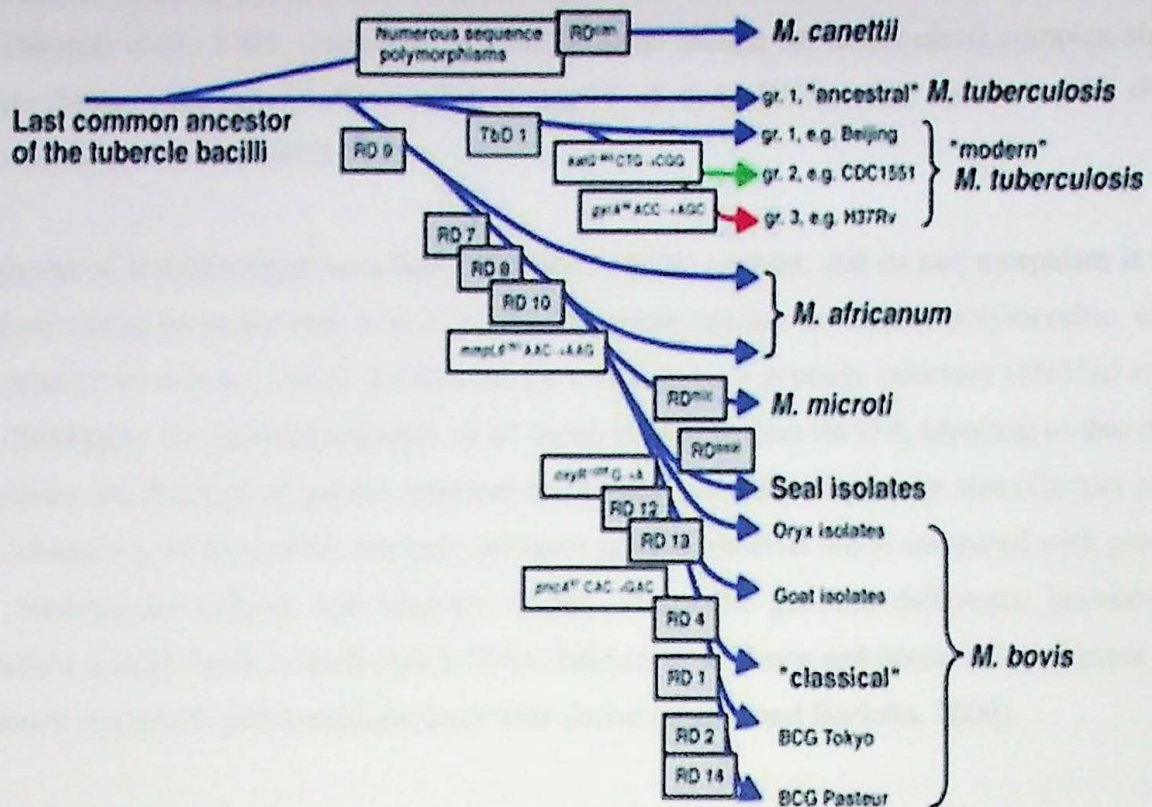


Figure 1: Evolutionary scheme of the members of the *M. tuberculosis* complex illustrating successive loss of DNA in certain lineages

Source: Brosch *et al.* (2002).

2.2. Pathogenesis and Immunology

Tubercle bacilli gain entrance to the animal body through respiratory, alimentary, genital, cutaneous and genital routes. The first two are being the most commonly observed routes of infection resulting in pulmonary and extra-pulmonary form of the disease, respectively. After infection the bacteria may localize in tissue related to the route of infection and associated lymph nodes (Menzies and Neill, 2000).

Mycobacterial infection triggers a Th1-induced cell-mediated immune response (CMI) which leads to release of cytokines such as tumor necrosis factor- α , Interleukin-12 (IL-12) and

Interferon gamma (IFN- γ). This pathway is essential to activate macrophages (Orme and Cooper 1999). Depending on the balance of cytokines involved, three outcomes are possible: 1) macrophages kill and eliminate the bacteria, 2) the bacteria lies dormant (latency), 3) the bacteria cannot be contained by the immune system and the disease develops to active TB (Welsh *et al.*, 2005).

Containment of the bacteria results in the formation of nonvascular nodular granulomas known as “tubercles”. Lesions show typically a centre of caseous necrosis with some degree of calcification surrounded by a cell wall of epithelioid cells, lymphocytes and neutrophils (Doherty *et al.*, 1996). Unlike in man, these primary lesions are rarely contained by the immune system in cattle and bacilli spread by lymphatic and haematogenous routes, resulting in tubercles in other organs (Neill *et al.*, 1994).

The initial CMI response is followed later in time by a humoral antibody response, which is caused by a shift of Th1 to Th2 cell activation (Dlugovitzky *et al.*, 2000). A state of anergy may occur in advanced stages of the disease and a CMI response is no more detected. Initial pathological changes are associated with the onset of CMI response (Cosivi *et al.*, 1998) (Figure 2). CMI response can be affected by the animal’s nutritional state (e.g. deficiency in energy, protein and micronutrients), by stress or concurrent diseases, which lead to a reduction of the host resistance (Doherty *et al.*, 1995; Pollock and Neill, 2002).

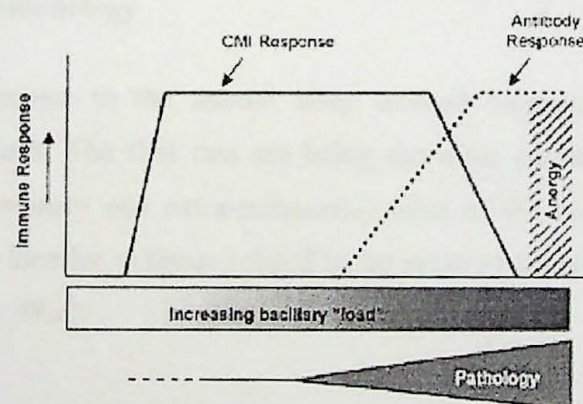


Figure 2: Temporal spectrum of immune responses in cattle following infection of *M. bovis*
Source: Pollock and Neill, (2002)

2.3. Epidemiology

2.3.1. Host range

Mycobacterium bovis has a broad host range, the largest of any member of the *M. tuberculosis* complex (Biet *et al.*, 2005). Domestic cattle are considered the true natural hosts of the bacterium and the principal reservoir of infection for other animals and man (de la Rua-Domenech, 2006). However, most mammalian species are susceptible to various degrees to BTB and the disease has been reported in human beings, domesticated animals and various free-ranging and captive wildlife species (de Lisle *et al.*, 2001; Biet *et al.*, 2005). The pathogen was isolated from, ovidae, suida, camelidae, bovidae, caprinae, cervidae, mustelidae, rodents, primates, marsupials, lagomorphs, perrissodactyls, procyonidae, canidae, felidae, ursidae and elephant (de Lisle *et al.*, 2002; Biet *et al.*, 2005; Nishi *et al.*, 2006; Delahay *et al.*, 2007; Naranjo *et al.*, 2008).

2.3.2. Distribution

Bovine tuberculosis is a worldwide disease (Fra'guas *et al.*, 2006). Out of 209 countries reported to OIE (Table 2), 49.3% (103/209) reported the detection of BTB, 37.8% (79/209) noted its absence and the remainder didn't report about the disease (Amanfu, 2006). Industrialized countries have eradicated or drastically reduced the prevalence of *M. bovis* infection in both animals and humans through animal tuberculosis control and elimination programmes, improvements in milk hygiene and pasteurization and awareness creation on the zoonotic and economic significance of *M. bovis* infection (Amanfu, 2006). In developing countries, however, animal TB is widely distributed, control measures are not applied or are applied sporadically, and pasteurization is rarely practiced (Bakshi *et al.*, 2005). Even though the distribution of BTB is globally, data on the prevalence of animal tuberculosis is generally scarce. It is difficult to measure the precise incidence of the disease in some countries and only qualitative assessment often not supported by laboratory or field investigations, are submitted (Amanfu, 2006).

In Africa, as in most developing countries, *M. bovis* infection largely remain uninvestigated and the data from Africa were insufficient to represent the true epidemiological picture of the diseases

(Ayele *et al.*, 2004), yet bovine tuberculosis is present in almost all African countries, affecting both domestic and wild animals. Moreover, despite its wide presence in Africa very little is known about risk factors for *M. bovis* infection in either cattle or human populations (Cleaveland *et al.*, 2007).

Table 2: The global distribution of bovine tuberculosis in animals

Continent	Detection of BTB	Absence of BTB	No report	Total
Africa	26(51%)	7(13.7%)	18(35.3%)	51(100%)
Americas*	21(48.8%)	19(44.2%)	3(7%)	43(100%)
Asia	20(44.4%)	20(44.4%)	5(11.2%)	45(100%)
Europe	35(68.6%)	15(29.4%)	1(2%)	51(100%)
Oceania	1(5,3%)	18(94.5%)	-	19(100%)
Total	103(49.3%)	79(37.8%)	27(12.9%)	209(100%)

* North and South America and the Caribbean Islands

Source: Amanfu, (2006).

2.3.3. Source of infection and Transmission pathway

Bovine tuberculosis infected cattle should always be considered as potential sources of infection (Menzies and Neill, 2000). Besides, maintenance and amplifier hosts if infected, they may act as a source of infection for other animals and man. Also infected humans can act as amplifier hosts of BTB, representing a potential source of *M. bovis* for animal and human contacts (de la Rua-Domenech, 2006). Human patients with genitourinary BTB can be a source of infection for cattle when they urinate in cowsheds or on pastures (Grange, 2001). *M. bovis* is usually excreted in respiratory discharges, faeces, urine, semen (Quinn *et al.*, 2004), genital discharges (lochia in cows with tuberculous endometritis) and milk from infected mammary glands (Biberstein and Hirsh, 1999).

The possible routes of infection include the respiratory, alimentary, congenital, cutaneous, venereal, percutaneous routes and via the teat canal (Menzies and Neill, 2000). The agent of bovine tuberculosis is mainly transmitted via the respiratory and alimentary routes. However, the

percutaneous and transplacental routes of infection are unusual and intrauterine infection of calves occur only when bovine tuberculosis is common and when the reproductive organ is infected (Biberstein and Hirsh, 1999; Menzies and Neill, 2000).

Inhalation is the most probable and principal route to bovine infection and is facilitated by close, prolonged contact between infected and healthy animals (Menzies and Neill, 2000). Intensive livestock farming promotes close contact between animals and in extensive livestock farming, close contact between animals occur at watering points, at night gathering places, vaccination and artificial insemination centers, dipping tanks, market places, during transportation, during concentration under trees or other shaded areas and salt supplementing sites favoring the spread of *M. bovis* in animals (Ayele *et al.*, 2004). Ingestion of *M. bovis* directly from infected animals or from contaminated pasture, water or utensils may also be very common (Ayele *et al.*, 2004). *M. bovis* survives in water and may enter the digestive tract during drinking. In addition, contact with soil is a potential means of transmission (Phillips *et al.*, 2003).

2.3.4. Risk factors

The ubiquitous distribution of *M. bovis* in farmed and to a large extent in the wild animal population, the trend towards intensification of animal production to meet the increase in demand for milk and meat, gathering of animals at watering points, markets and in corrals over night and lack of control measures in most African countries are some factors which are likely to facilitate *M. bovis* infection in the African animal population (WHO, 1993).

I. Agent risk factors

Mycobacterium bovis is moderately resistant to heat, desiccation and many of the acid and alkaline disinfectants (Radostits *et al.*, 2007); this resistance is due to hydrophobic lipid surface found in their cell wall. It can survive for several months in the environment, in moist soil and in darkness and remains viable for long periods (>6 months) in frozen tissues (Jahans and Worth, 2006). They also remain viable for long period in the dark, putrefying material, faeces, sputum and soil (Sharma and Adlakha, 1996; Biberstein and Hirsh, 1999). Tubercle bacilli survives in

acid milk for 15 days and in milk products such as cheese and butter for weeks but it gets destroyed when milk is heated for 30 minutes at 65⁰C, and cream is heated at 85 ⁰C for 2 minutes and, it can also survive for over 2 years in frozen carcasses (Seifert, 1996). It has been also reported *M. bovis* can survive in cow feces for more than 5 months and in soil for up to 2 years depending on weather conditions (Courtenay *et al.*, 2006). In developing countries manure fertilization of arable land is a common practice, which might represent a potential source of infection for animals and humans through contaminated pastures and vegetables (Ayele *et al.*, 2004). However, the tubercle bacilli of various types can be killed at 60 ⁰C in 15 minutes (Sharma and Adlakha, 1996). They are also susceptible to ionic detergents and are killed by sunlight, ultraviolet irradiation, and pasteurization (Biberstein and Hirsh, 1999).

II. Host risk factors

Genetic resistance of hosts to *M. bovis* infection has never been conclusively demonstrated (Morris *et al.*, 1994), but zebu (Brahman) types of cattle are thought to be much more resistant to TB than the European cattle. The effects on these cattle are much less severe but under intensive feedlot conditions a morbidity rate of 60% and a depression of weight gain can be experienced in tuberculous zebu cattle (Radostits *et al.*, 2007). A study conducted in central Ethiopia by Ameni and colleagues, suggested that a higher susceptibility to bovine TB in Holsteins (more than twice) than zebras under identical field husbandry conditions (on pasture). Besides, the severity was also significantly higher in Holsteins than either zebus or crosses (Ameni *et al.*, 2007).

The higher prevalence of BTB in dairy than beef cattle may reflect closer confinement, longer life spans, and greater productivity stress among dairy cows. Exemption from pregnancy and lactation may explain the lower disease prevalence in bulls than cows (Biberstein and Hirsh, 1999). Risk of a positive reaction varies with an animal's age and body condition (Cook *et al.*, 1996). Besides, immunological suppression in the periparturient period can produce anergic reactors, which may act as a constant source of infection for cattle-to-cattle transmission (Foster *et al.*, 2002).

III. Husbandry and environmental factors

Husbandry and environmental factors that play role in predisposing animals to infection include housing system, feeding conditions and presence of wild reservoirs of *M. bovis* infection for grazing cattle (Quinn *et al.*, 2004). Intensification predisposes to the disease because animals are closer; the greater is the chance that the disease will be transmitted. For example, the disease incidence is high in intensive dairy farms than in beef ranches (Morris *et al.*, 1994) whereas in beef cattle the degree of infection is usually low. The existence of a widespread intermediate host is also reported as the greatest contributor to infection in cattle (Phillips *et al.*, 2003).

Malnutrition, pregnancy and concurrent infections may depress the immune responsiveness in some cases and they may be important factors in cattle herds (Collins, 1994). Circumstantial evidence suggests that an adequate intake of mineral, vitamin and protein reduces the susceptibility of cattle to BTB infection (Griffin *et al.*, 1993). Although weather patterns have been implicated in the susceptibility of herds, it is concluded that some reduction in the susceptibility of cattle to *M. bovis* infection can be achieved by modifications to the management system to minimize risk factors (Foster *et al.*, 2002).

Cattle movement, purchase, grazing field boundaries or short swards, spreading cattle slurry on the fields are a potential risk for transmission of the disease between cattle. Recent introductions from market, Provision of water from mud holes in dry river beds during dry season, and the presence of monkeys or warthogs are risk factors associated with BTB infection of cattle identified in transhumance areas of Uganda (Oloya *et al.*, 2007). Herds with the following risk factors had a significantly greater prevalence of intradermal test positives: >50 cattle in the herd; herds housed inside at night and herds in contact with wildlife. Furthermore, villages that experienced annual flooding had a higher prevalence of infection (Cleaveland *et al.*, 2007). The herds of those households with a reported human TB cases are more likely to have a tuberculin positive animal than herds in households without a reported human TB case (Cook *et al.*, 1996).

2.4. Economic importance

Bovine tuberculosis represents a serious health threat for livestock, wildlife and humans (Tschopp *et al.*, 2009). But the disease is also an important economical and financial burden to society, linked with economic losses: loss of productivity of infected animals (e.g. reduced milk yields and meat production, reduced fertility), animal market restrictions, control and eradication programs costs, human health costs, loss from tourism sector etc. (Morris *et al.*, 1994; Seifert, 1996; Zinsstag *et al.*, 2006; Radostits *et al.*, 2007). The socio-economic impact of BTB to the agriculture and health sector in Turkey has been estimated between 15 and 59 million US\$ per year (Barwinek and Taylor, 1996). In Argentina, the annual loss due to BTB is approximately US\$ 63 million (Cosivi *et al.*, 1998). Even in some industrialized countries, where BTB has been eradicated by expensive schemes for control, eradication and compensation for farmers, the disease still has a major economic impact, mainly due to the existence of a permanent wildlife reservoir that reduces the efficiency of control strategies. In UK, where badger and other wildlife such as deer remain an important source of infection for livestock, approximately £100 million is spent annually in efforts to control the disease (Matthews *et al.*, 2006).

In Africa, the economic losses associated with livestock infected with BTB have not been examined sufficiently, or have not been studied at all (Ayele *et al.*, 2004). There are also costs associated with human TB due to *M. bovis*. This includes cost of treatment, hospitalization, loss of working days and payment for sick leave (WHO, 1994; Barwinek and Taylor, 1996). For the public health sector, WHO estimates total TB control costs in Ethiopia of US\$14.2 million per year and US\$129 per patient (WHO, 2004).

2.5. Zoonotic importance

The current increasing incidence of tuberculosis in humans, particularly in immunocompromised humans, has given a renewed interest in the zoonotic importance of *M. bovis*, especially in developing countries and, the ease and frequency of the spread of tuberculosis from animals to humans in an uncontrolled environment makes this an important zoonosis (Radostits *et al.*, 2007).

Global prevalence of human TB due to *M. bovis* has been estimated at 3.1% of all human TB cases accounting for 2.1% of pulmonary and 9.4% of extra pulmonary TB cases (Cosivi *et al.*, 1998). Studies have shown that *M. bovis* accounted for 0.5-1.5% of all culture confirmed TB cases in UK (de La Rua-Domenech *et al.*, 2006), for 1-6% of human TB in Africa (Cadmus *et al.*, 2006; Kazwala *et al.*, 2001), and for 5-10% of human TB in Latin America (Haddad *et al.*, 2004).

Transmission of *M. bovis* can occur between animals, from animals to humans and vice versa and rarely, between humans (Figure 3) (Anaelom *et al.*, 2010). *M. bovis* infection is transmissible from cattle to humans directly by aerogenous route (WHO, 1994) and through direct contact with material contaminated with nose and mouth secretions from an infected herd of cattle (Thoen *et al.*, 2006). Research findings revealed that the risk group are individuals persons in contact with potentially infected animals such as veterinarians, abattoir workers, meat inspectors, autopsy personnel, farmers, milkers, animal keepers (as well as those in the zoo), animal dealers, laboratory personnel and owners of potential tuberculous pets (e.g. monkeys) (Une and Mori, 2007; Anaelom *et al.*, 2010). Indirectly, man can acquires the disease from animal sources by ingestion of meat and meat products from slaughtered infected cattle and consumption of unpasteurized infected milk (Cosivi *et al.*, 1998; Thoen *et al.*, 2006; Radostits *et al.*, 2007). Ingestion of unpasteurized contaminated milk products poses a greater risk than ingestion of infected meat products because highly infected carcasses are condemned; parts of carcasses that are processed as meat products are inspected and thoroughly cooked (Anaelom *et al.*, 2010). Conversely, people suffering from *M. bovis* tuberculosis can retransmit the infection to cattle; however, this is not common (Anaelom *et al.*, 2010).

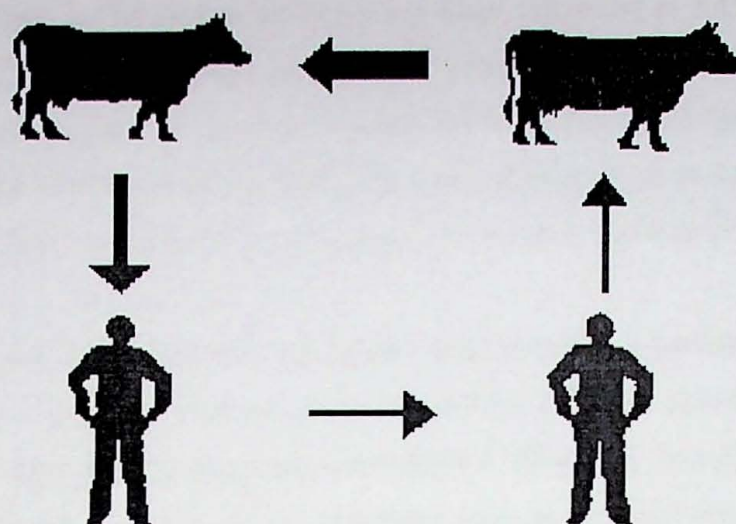


Figure 3: Cycle of *M. bovis* transmission between cattle and humans. The thickness of the arrows suggests level of probability

Source: Anaelom *et al.* (2010).

2.5.1. Factors favoring infection of human by *M. bovis* in Africa

I. Close physical contact

Close physical contact between humans and potentially infected animals is present in some communities, especially in developing regions. For example, in many African countries cattle are an integral part of human social life; they represent wealth and are at the center of many events and, therefore, gatherings. In addition, with 65% of African, 70% of Asian, and 26% of Latin American and Caribbean populations working in agriculture, a significant proportion of the population of these regions may be at risk for bovine TB (Cosivi *et al.*, 1998).

II. Poor food hygiene practices

Consumption of milk contaminated by *M. bovis* has long been regarded as the principal mode of TB transmission from animals to humans (Anaelom *et al.*, 2010). In regions where bovine TB is common and uncontrolled, milkborne infection is the principal cause of cervical lymphadenopathy (scrofula) and abdominal and other forms of nonpulmonary TB. Although

proper food hygiene practices could play a major role in controlling these forms of TB, such practices are often difficult to institute in developing countries (Cosivi *et al.*, 1998).

In all countries of sub-Saharan Africa, there is active competition between large-scale, often state-run, processing and marketing enterprises and the informal sector. The informal sector can ignore standards of hygiene and quality, and producers often sell directly to the final consumers. In addition, an estimated 90% of the total milk produced is consumed fresh or soured (Cosivi *et al.*, 1998). *M. bovis* was isolated from raw milk (Ameni *et al.*, 2003b; Ayele *et al.*, 2004). Thus, serious public health implications of potentially contaminated milk and milk products should not be underestimated.

III. Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS)

The incidence of BTB in humans has risen as a result of the impact of the HIV/AIDS epidemic (Cosivi *et al.*, 1998; Ayele *et al.*, 2004; Zinsstag *et al.*, 2006). According to recent WHO global estimates, of the 9.4 million incident cases in 2009, an estimated of 1.1 million (12%) were HIV-positive. Of these HIV-positive TB cases, approximately 80% were in the African Region (WHO, 2010).

Tuberculosis and other mycobacterial infections are major opportunistic infections in HIV/AIDS infected individuals, while HIV/AIDS is a major predisposing factor for tuberculosis including reactivation (Cosivi *et al.*, 1998). The occurrence of both infections in one person makes TB infection very likely to progress to active disease. Persons infected with both pathogens have an annual risk of progression to active TB of 5% to 15%, depending on their level of immunosuppression; approximately 10% of non-HIV infected persons newly infected with TB become ill at some time during their lives. In the remaining 90%, effective host defenses prevent progression from infection to disease (Raviglione *et al.*, 1995). It is commonly believed that *M. bovis* is less virulent than *M. tuberculosis* in humans and therefore less likely to lead to overt postprimary disease and that human-to-human transmission leading to infectious disease is rare. However, if the apparent difference in virulence is the result of differences in responsiveness of the host defense mechanisms, HIV-induced immunosuppression could well lower host defenses leading to overt disease after infection (Cosivi *et al.*, 1998).

2.6. Bovine Tuberculosis in Ethiopia

2.6.1. Magnitude and Distribution

BTB is endemic in cattle in Ethiopia; the disease is wide spread in both humans and cattle, and has been reported from different field and abattoir studies (Asseged *et al.*, 2004; Ameni *et al.*, 2007; Shitaye *et al.*, 2007). However, the prevalence of the disease is not well established in livestock and most studies focused mainly on Central Ethiopia, whereas large areas in the country remain un-investigated, even if very few study have been conducted outside the Central Ethiopia, it does not give the overall nationwide epidemiological picture.

A recent study, Tschopp *et al.* (2010), found that in Hamer Woreda, South Omo (Southern Ethiopia) BTB prevalence as low as 0.8% in cattle. Similarly, a study conducted on 2216 cattle from various region of Ethiopia indicated that a prevalence of 3%, with the highest found in Meskan Mareko, in Central Ethiopia (7.9%) and the lowest in Woldia, North Wollo (1.2%) (Tschopp *et al.*, 2009). Ameni *et al.* (2003a) found an individual animal prevalence of 7.9% using CIDT in the Wuchale district. A large scale study involving 5,424 cattle carried out in Central Ethiopia showed that the overall prevalence in cattle was 13.5%, with higher prevalence found in Holstein (22.2%) compared to local zebus (11.6%) (Ameni *et al.*, 2007). In high density herds maintained under intensive farming conditions, BTB prevalence was found as high as 50% of Holstein cattle at the Holeta National Insemination Centre (Ameni *et al.*, 2007).

High prevalence of BTB (23.7%) was reported by Elias *et al.* (2008) in Addis Ababa dairy farm. Another study in the same area, a prevalence rate of 34.1% was recorded (Tsegaye *et al.*, 2010). A prevalence rate as high as 50% has been reported in dairy cattle in eastern Shoa (Ameni and Roger, 1998) and 38.5% in goats in Adami Tulu district (Tafesse, 2006) using the SIDT test. Ameni *et al.* (2003b) reported a prevalence rate of 46.8% in cattle from dairy farms of Ethiopia.

Mycobacterium bovis was also isolated from abattoir surveys. Asseged *et al.* (2004) found 1.5% of 1350 examined cattle in Addis Ababa abattoir displaying tuberculous lesions; *M. bovis* was isolated from 50% of these lesions. Similarly, *M. bovis* was also isolated in an abattoir survey

from Hossana where 4.5% of the animals showed tuberculous lesions (Teklul *et al.*, 2004). These studies also showed that the efficiency of routine abattoir inspection was very low. In Asseged *et al.* (2004) study only 55% of the tuberculous cattle could be detected by meat inspection, whereas Teklul *et al.* (2004) noticed that only 29.4% of the carcasses with lesions could be detected during routine meat inspection. An investigation of the prevalence of BTB in various parts of Ethiopia based on cases identified at slaughterhouses indicated that, out of approximately 32,800 inspected cattle, 4.7% showed suspect tuberculous lesions (Berg *et al.*, 2009).

2.6.2. Mycobacterium species and strains in Ethiopia

There are several reports indicating the isolation of the *M. bovis* and *M. tuberculosis* from clinical and tissue samples (in animals only) obtained from humans and animals in different parts of Ethiopia (Kidane *et al.*, 2002; Ameni *et al.*, 2007; Shitaye *et al.*, 2007; Berg *et al.*, 2009; Ameni *et al.*, 2010; Tsegaye *et al.*, 2010). Ameni and colleagues reported the results of strain typing of isolates for the first time in the country from samples of animals in Holeta dairy farm. Spoligotyping of 41 isolates from 17 cows gave an identical and a unique spoligotype pattern that was represented by the binary number 110000010111111011111110001000000000100000, where 1 indicates the presence of a spacer and 0 represents a loss. That spoligotype pattern had not been reported to the *M. bovis* database, and it was designated as *EMbs1* (Ethiopian *M. bovis* strain 1) or SB1176 (Ameni *et al.*, 2007).

A comprehensive investigation of mycobacterium species and strains in Ethiopia has been made by Berg and colleagues (2009). Characterization of mycobacterium isolates, collected from different part of Ethiopia (Woldiya, Gimbi, Butajira and Jinka), by molecular typing indicated that a number of *M. bovis*, *M. tuberculosis* and non-tuberculous mycobacteria (NTM) were identified (Table 3). Eleven different species of NTM were characterized by the author; these are *M. acapulcensis*, *M. colombiense*, *M. engbaekii*, *M. fortuitum*, *M. gordonae*, *M. intracellulare*, *M. monacense*, *M. mucogenicum*, *M. nonchromogenicum*, *M. peregrinum* and *M. vaccae*. Up on further characterization of *M. bovis* using spoligotyping seven different spoligotype patterns were identified, theses are designated as SB1176, SB1476, SB0133, SB0134, SB1488, SB1489 and SB1477 of which four had not been registered before in the international *M. bovis* spoligotype

database at www.Mbovis.org. Similarly, eight *M. tuberculosis* strains were isolated from cattle, and they originated from four out of the six abattoirs (Berg *et al.*, 2009).

Another work by Tsegaye *et al.* (2010) in Addis Ababa city dairy farms, of the 11 isolates from tissue specimen culture, 8 isolates were confirmed as *M. bovis* and other three were identified as *M. tuberculosis*. The eight *M. bovis* isolates exhibited three different spoligotype patterns. The three *M. tuberculosis* isolates were identified as two different spoligotyping patterns. The three *M. bovis* spoligotype patterns were SB0133, SB0134 and SB1176. More recently another study conducted in Kombolcha abattoir by Ameni and colleagues indicated that from the 27 isolates showed evidence for RD4 deletion typing; 25 isolates were *M. bovis* and two were *M. tuberculosis*. Up on spoligotyping, the isolates were grouped into six clusters of *M. bovis* strains (SB1176, SB0133, SB0912, SB1176, SB1490, SB1491 and SB1492) and one cluster of *M. tuberculosis* (SIT262). Of the six clusters of *M. bovis*, three clusters were new (SB1490, SB1491 and SB1492) (Ameni *et al.*, 2010).

Another recent study by Biffa and colleagues, twelve spoligotypes were identified from 34 distinct strains. Interestingly, six new spoligotypes of *M. bovis* were reported and subsequently registered in the database with international identification codes of SB1517, SB1518, SB1519, SB1520, SB1521 and SB1522 (Biffa *et al.*, 2010). On the basis of the Spoligotype Evolutionary Index, SEI (a numeric expression approach to make standardized comparison of spoligotype evolution), *M. bovis* isolates from Ethiopia were relatively more heterogeneous (SEI = 3.2) compared to isolates from other countries. This might be attributed to extensive livestock movement linked to trading or seasonal migration, high degree of livestock mingling, and also diversities of the country's agricultural and livestock ecosystems, in addition to lack of disease control measures that led to high infection prevalence (Biffa *et al.*, 2010).

In general, the most commonly reported strain of *M. bovis* in several studies and found localized in Ethiopia is SB1176 (Ameni *et al.*, 2007; Berg *et al.*, 2009; Ameni *et al.*, 2010; Biffa *et al.*, 2010; Tsegaye *et al.*, 2010).

Table 3: Summary of collected lesions and molecular typed AFB isolated from cattle slaughtered at six Ethiopian abattoirs

Study Site	Animals Investigated	Lesioned Animals	Culture and ZN positive	<i>M. bovis</i>	<i>M. tuberculosis</i>	NTM*
Butajira	4606	281	26	4	2	12
Jinka	3471	278	54	13	2	19
Gimbi	3250	413	36	10	3	13
Gonder	14314	261	20	11	0	2
Woldiya	4338	140	19	4	1	7
Addis Ababa	2800	51	16	16	0	0
All abattoirs	32779	1524	171	58	8	53

*NTM = Typed as Mycobacterium species not from the *M. tuberculosis* complex

ZN= Ziehl-Neelsen stain

Source: Berg *et al.* (2009).

2.6.3. Zoonotic implication

Ethiopia is a country where the impact of BTB is particularly important and it is amongst the three African countries with the highest burden of human TB cases (WHO, 1997). Similarly, the prevalence of BTB in Ethiopia is high, reach up to 50% in peri-urban (intensive) dairy production systems (Ameni and Roger, 1998; Kiros, 1998). The high prevalence of TB in cattle, the close contact of cattle and humans in rural areas, the habit within the community (consuming raw milk and milk product and eating raw or undercooked meat (e.g. *kitfo* and *kurt*)) and the increasing prevalence of HIV may all increase the potential for transmission of mycobacteria between cattle and their owner. Ameni *et al.* (2003a) showed that in Central Ethiopia, only 3% of the interviewed people boiled milk, and only 1% consumed cooked meat. Therefore, it was speculated that bovine tuberculosis caused by *M. bovis* might be the reason for these high extrapulmonary tuberculosis rates (Ameni *et al.*, 2003a)

It is noteworthy, 36% of incident TB cases in Ethiopia are extrapulmonary (WHO, 2008). This is exceptionally high, considering that, 15% of incident TB is extrapulmonary in most high burden

countries (WHO, 2008). The relatively high proportion of extrapulmonary cases among Ethiopian TB patients signifies the role of mycobacteria including *M. bovis*, and of non-respiratory pathways of exposure. In a recent study carried out in rural Ethiopia, it has been demonstrated that the state of proportion of extrapulmonary TB cases due to *M. bovis* was greater than that of *M. tuberculosis* (Fetene *et al.*, 2011). Kidane *et al.* (2002) also reported *M. bovis* as a cause for 17.2% (6/35) of the cervical lymphadenitis cases from rural Ethiopia. Similarly, Shitaye *et al.* (2007) reported that 16.7% of 42 human isolates were identified as *M. bovis*. These findings show that the role of *M. bovis* in causing human tuberculosis seemed to be significantly important. Strikingly, *M. tuberculosis* was isolated from eight cattle, an unusual finding that suggests human to animal transmission (Berg *et al.*, 2009).

2.7. Diagnosis

2.7.1. Clinical examination

Bovine tuberculosis may be diagnosed by its clinical symptoms (Seifert, 1996). The general signs are weakness, erratic appetite, dyspnea, emaciation, and low grade fluctuating fever. Enlarged superficial lymph nodes provide a useful diagnostic sign. When lungs are extensively involved, there is commonly an intermittent cough. The principal sign of TB commonly is chronic wasting or emaciation that occurs despite good nutrition and care (Thoen and Bloom, 1995). These signs are not unique to bovine TB. Although normally a chronic debilitating disease, bovine TB can assume a more acute, rapidly progressive course (AHA, 2009). Nevertheless, other techniques are required to ensure the diagnosis (OIE, 2004).

2.7.2. Gross and histopathology

A tentative or presumptive diagnosis of bovine tuberculosis can be made following the macroscopic detection at necropsy of typical lesions (Corner, 1994). TB causes abscess-like lesions commonly referred to as granulomas or tubercles (AHA, 2009). Lesions in cattle may vary in size from 1 mm to more than 10 cm in diameter. Most lesions appear as firm or hard,

white, grey or yellow nodules. The cut surface usually shows a yellowish, caseous centre, which is dry and firm. Calcification is common, particularly in lymph nodes, and on sectioning the lesion, a gritty sensation and grating sound indicate its occurrence. Conglomerate tubercles, formed by the growth and coalescence of one or more adjacent tubercles, may occur over the pleural or peritoneal surfaces. Metastases give rise to myriad tubercles of the same size, usually 2-3 mm in diameter. Old lesions may be encapsulated by connective tissue, heavily calcified and inspissated (very dense) (AHA, 2009).

Careful examination of as few as 6 pairs of lymph nodes, the lungs and the mesenteric lymph nodes can result in 95% of cattle with macroscopic lesions being identified. However, this method is not useful to determine the significance of cattle that give a positive reaction in diagnostic tests but do not have visible lesions. Non visible lesion (NVL) reactors may be due to early infection, poor necropsy technique or infection with mycobacteria other than *M. bovis* (Corner, 1994). Therefore, postmortem examination should be supported by histopathology and bacteriological examination of lesions (OIE, 2004) for definitive diagnosis of BTB.

Another important diagnostic method for detection of tuberculosis is histopathologic examination conducted following postmortem (Jolley *et al.*, 2007). For histopathology a fixed samples (in 10% formalin) of lesion are required (Quinn *et al.*, 2004) and histologic sections are stained with hematoxylin-eosin (Biberstein and Hirsh, 1999). Microscopic appearance of a typical bovine tuberculous lesion consists of a peripheral zone of mononuclear cells, fibroblasts and giant cells with central caseous necrosis (Quinn *et al.*, 2004).

2.7.3. Tuberculin test

Tuberculin test is standard test recommended by Office International des Epizooties (OIE) to diagnose BTB in cattle in which purified protein derivative (PPD) is injected intradermally (OIE, 2004). It is based on eliciting a delayed-type hypersensitivity (DTH) response to the intradermal injection of tuberculin, a crude protein extract from supernatants of mycobacterial cultures (de la Rua-Domenech *et al.*, 2006). The tuberculin test was introduced by M'Fadyean in 1899 (Silva, 2001). PPD is produced from Mycobacterium that grows in liquid media as a culture filtrate,

which is further processed by chemical fractionation, two strains namely *M. bovis*, AN5 and vallee strains and the avian PPD is produced from *M. avium*, D4ER or TB 56 strains are used to produce PPD (Monaghan *et al.*, 1994).

Tuberculin test can be performed as single intradermal, short thermal, stormont, and comparative test (Radostits *et al.*, 2007). Of these, the comparative test is most commonly used (Radostits *et al.*, 2007; OIE, 2004). For screening purpose the single intradermal test has been widely used at a dose of 0.1 or 0.2 ml of bovine or human PPD in the cervical or caudal area (O'reilly and Daborn, 1995). Animals, which have been sensitized by non-pathogenic environmental strains of mycobacteria, may react positively to PPD-B, due to the presence of antigens common to virulent and non-virulent mycobacterial strains. Where this occurs, discrimination between cattle infected with *M. bovis* and those exposed to environmental strains is done using the comparative intradermal skin test (Pollock *et al.*, 2005). The comparative intradermal skin test is that in which a DTH response to PPD of tuberculin from *M. bovis* (PPD-B) and *M. avium* (PPD-A) is assessed and compared (Thom *et al.*, 2006).

In spite of its wide use, intradermal tuberculin reactions present some important limitations, related to their sensitivity and specificity (Fra'guas *et al.*, 2006). Monaghan *et al.* (1994) suggested that the sensitivity varies from 68-95% while specificity is as high as 96-99%. Radostits *et al.* (2007) stated that recently infected animals until 6 weeks after infection and advanced cases of tuberculosis show state of anergy (false negative). Desensitization resulting from injection with PPD during the preceding 60 days (Radostits *et al.*, 2007) and immunosuppression during the early postpartum period is also an additional reason. The reason for this may be due to depletion of T-lymphocytes from the skin into the circulation and then into colostrum (Radostits *et al.*, 2007). Tuberculin skin testing lacks sensitivity, can be confounded by exposure to NTM, and cannot be repeated for 60 days due to desensitization (Palmer *et al.*, 2006). When skin-test reactive animals are slaughtered and lesions characteristic for tuberculosis are not found (NVL reactors) (Monaghan *et al.*, 1994). Therefore the reliability of the test may be questioned and confirmatory tests may be required. Calves born from infected cows may have acquired colostral immunity for about 4 weeks and react false positive to tuberculin test (Barwinek and Taylor, 1996).

2.7.4. Blood-based laboratory test

Besides the classical tuberculin test, a number of blood tests have been used. In this regard, the gamma-interferon assay and the lymphocyte proliferation assay measure cellular immunity, while the ELISA measures humoral immunity (OIE, 2004). Due to the cost and the more complex nature of laboratory-based assays, they are usually used as ancillary tests to maximize the detection of infected animals (parallel testing), or to confirm or negate the results of an intradermal skin test (serial testing). There is also evidence that when an infected animal is skin tested, an enhanced blood test can occur during the following week. This allows for better separation of *in-vitro* blood test responses leading to greater test accuracy. The test may be useful in wildlife and zoo animals (OIE, 2010).

1. Lymphocyte proliferation assay

This type of *in-vitro* assay compares the reactivity of peripheral blood lymphocytes to tuberculin PPD (PPD-B) and a PPD from *M. avium* (PPD-A) (OIE, 2010). The assay can be performed on whole blood or purified lymphocytes from peripheral blood samples. The method involves incubating lymphocytes in the whole blood diluted with tissue culture media for 3-5 days, and then using radioactive nucleosides to detect the level of cell proliferation (WHO, 1994; Wood and Rothel, 1994). These tests endeavour to increase the specificity of the assay by removing the response of lymphocytes to 'nonspecific' or cross-reactive antigens associated with non-pathogenic species of mycobacteria to which the animal may have been exposed. Results are usually analysed as the value obtained in response to PPD-B minus the value obtained in response to PPD-A. The B-A value must then be above a cut-off point that can be altered in order to maximize either specificity or sensitivity of the diagnosis. The assay has scientific value, but is not used for routine diagnosis because the test is time-consuming and, the logistics and laboratory execution are complicated (it requires long incubation times and the use of radio-active nucleotides) (OIE, 2010).

II. Gamma interferon (γ -IFN) assay

In this test, the release of a cytokine gamma interferon (IFN- γ) is measured in a whole-blood culture system. The assay is based on the release of IFN- γ from sensitized lymphocytes during a 16-24hours incubation period with specific antigen (PPD-tuberculin) (Wood *et al.*, 1990; OIE, 2010). The test makes use of the comparison of IFN- γ production following stimulation with avian and bovine PPD. The detection of bovine IFN- γ is carried out with a sandwich ELISA that uses two monoclonal antibodies to bovine gamma-interferon (OIE, 2010). In some areas, especially where 'nonspecificity' is prevalent, some concerns about the accuracy have been expressed. However, because of the IFN- γ test capability of detecting early infections, the use of both tests in parallel allows detection of a greater number of infected animals before they become a source of infection for other animals as well as a source of contamination of the environment (Gormley *et al.*, 2006). The use of defined mycobacterial antigens such as ESAT 6 and CFP-10 shows promise for improved specificity (OIE, 2010).

III. Enzyme-Linked Immuno-Sorbent Assay (ELISA)

Different enzyme-linked immunosorbent assay (ELISA) tests have been developed recently for the detection of antibodies to *M. bovis* (Silva, 2001) and have been suggested as an alternative test for field diagnosis of tuberculosis but should not be considered as a reliable confirmatory test for bovine tuberculosis diagnosis at the slaughter (Fra'guas *et al.*, 2006), because of the low sensitivity they have, due to the large number of protein antigens present in mycobacterium and due to the variable response to mycobacterial infections (Wood and Rothel, 1994). They are only used as a supplement to pick out some of the anergic cattle which failed to respond to the single intradermal test (Radostits *et al.*, 2007) or to complement the in-vitro cellular assay. They offer the important advantages of ease of use, assay speed and relatively low cost (Jolley *et al.*, 2007). Serological assays provide an important and needed tool for large volume testing for exposure to *M. bovis* and diagnosis of tuberculosis in wild animals (OIE, 2010).

2.7.5. Bacteriology

Bacteriological examinations may consist of the demonstration of acid-fast bacilli by microscopic examination, which provides presumptive confirmation. The isolation of mycobacteria on selective culture media and their subsequent identification by cultural and biochemical tests or DNA techniques, such as PCR, confirms infection. Animal inoculation, which has been used in the past for confirming infection with *M. bovis*, is now rarely used because of animal welfare considerations (OIE, 2010). Handling and manipulation of infectious material, both tissue specimens and cultures, should be done in a Class I or Class II biosafety cabinet to protect the operator from infection (AHA, 2009; OIE, 2010).

I. Microscopic examination

Mycobacterium bovis can be demonstrated microscopically on direct smears from clinical samples and on prepared tissue materials (OIE, 2010). The acid fastness of *M. bovis* is normally demonstrated with the classic Ziehl-Neelsen stain, but a fluorescent acid-fast stain may also be used. Immunoperoxidase techniques may also give satisfactory results. In the smear, the organisms appear red rods against a blue background (Ziehl-Neelsen staining), while in the fluorochrome procedures, the acid-fast organisms appear as fluorescent rods, yellow to orange (WHO, 1998). The presumptive diagnosis of mycobacteriosis can be made if the tissue has characteristic histological lesions. As lesions are often paucibacillary, the presence of acid-fast organisms in histological sections may not be detected, although *M. bovis* can be isolated in culture (OIE, 2010).

II. Culture

Traditional mycobacterial culture remains the gold standard method for routine confirmation of infection (OIE, 2010). Mycobacteria are slow-growing compared with other bacteria (AHA, 2009). The success of *M. bovis* isolation depends on the type of media used; procedures applied for decontamination and concentration of the specimen as well as the incubation conditions (Corner, 1994). The most commonly used media are either the egg based (Löwenstein-Jensen and

Stone Brink's media) or agar-based medium enriched with blood and/or serum such as the modified Middle Brook 7H11 (WHO, 1998). To process specimens for culture, the tissue is first homogenised using a mortar and pestle, stomacher or blender, followed by decontamination with either detergent, an alkali (2-4% sodium hydroxide) or an acid (5% oxalic acid). The alkali or acid mixture is shaken for 10-15 minutes at room temperature and then neutralised. The suspension is centrifuged, the supernatant is discarded, and the sediment is used for culture and microscopic examination. For primary isolation, the sediment is usually inoculated on to a set of solid egg-based media, such as Lowenstein-Jensen, Coletsos base or Stonebrinks; these media should contain either pyruvate or pyruvate and glycerol. Cultures are incubated for a minimum of 8 weeks (and preferably for 10-12 weeks) at 37°C with or without carbon dioxide. The media should be in tightly closed tubes to avoid desiccation. Slopes are examined for macroscopic growth at intervals during the incubation period. When growth is visible, smears are prepared and stained by Ziehl-Neelsen staining (OIE, 2010).

III. Nucleic acid based methods

Rapid identification of isolates to the level of *M. tuberculosis* complex can be made by Gen Probe TB complex DNA probe or PCR targeting 16S-23S rRNA, the insertion sequences IS6110 and IS1081, and genes coding for *M. tuberculosis* complex specific proteins, such as MPB70 and the 38 kDa antigen have been used (OIE, 2010). Specific identification of an isolate as *M. bovis* can be made using Polymerase chain reaction (PCR) targeting a mutation at nucleotide positions 285 in the *oxyR* gene, 169 in the *pncA* gene, 675/756/1311/1410 and 1450 of the *gyrB* gene and presence/absence of RDs (Niemann *et al.*, 2000; Parsons *et al.*, 2002; Huard *et al.*, 2003). Alternatively molecular typing techniques, such as Spoligotyping, Restriction Fragment Length Polymorphism analysis with the Direct Repeat and Polymorphic Guanine Cytosine Rich sequence probes can identify *M. bovis* isolates and provide some molecular-typing information on the isolate that is of epidemiological value (Kamerbeek *et al.*, 1997).

Polymerase chain reaction has been widely evaluated for the detection of *M. tuberculosis* complex in clinical samples (mainly sputum) in human patients and has recently been used for the diagnosis of tuberculosis in animals (Huard *et al.*, 2003; OIE, 2010). False-positive and false-

negative results, particularly in specimens containing low numbers of bacilli, have reduced the reliability of this test. Variability in results has been attributed to the low copy number of the target sequence per bacillus combined with a low number of bacilli, decontamination methods, DNA extraction procedures, techniques for the elimination of polymerase enzyme inhibitors, internal and external controls and procedures for the prevention of cross-contamination. Although direct PCR can produce a rapid result, it is recommended that culture be used in parallel to confirm a viable *M. bovis* infection (OIE, 2010).

Several genes and insertion sequences have been targeted in attempts to develop PCR assays to differentiate between mycobacteria in general and between members of the *M. tuberculosis* complex in particular (Kurabachew *et al.*, 2004). Multiplex Polymerase chain reaction (m-PCR) were used to differentiate the genus *Mycobacterium* or, *M. tuberculosis* complex from *M. avium*, *M. intracellulae* and other mycobacterial species (Jahans and Worth, 2006). A single-step m-PCR assay for distinguishing (1) between the *Mycobacterium tuberculosis* complex and mycobacteria other than tuberculosis targeted the 16S and the 23S rDNA and (2) between *M. tuberculosis* and *M. bovis* species targeted the *oxyR* gene have been developed (Kurabachew *et al.*, 2004). Besides, a single tube m-PCR has been developed and said valuable where bovine and human tuberculosis coexist, and the distinction of *M. bovis* from *M. tuberculosis* is required for monitoring the spread of *M. bovis* to humans (Bakshi *et al.*, 2005).

A variety of DNA-fingerprinting techniques has been developed to distinguish the *M. tuberculosis* complex isolates for epidemiological purposes (OIE, 2010). These methods can distinguish between different strains of *M. bovis* and will enable patterns of origin, transmission and spread of *M. bovis* to be described (Durr *et al.*, 2000; Haddad *et al.*, 2004). The most widely used method is spoligotyping (from 'spacer oligotyping'), which allows the differentiation of strains inside each species belonging to the *M. tuberculosis* complex, including *M. bovis*, and can also distinguish *M. bovis* from *M. tuberculosis* (Kamerbeek *et al.*, 1997). The use of a standard nomenclature for the spoligotypes according to the database Mbovis.org (<http://www.mbovis.org>) is encouraged to allow international comparison of profiles. Often a combination of techniques may be used to gain the maximum discrimination between strains (OIE, 2010).

2.8. Prevention and control

Reasons for eradicating bovine TB include the risk of infections to the human population, loss in productivity due to infected animals, and animal market restrictions set by countries with advanced eradication programmes (Cousins, 2001). Developing countries such as those in Africa need to control BTB, as agriculture remains the back bone of many of these nations (Ayele *et al.*, 2004). In developed countries, BTB has been eradicated by means of costly test and slaughter programs. This approach is unthinkable in developing countries, which should opt for different, country adapted control strategies. In developing countries, from an animal health perspective, it is most probably not cost effective to launch a control/eradication program at this stage (e.g. test and slaughter, farmer compensation). However, measures can be readily taken on the human health sector in the absence of data on animal BTB: pasteurization of milk, strengthening of meat inspection, public health education and strengthening awareness about the transmission of BTB (handling of animals, consumption of raw animal products), and BCG vaccination of people (Cosivi *et al.*, 1998; LoBue, 2006).

Vaccination of animals against BTB would be important in reducing the prevalence of the disease to an acceptable level before the test and slaughter policy in domesticated animals is applied. It is also applied in wild and feral reservoirs of the disease where test and slaughter programs (stamping out) and control of animal movement have failed to achieve elimination of the disease (OIE, 1996). In countries where there is no test and slaughter control scheme, BCG vaccination may be used to reduce the spread of infection in cattle; however, there is no solid knowledge of long-term reduction in prevalence and safety for human beings and the environment. Before embarking on a vaccination programme, the vaccination schedule must be optimized for local conditions (OIE, 2010).

Husbandry practices such as raising of closed herds, limiting the stocking densities in cattle housing to recommended maxima, provision of adequate ventilation, regular cleaning of drinking troughs, fencing of natural water sources, limiting contact between cattle and wildlife, keeping cattle away from slurry spreading, avoiding of feeding calves with milk from confirmed or

inconclusive reactor cows and pre- or post-purchase tuberculin testing will help to prevent transmission of infection (Phillips *et al.*, 2003).

Test-and-slaughter policy is effective only in areas with relatively low BTB prevalence and effective control of animal movement (Barwinek and Taylor, 1996). In most developing and some European countries where test and slaughter could have been impractical, varying forms of test and segregation have been used with the test-and-slaughter program applied only in the final stages of eradication (WHO, 1993). Reactors to the tuberculin skin test with no grossly visible lesions on necropsy are a problem. In developing countries, a program based on slaughterhouse surveillance and trace back of tuberculous animals to herd's origin is most appropriate since it is technically, economically and socially feasible (LoBue, 2006).

In humans, the danger of infection can be reduced through milk pasteurization or other effective heat treatment of milk prior to sale for human consumption or for further processing and post-mortem inspection to detect lesions and confiscation of the affected organs or whole carcasses (Aranaz *et al.*, 2004). Besides, public education regarding the risks of consumption of unpasteurized dairy products and the precautions that should be taken in dressing or processing animal carcasses or when cooking meat from animals that are particularly susceptible to the disease may be useful in reducing the risks of transmission through ingestion or direct inoculation (LoBue, 2006).

3. MATERIALS AND METHODS

3.1. Study area

The study was carried out in and around Mekelle city (Figure 4) of Tigray Regional State, located in Northern part of Ethiopia. Mekelle, the capital of the National Regional State of Tigray, has recently stood as a special zone. It lies 783 km north of Addis Ababa. Geographically, Mekelle city is located in the northern highlands of Ethiopia, covering an area of 28km². It is found in 39°38'33" east longitude and 33° 32' 30" north latitude. The climatic condition of the area includes "Dega", (Temperate/Highland) (15%), "Weinadega" (Subtropical /Medium land) (50%) and "Kola" (Tropical/Low land) (5%). The altitude of Mekelle ranges from 2000 to 2270 meter above sea level. It has mean erratic average annual rainfall of 479-650mm of which 84% falls down during the long rain season that extends from June to August ("*Kiremt*"), the remaining (16%) during the short rain season that extends from March to May ("*Belg*") which is associated with north and south oscillation of inter-tropical convergence zone. The annual minimum and maximum temperature is 11.8°C and 29.94°C, respectively, and the mean humidity is 75.4 % (Bureau of Planning and Economic Development of Tigray Region, 1998).

Based on figures from the Central Statistical Agency, Mekelle has an estimated total human population of 169,207 (CSA, 2005). According to Mekelle city Urban Agriculture annual report of 2008/09, Mekelle has 41379 cattle, 6559 sheep, 4644 goats, 122 camels, 3557 donkey, 1347 horses, 224 mules, 59310 poultry and 900 beehives. In study area there are two production system namely intensive dairy farms and extensive type. The intensive dairy farms are almost small scale with exception of few dairy farms. Farmers owning dairy cows sell raw milk to the community (personal observation). Cattle used to produce milk and meat in urban area whereas in the peri-urban area cattle are used mainly to generate draught power and milk production. The main types of feed used in the area are hay, crop residue, wheat bran, byproduct of local beverage called "*atella*" and alfalfa (green feed).

In Mekelle there are two abattoirs; one municipality and one export (Abergelle Slaughterhouse) abattoir. Mekelle municipality abattoir, the oldest and largest abattoir in the region, is located between public premises. The abattoir was established in 1964 and is the only source of inspected beef to all Mekelle city dwellers. Usually cattle are slaughtered every day starting from 3 pm with the exception of Tuesday and Thursday. On an average around 30 animals are slaughtered per day at the municipality. The abattoir has only one slaughter hall and all the procedures including stunning, skinning, evisceration, removal of rumen contents, washing of offals are usually performed in one hall which favors contamination of carcasses.

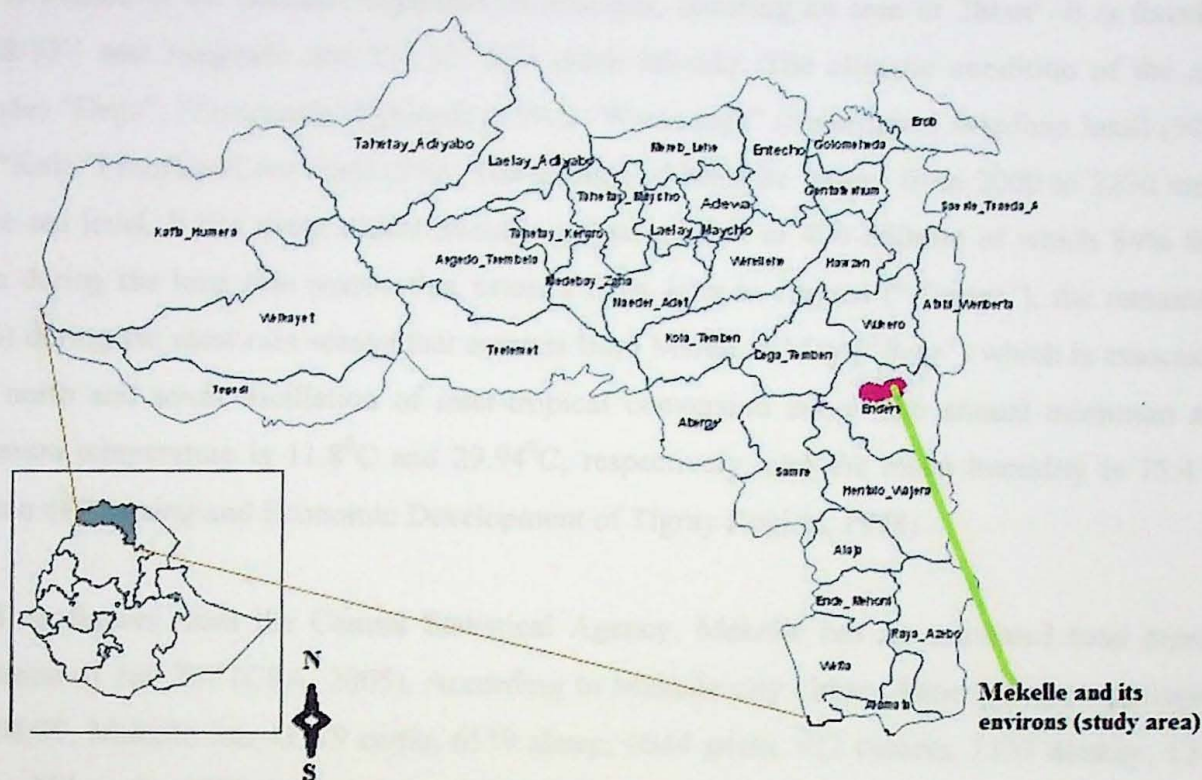


Figure 4: Map of the selected study area

3.2. Study population

Comparative intradermal tuberculin test and abattoir-based surveillance was conducted on 480 and 768 cattle, respectively. The study animals for skin test were those cattle which are originated from Mekelle and its environs. However, the cattle used for abattoir-based study originated from

different areas of Tigray frequently from Eastern and Southern zone, and neighboring regions such as Afar Regions and North Wollo areas.

3.3. Study design

The study design used in this study was cross-sectional study design. This study was conducted from September 2010 to May 2011 using comparative intradermal tuberculin test on 480 cattle, and abattoir based survey on 768 slaughtered cattle. Collection of milk samples from tuberculin test reactor milking cows and, suspected TB lesion tissue samples collected from slaughtered cattle at municipal abattoir and from tuberculin reactor animal which were purchased afterward followed by laboratory investigation of the agent. A questionnaire survey was used in for livestock owners/herder to assess the associated risk factors in this study. Besides, secondary data on the human health records (retrospective case) in Mekelle hospital were used to assess the status of human tuberculosis in the study area from September 2005 to August 2010.

3.4. Sampling method and Sample size determination

3.4.1. Comparative intradermal tuberculin test

Mekelle city is divided in to seven local administrations. A list of households/farms owning dairy cattle in each of the seven local administrations was obtained from Mekelle city Urban Agriculture. Thus, households/farms were used as sampling frames. The sampling method involved was a two-stage cluster sampling method. The primary stage was sampling of households in smallholder and intensive dairy farms. In secondary stage was sampling of animals within households or farms which were selected. Both the primary and secondary units were selected using the systematic random sampling. In this study 30% of the total households owning cattle were included. Accordingly, 120 households were selected in this study. Proportional allocation was used to distribute the household/farms evenly among the seven local administrations.

Sample size was determined using cluster sample formula (Thrusfield, 2005).

$$Ts = \frac{g \times 1.96^2 \times P_{exp} \times (1 - P_{exp})}{g \times d^2 - 1.96^2 \times V_c}$$

Where, Ts = total sample size

P_{exp} = expected prevalence

g = number of cluster

d = desired absolute precision

V_c = between cluster variance

As there has been no previous study conducted on prevalence rate of BTB in the study area, the expected prevalence was assumed to be 50%. A total of 120 clusters from seven local administrations were pre-determined to be selected, with 95% confidence level, 5% desired absolute precision (d²). The between cluster variance to be 0.01 (i.e. since between cluster variance is not available, the variance of different studies conducted in Ethiopia was taken). Thus, giving (Ts) 440 cattle and 3.6 cattle per cluster. In practical field condition, 4 cattle from each cluster were sampled and hence the total cattle tested were 480.

3.4.2. Abattoir survey

Systematic random sampling was used for the determination of the magnitude of BTB in the slaughterhouse. The sample size required also obtained using the formula given by Thrusfield (2005) for systematic random sampling.

$$n = \frac{1.96^2 \times P_{exp} (1 - P_{exp})}{d^2}$$

Where, n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

As previous study has not been conducted on abattoir based prevalence of BTB in the study area, the expected prevalence was assumed to be 50%. Therefore, the minimum sample size calculated at 50% prevalence rate with a desired precision of 5% and 95% confidence interval was 384 animals. However, in order to increase our chances of finding several isolates for further molecular characterization, the carcasses of 768 animals were inspected.

3.5. Study methodology

3.5.1. Comparative intradermal tuberculin test

The comparative intradermal tuberculin test was conducted on 480 cattle using both avian and bovine purified protein derivates (PPD) (Lelystad Biological BV., Lelystad, Netherlands). Intradermal injections of 0.1 ml (2500 IU/ml) bovine PPD and 0.1 ml (2500 IU/ml) avian PPD were administered in two shaved sites. Young stocks ≤ 6 months of age, and cows in late pregnancy and those recently calved excluded from the study for fear of immune dysfunction (OIE, 2004). All cattle belonging to the selected households were individually identified by names which were previously given by their owners and/ code number painted on their body on the day of tuberculin inoculation. Their age, body condition score (BCS), sex, lactation status, reported pregnancy status and skin test measurements were recorded on preformed format (Annex 2). Body condition scoring was done according to Nicholson and Butterworth (1986). Body condition scores 0 and 1 for exotic and crosses and 1 (L-), 2 (L) and 3 (L+) for zebu were considered as poor, BCS 2 and 3 for exotic and crosses and 4 (M-), 5 (M) and 6 (M+) for zebu were considered as medium and BCS 4 and 5 for exotic and crosses and 7 (F-), 8 (F) and 9 (F+) for zebu were considered as good (Annex 5).

The management of the farm was categorized on the bases of the method used by Ameni *et al.* (2003b) and Ameni *et al.* (2003c) with minor modification. In order to evaluate the effect of management, information on the degree of ventilation, neatness of the floor, degree of exposure to the sunlight, stocking rate, neatness of watering and feeding were collected. Accordingly, those herds with clean house, frequent waste disposal, non slippery floor, good aeration, getting concentrate feed and clean water were categorized under good management system. Whereas herds with unclean and poor construction of house, poor waste disposal, slippery or/and muddy floor, without any concentrate supplement, supplied with unclean water and crowded stocking rate were classified under poor management category. On the other hand those herds sharing one or two of the criteria set for either of the two management system were categorized under medium management system.

I. Skin test procedures

For the test procedure, recommendation of the Office International des Epizooties (OIE, 2010) was followed. At the center of the neck of a test animal, an area was shaved, cleansed with a swab of 70% alcohol and dried. A fold of skin was pinched with tips of fingers; its thickness was measured with caliper and recorded in CIDT recording sheet (Annex 2). Using tuberculin syringe, 0.1 ml of avian tuberculin was injected into the skin intradermally in the upper site. Similarly, fold of skin was pinched, its thickness measured and 0.1 ml bovine tuberculin was injected intradermally in the lower site (12 cm apart). Both injection sites were encircled with indelible ink of different colors. A correct injection was confirmed by palpating a small pea-like swelling at each site of injection. The results were recorded after 72 hours post-inoculation by measuring the thickness of skin fold using the tuberculin caliper.

II. Interpretation

The interpretation was made using two different diagnostic thresholds: 1) an animal was considered positive if the bovine PPD reaction minus the avian tuberculin reaction was greater than 2 mm (Ameni *et al.*, 2008) and 2) an animal was considered positive if the bovine minus the avian reaction was greater than 4 mm (OIE, 2010). When differences in skin thickness were greater at the site of injection for avian PPD than for bovine PPD, then the animal was considered positive for *M. avium* or other atypical mycobacteria; but when the change in skin thickness is increased at both injection sites, difference in thickness of the two sites is considered and results interpreted according to OIE, (2010) as follows:

- The reaction was usually considered to be positive if the increase in skin thickness at the bovine site of injection is more than 4 mm greater than the reaction shown at the site of the avian injection. If there was inflammation and edema at the injection site regardless of measurement, the result was considered positive.
- The reaction was considered to be inconclusive if the increase in skin thickness at the bovine site of injection is from 1 to 4 mm greater than the avian reaction.

- The reaction was considered to be negative if the increase in skin thickness at the bovine site of injection was less than or equal to the increase in the skin reaction at the avian site of injection.

3.5.2. Postmortem examination of tuberculin reactor cattle

Out of the total 54 tuberculin reactor cattle 4 were purchased (based on the willingness of the owner to sell positive animals) and slaughtered for detailed postmortem examination of suspected tuberculous lesions. Postmortem examination was conducted through procedure described by Corner (1994). Tissue sample which harbor tuberculous lesion were carefully removed and collected in universal bottle for further isolation and molecular characterizations of the causative agents.

3.5.3. Detailed postmortem examination in Abattoir

Animal identification and epidemiological information such as breed, sex, body condition and others was carried out during antimortem inspection of selected animal in the abattoir. All superficial lymph nodes was visualized and palpated to appreciate any abnormality especially for swelling. Detailed postmortem examination was performed following the procedure described by Corner (1994). All lymph nodes mainly mandibular, retropharyngeal, left and right bronchial, mediastinal, mesenteric, inguinal and other tissues such as the lungs, liver, and kidneys were examined in detailed during postmortem under a bright light source. All the lobes of the lung were inspected and palpated. Incision was made onto the parenchyma of the lung only when it was suspected to harbor tuberculous lesions. The tissues were cut into slices of 2 cm using separate sterile surgical blades. The slices were then examined for the presence of suspected tuberculous lesions. The suspected tissue samples were collected for mycobacteriological investigation using culture. The animal was classified as positive on postmortem examination when any tuberculous lesion was found and negative if no suspected tuberculous lesion is found in the inspected tissues (Annex 3).

3.5.4. Isolation and identification of mycobacteria

I. Sample collection and transportation

For laboratory investigations, samples were collected both from live and slaughtered animals. From slaughtered animals, 61 tissue samples containing tuberculous lesion were collected from 49 carcasses for isolation of the causative mycobacterial species. These tissues sample were collected aseptically and placed in a 50 ml capacity universal bottle with screw caps containing 5 ml of sterile 0.85% saline water and the samples were kept at -20 °C (frozen) until they were transported to Aklilu Lemma Institute of Pathobiology (ALIPB) for mycobacteriological culture and molecular typing.

Milk samples were also collected from 31 tuberculin positive lactating cow for bacteriological examination. Approximately 30 mL of milk was drawn from the 4 quarters of each the CIDT test positive cows under aseptic conditions at the end of milking using a sterile disposable plastic container and were stored at 2-8 °C (Ameni *et al.*, 2003b) until they are transported to ALIPB.

II. Culture and direct microscopy

All specimens collected from tuberculin positive cattle and abattoir were processed and prepared for mycobacterial culture at the ALIPB in a biological safety cabinet. The procedure used for culturing of the tissue specimens, as recommended by OIE, the tissue was homogenised using a mortar and pestle, followed by decontamination with 4% NaOH, centrifugation 3000 rpm for 10 minutes at room temperature. After the supernatant was discarded then neutralized using 10% HCL. Finally, the sediment was inoculated on to two Lowenstein-Jensen, one contain pyruvate which favoring growth *M. bovis* and the other containing glycerol which favoring growth *M. tuberculosis*. Cultures were incubated at 37°C and followed up for a minimum of 8 weeks (Ameni *et al.*, 2006; Cadmus *et al.*, 2006; OIE, 2010).

The milk samples were first centrifuge at 3000 rpm for 10 minutes at room temperature. The supernatant was decanted and the sediment was decontaminated with 4% NaOH, centrifuged

again, and neutralized with 10% HCL. After neutralization, each sediment was inoculated onto 2 Lowenstein-Jensen media (one with pyruvate and the other with glycerol). The culture was incubated at 37°C. Observation for bacterial growth was made every week for 12 weeks (WHO, 1998). Positive cultures were subcultured onto another set of media and incubated for another 3 to 4 weeks for further identification. When growth was visible, smears were prepared and stained by Ziehl-Neelsen technique to check for the presence of acid fast bacilli (WHO, 1998).

III. Multiplex Polymerase chain reaction (m-PCR)

For m-PCR, the procedure described by Wilton and Cousins (1992) was followed. This analysis differentiates genus *Mycobacterium* from other bacteria, and within the genus *Mycobacterium* it differentiates *M. tuberculosis* complex from *M. avium* complex, and other mycobacterial species. Heat killed AFB positive samples were used as source of DNA template. DNA amplifications was done in thermocycler with 20 µl reaction volumes consisting: 5 µl of genomic DNA as a sample template, 8 µl HotstarTaqMasterMix (MgCL₂, dNTP, Taq polymerase and PCR buffer) (Qiagen, USA) for each sample, 0.3 µl of forward and reverse primers per each sample and 5.2 µl per sample of Qiagen water. The primers used were MYCGEN-F, 5'AGA GTT TGA TCC TGG CTC AG 3' (35 ng/µl); MYCGEN-R, 5'-TGC ACA CAG GCC ACA AGG GA 3', (35 ng/µl); MYCAV-R, 5'-ACC AGA AGA CAT GCG TCT TG 3', (35 ng/µl); MYCINT-F, 5'-CCT TTA GGC GCA TGT CTT TA 3', (75 ng/µl); TB1-F, 5'-GAA CAA TCC GGA GTT GAC AA 3', (20 ng/µl); TB1-R, 5'-AGC ACG CTG TCA ATC ATG TA 3', (20 ng/µl). The PCR reaction was carried out using Thermal Cycler (VMR Thermocycler 732-1200 Leicestershire, UK) based on the following amplification programme: 95°C for 10 minutes for enzyme activation; 95°C for 1 minute for denaturation; 61°C for 0.5 minute for annealing; 72°C for 2 minutes for extension, involving 35 cycles all in all; and final extension at 72°C for 10 minutes. *M. avium* and *M. bovis* strain 2122/97 were used as positive controls while H₂O (Qiagen, USA), was used as a negative control. The PCR product was then electrophoresed in 2% agarose gel in TAE running buffer 10X. Ethidium Bromide at a concentration of 0.5 µg/ml in 2% agarose gel, 100bp DNA ladder, and orange 6x loading dye were used in gel electrophoresis. All members of the genus *Mycobacterium* produce a band of 1030bp. On the other hand, *M. avium* or subspecies such as *M.*

avium subsp. paratuberculosis, *M. intracellularae* and members of *M. tuberculosis* complex produce a band, 180bp, 850bp and 372bp, respectively.

3.5.5. Questionnaire survey

The role of various risk factors for the occurrence and spread of bovine tuberculosis among cattle and, between cattle and human were assessed by a questionnaire. 120 cattle owners/farm worker were interviewed on the same day of their cattle were tested for BTB. Questionnaires included closed and open questions on livestock husbandry/management and household characteristics, such as herd size and structure, presence of other livestock, presence of wild life in the surrounding area, mixing of cattle with other livestock, watering and feeding system and reproduction. Furthermore, questions were asked related to human consumption habit, contact between humans and cattle, TB knowledge and history of TB status in the household. A household was considered positive for TB if at least one member in the last five years had been diagnosed with pulmonary TB or showed clinical signs of extra pulmonary TB (EPTB) (e.g. cervical lymphadenitis). Annex 1 shows format of the questionnaire used.

3.5.6. Retrospective case record analysis

Annual records of TB patients of Mekelle Hospital that were admitted from September 2005 to August 2010 were documented. The total number of TB patients admitted every year, their age, the TB category identified and HIV status were carefully documented to assess the extent and importance of tuberculosis in the study area.

3.8. Data analysis

The collected data were entered into Microsoft Excel data sheets and analyzed using STATA 11 statistical software (STATA Corporation, College Station, Texas, USA). The prevalence was calculated by dividing the proportion of cattle found infected (either positive reactors or harbouring tuberculous lesions) by the total number of cattle tested or whose carcasses was inspected multiplied by 100. Likewise, awareness of cattle owners was determined by dividing

the proportion of people who knew BTB by the total number of respondents. The difference between the effects of different risk factors on prevalence was analyzed using the Pearson chi-square (χ^2) test. Logistic regression analyses was used to assess the strength of association and odds ratio (OR) was also calculated to quantify the association of different factors with the prevalence of bovine TB. A statistically significant association between variables was said to exist if the calculated $P < 0.05$ and the 95% confidence interval (CI) for OR doesn't include 1.

4. RESULTS

4.1. Comparative intradermal tuberculin test

4.1.1. Herd level prevalence and risk factors

Based on the >4mm OIE recommended cut-off value, the herd prevalence was 34.2% (41/120) (95% confidence interval [CI]: 25.5%-42.8%) with and 20.0% (24/120) (95% CI: 12.7%-27.3%) without considering doubtful reactors as bovine TB infected herds (Table 4). A herd was considered as positive if at least one positive animal found in the herd. Using >2 mm cut-off value, 30 herds have been found positive, hence the herd prevalence rate obtained was 30.8% (95% CI: 22.4%-39.2%) (Table 4). However, using the OIE recommended cut-off value, only positive reactors (without considering doubtful) were considered in investigating the possible risk factors.

The herd level risk factors such as type of farming, herd size, management, owner TB infection status (past 5 years or present), presence of livestock other than cattle and presence of wildlife in nearby surrounding of the farm are shown in Table 5. The prevalence of bovine TB varied significantly among different type of farming ($\chi^2=4.37$; $P=0.036$), herd size ($\chi^2=19.47$; $P=0.000$) and management ($\chi^2=25.04$; $P=0.000$) (Table 5). On univariate analysis, cattle herd in intensive type of production were three times (OR=3.67; 95% CI:1.02-13.20) to be reactive than cattle herd kept under extensive production type and cattle herd managed under poor management conditions were four times more likely (OR=4.28; 95% CI: 0.45-40.15) to become infected with BTB than cattle herd kept under good management conditions (Table 6). The positivity to tuberculin test was increased as herd size increased. Those herds which had 6-10 and >10 cattle were two times (OR=2.07; 95% CI: 0.56-7.62) and eleven times (OR=11.25; 95% CI:3.12-40.4) become more reactive than those herds which had less than five cattle in their farm. Similarly, multivariate logistic regression analysis also indicated that (Table 6), those herds which had 6-10 and >10 cattle were around two times (OR=1.95; 95%CI: 0.39-9.76) and five times (OR=5.89; 95% CI: 1.29-26.77) more tuberculin reactive than those herds which had less than five cattle.

Table 4: Herd and individual animal prevalence of BTB by >4mm and >2mm cut-off value

	Total tested	By >4mm cut-off		By >2mm cut-off	
		Positive	Prevalence (95%CI)	Positive	Prevalence (95% CI)
Animal level	480	54	11.2% (8.4%-14.1%)	77	16.04%(12.7%-19.3%)
Herd level	120	24	20% (12.7%-27.3%)	30	30.8%(22.4%-39.2%)

Table 5: Association of different recorded variables and CIDT test results at herd level

Variable	No. of herds		χ^2 - value	P-value
	Tested	Positive (%)		
Type of farming			4.375	0.036*
Intensive	84	21(25.0)		
Extensive	36	3(8.3)		
Herd size			19.47	0.000*
≤ 5	49	4(8.1)		
6-10	45	7(15.5)		
>10	26	13(50.0)		
Owner's health status			3.105	0.078
Sick with TB	13	5(38.4)		
Not sick with TB	107	19(17.7)		
Management			25.048	0.000*
Good	6	1(16.6)		
Medium	75	5(6.6)		
Poor	39	18(46.1)		
Presence of livestock			3.119	0.077
Present	82	20(24.4)		
Absent	38	4(10.5)		
Presence of wildlife			0.534	0.465
Present	57	13(22.8)		
Absent	63	11(17.4)		

* Statistically significant

Table 6: Univariate and multivariate logistic regression analyses of risk factors for CIDT test result at herd level

Risk Factors	No. of herd		Crude OR* (95%CI)	Adjusted OR** (95%CI)
	Tested	Positive (%)		
Type of farming				
Intensive	84	21(25.0)	3.67(1.02-13.20)	2.45(0.48-12.34)
Extensive	36	3(8.33)	1	1
Herd size				
≤5	49	4(8.16)	1	1
6-10	45	7(15.56)	2.07(0.56-7.62)	1.96(0.39-9.76)
>10	26	13(50.00)	11.25(3.12-40.4)	5.89(1.29-26.77)
Owner's health status				
Sick with TB	13	5(38.4)	2.8(0.85-9.83)	2.96(0.47-18.69)
Not sick with TB	107	19(17.7)	1	1
Management				
Good	6	1(16.67)	1	1
Medium	75	5(6.67)	0.35(0.03-3.67)	1.33(0.07-24.31)
Poor	39	18(46.15)	4.28(0.45-40.15)	18.38(0.97-349.5)
Presence of livestock				
Present	82	20(24.4)	2.74(0.87-8.68)	3.33(0.84-13.32)
Absent	38	4(10.5)	1	
Presence of wildlife				
Present	57	13(22.8)	1.39(0.57-3.43)	3.45(0.83-14.37)
Absent	63	11(17.4)	1	

4.1.2. Animal level prevalence and risk factors

Out of 480 CIDT tested cattle, using the >4 mm cut-off, 96 and 54 were found positive for B1B infection with and without including doubtful reactors respectively. Therefore, the individual

animal prevalence of BTB obtained was 11.25% (95% CI: 8.41%-14.1%), if doubtful animals were considered as negative (Table 4). On the other hand, if doubtful reactors were considered as positive the prevalence of BTB would be 18.3% (95% CI: 16.4%-23.6%). However, for risk factor investigation the former BTB prevalence were used. Using >2 mm cut-off, a prevalence rate of 16.04% (77/480) (95% CI: 12.7%-19.3%) was recorded. Seven cattle were positive for *M. avium*-complex (MAC) resulting in a prevalence of 1.45% (95% CI: 0.38%-2.53%).

A prevalence rate of 11.8% and 10.9% in urban and peri-urban were recorded, respectively. But found statistically insignificant ($P=0.540$) (Table 8). Of the many animal characteristics were considered (Table 7), sex ($\chi^2=8.1$; $P=0.004$), breed ($\chi^2=15.03$; $P=0.001$) and body condition (χ^2 value=6.65; $P=0.036$) were significantly associated with the prevalence of BTB. In contrast, the skin test prevalence among different age group ($P=0.292$), lactation ($P=0.321$), pregnancy ($P=0.722$) and parity class ($P=0.094$) was not statistically significant. On univariate analysis (Table 9), being female were four times (OR=4.81; 95% CI: 1.47-15.77) become at risk for BTB infection than male. Exotic (OR=6.13; 95% CI: 2.16-17.41) and cross (OR=6.61; 95% CI: 1.76-24.79) breed cattle were six times more likely to become infected with bovine TB than Zebu. Cattle with poor body condition were two times reactive (OR=2.65; 95% CI: 1.19-5.88) to the test than good body conditioned animals. Similarly, in multivariate logistic regression analysis (Table 9) animal with poor body condition were four times (OR=4.0; 95% CI: 1.36-11.68) more likely at higher risk for BTB infection.

On the other hand, table 8 shows the prevalence of BTB were significantly associated with the different environmental risk factors like herd size ($\chi^2=85.05$; $P=0.000$) and farming system ($\chi^2=12.66$; $P=0.002$). On univariate analyses (Table 10), cattle kept in herds which had >10 cattle were thirteen times (OR=13.53; 95% CI: 6.0-30.49) at higher risk of infection with TB than those cattle kept with herds had less than five cattle. Cattle kept under intensive farming system were more than four times as likely to present as tuberculin positive, i.e., infected with *M. bovis*, than cattle managed under extensive type of production (OR=4.79; 95% CI=1.87-12.31). On multivariate logistic regression analyses (Table 10), cattle kept with >10 cattle were 17 times (OR=17.9; 95% CI: 6.7-47.74) more likely to infected with BTB than those which had less than five animals.

Table 7: Association of host risk factors affecting reactivity of cattle to tuberculin test

Variables	No. of animals		χ^2 -value	P-value
	Examined	Positive (%)		
Sex			8.1	0.004*
Male	97	3(3.09)		
Female	383	51(13.32)		
Age (Yrs)			3.73	0.292
<2	52	6(11.5)		
2≤x≤ 5	207	26(12.5)		
>5-x≤ 9	120	16(13.3)		
>9	101	6(5.9)		
Breed			15.03	0.001*
Local	145	4(2.7)		
Cross	38	6(15.8)		
Exotic	297	44(14.8)		
Body condition			6.65	0.036*
Good	134	9(6.7)		
Medium	184	19(10.3)		
Poor	162	26(16.0)		
Lactation			0.985	0.321
Lactating	212	31(14.6)		
Non lactating	170	19(11.2)		
Pregnancy			0.126	0.722
Pregnant	205	28(13.6)		
Non pregnant	177	22(12.4)		
Parity class			6.402	0.094
Heifer	118	16(13.9)		
Parity 1 and 2	116	9(7.8)		
Parity 3-5	119	22(18.5)		
Parity ≥6	32	3(9.4)		

* Statistically significant

Table 8: Association of different environmental risk factors affecting reactivity of cattle to CIDT test result

Variables	No. of animals		χ^2 -value	P-value
	Examined	Positive (%)		
Herd size			85.05	0.000*
≤ 5	196	8(4.1)		
6-10	180	8(4.4)		
>10	104	38(36.5)		
Farming system			12.66	0.000*
Extensive	145	5(3.4)		
Intensive	335	49(14.6)		
Study area			0.376	0.540
Urban	320	38 (11.8)		
Peri-urban	160	16 (10.0)		

*Statistically significant

Table 9: Univariate and multivariate logistic regression analyses of host risk factors for CIDT test result in individual animal level in cattle raised in and around Mekelle

Risk factors	No. (%) of animals		Crude OR (95%CI)*	Adjusted OR (95%CI)**
	Examined	Positive		
Sex				
Male	97	3(3.09)	1	1
Female	383	51(13.32)	4.81(1.47-15.77)	9.99(0.421-23.7)
Age (years)				
<2	52	6(11.5)	1	1
2≤x≤ 5	207	26(12.5)	1.10(0.43-2.83)	1.41(0.39-5.08)
>5-x≤ 9	120	16(13.3)	1.18(0.43-3.21)	0.75(0.15-3.85)
>9	101	6(5.9)	0.48(0.15-1.58)	0.39(0.05-2.88)
Breed				
Local	145	4(2.7)	1	1
Cross	38	6(15.8)	6.61(1.76-24.79)	1.07(0.15-7.84)
Exotic	297	44(14.8)	6.13(2.16-17.41)	0.51(0.08-2.96)
Body condition				
Good	134	9(6.7)	1	1
Medium	184	19(10.3)	1.59(0.69-3.65)	2.21(0.78-6.29)
Poor	162	26(16.0)	2.65(1.19-5.88)	4.0(1.36-11.8)
Lactation				
Lactating	212	31(14.6)	1	1
Non lactating	170	19(11.2)	0.73(0.39-1.35)	0.40(0.13-1.21)
Pregnancy				
Pregnant	205	28(13.6)	1	1
Non pregnant	177	22(12.4)	0.89 (0.49-1.63)	1.79(0.35-1.80)
Parity class				
Heifer	118	16(13.9)	1	1
Parity 1 and 2	116	9(7.8)	0.52(0.22-1.23)	0.29(0.07-1.15)
Parity 3-5	119	22(18.5)	1.4(0.69-2.83)	1.01(0.23-4.38)
Parity ≥6	32	3(9.4)	0.64(0.17-2.35)	0.69(0.08-5.53)

Table10: Univariate and multivariate logistic regression analyses of environmental risk factors for CIDT test result in individual animal level in cattle

Risk factors	No. (%) of animals		Crude OR (95%CI)*	Adjusted OR (95%CI)**
	Examined	Positive		
Herd size				
≤ 5	196	8(4.1)	1	1
6-10	180	8(4.4)	1.09(0.40-2.97)	0.48(0.13-1.79)
>10	104	38(36.5)	13.53(6.0-30.5)	17.9(6.70-47.74)
Farming system				
Extensive	145	5(3.4)	1	
Intensive	335	49(14.6)	4.79(1.87-12.3)	1.28 (0.30-5.40)
Study area				
Urban	320	38(11.8)	1	
Peri-urban	160	16(10.0)	0.82(0.44-1.53)	4.43(0.64-11.95)

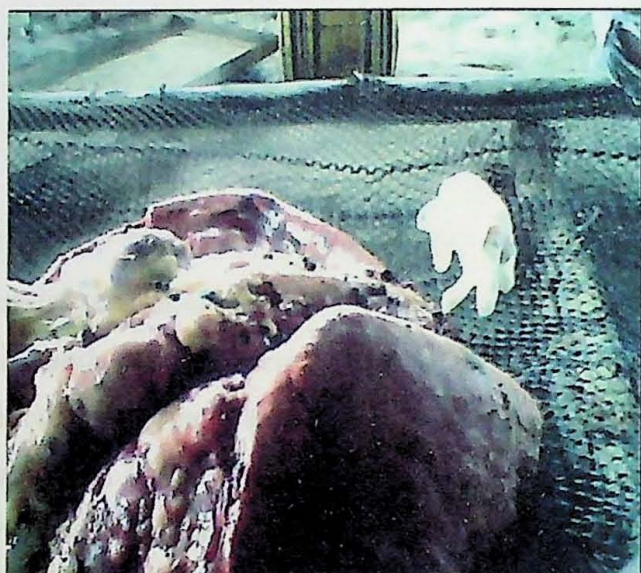
4.1.3. Necropsy findings of tuberculin reactor cattle

Out of the 54 CIDT reactor cattle, four sacrificed for detail postmortem examination. On antimortem examination, overt sign such as respiratory distress, frequent coughing, weight loss, docile and sluggish were observed in one of them. The rest three cattle had similar physical characteristics (apparently no detectable clinical sign). Upon postmortem examination, gross pathological lesions typical of bovine TB were detected in all scarified cattle 100% (4/4) of the reactor animals studied. Generalized type of tuberculous lesion was found in one of the sacrificed cow whereas; in the rest two cow and one bull localized tuberculous (granlomatous) lesion were detected. Some of the gross lesions are presented in figure 5. About 57% of the lesions were found in the thoracic cavity, followed by retropharyngeal (14.30%) and mesentric (14.30%) lymph nodes. The tropism or frequency of TB lesions was observed in bronchial and mediastinal lymph nodes, followed by retropharyngeal and mesentric lymph nodes (Table 11).

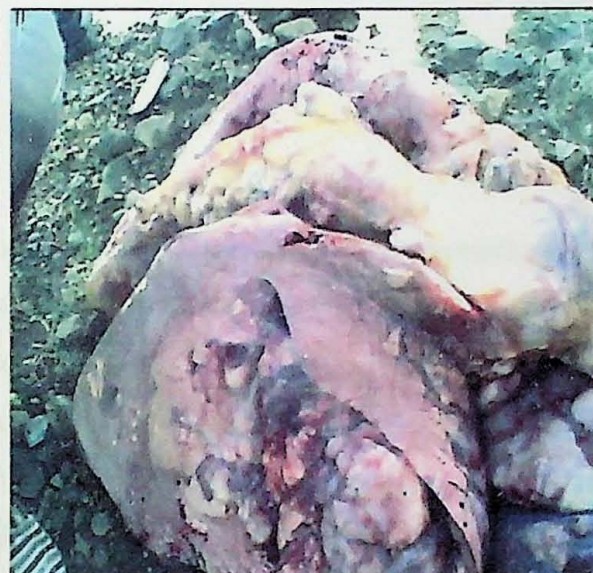
Table 11: Distribution and frequency of tuberculous lesions in four slaughtered CIDT test positive cattle

Infected organ	Frequency	Percent (%)
Retropharyngeal LN	2	14.30
Bronchial LN	3	21.43
Mediastinal LN	3	21.43
Lung	1	7.14
Visceral surface of rib	1	7.14
Mesenteric LN	2	14.30
Prescapular LN	1	7.14
Suprammary LN	1	7.14
Total	14	100

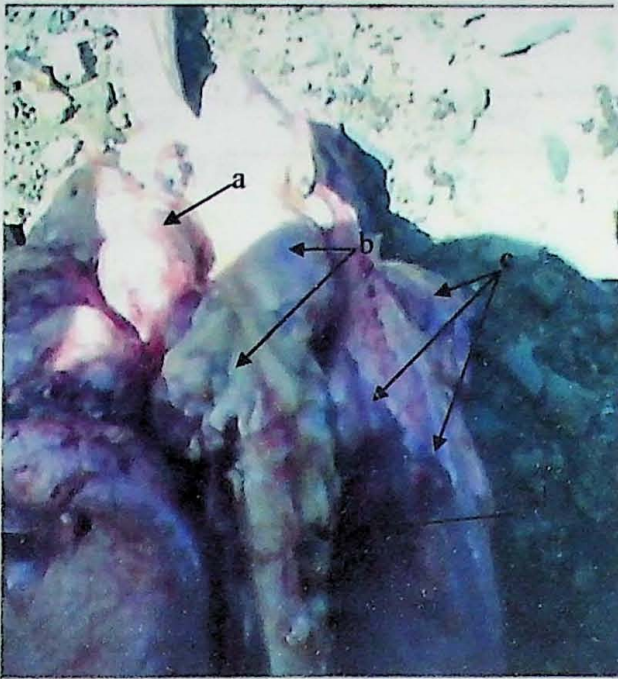
LN=lymph nodes



1. Diaphragmatic view of lung; extensively damaged with coalesced tubercle lesions and swollen associated lymph nodes.



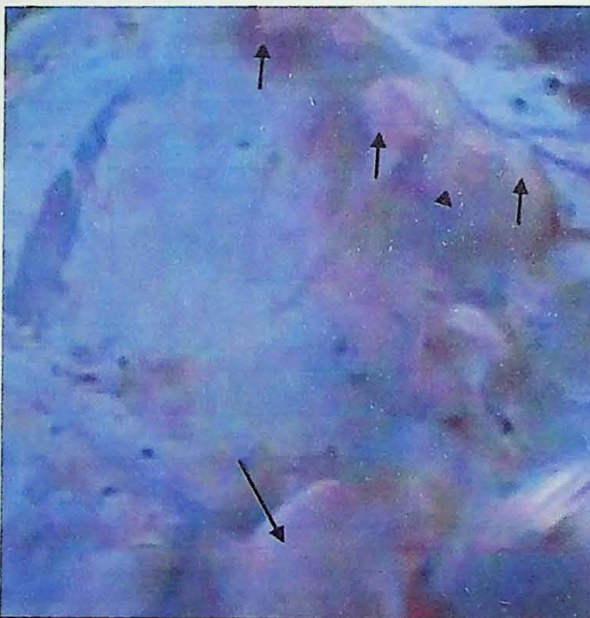
2. Lateral view of lung: extensively damaged with coalesced tubercle lesions and swollen associated lymph nodes.



3. Diaphragmatic view of lung: Granulomatous swelling in bronchial (a), cranial (b) and caudal (d) mediastinal LN and lung (c) coalesced granuloma (indicated by arrow)



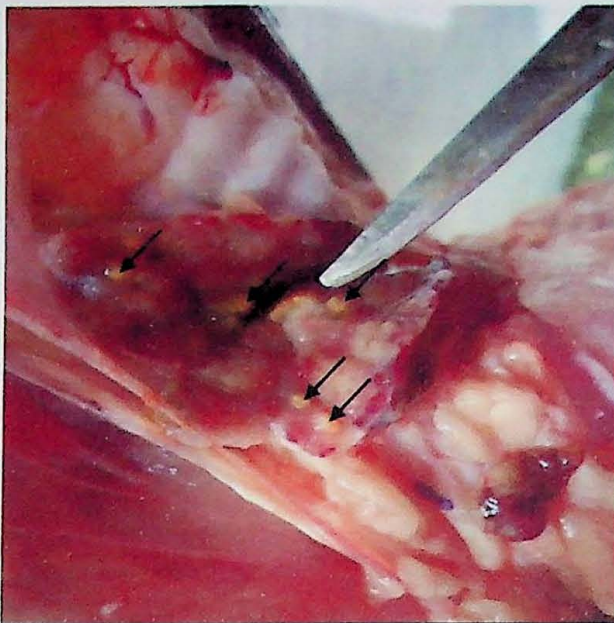
4. Sectioned tubercle granuloma in bronchial LN with caseous necrosis at the center (indicated by arrow).



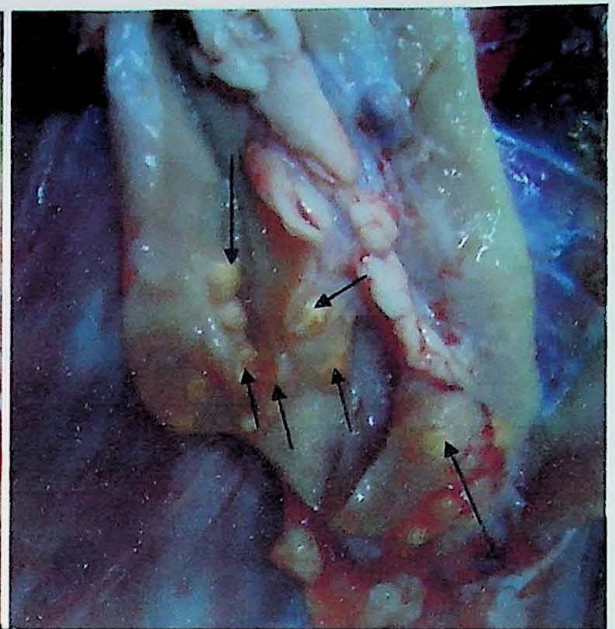
5. Granulomatous swelling in mesenteric lymph nodes (indicated by arrow)



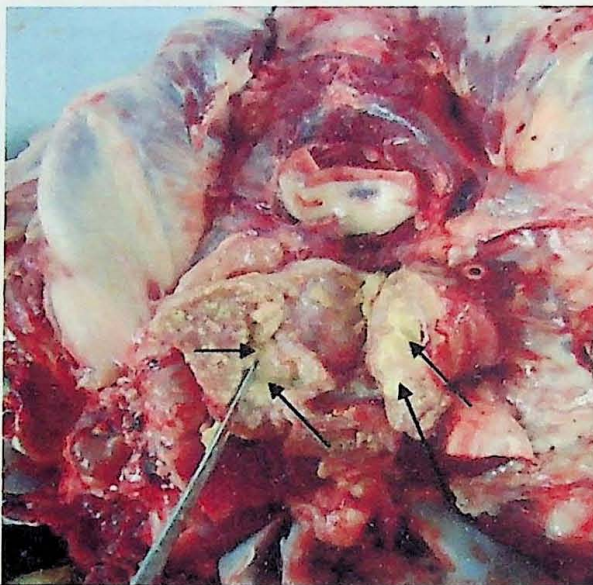
6. Tubercle granuloma (arrow) in rib (thoracic cavity)



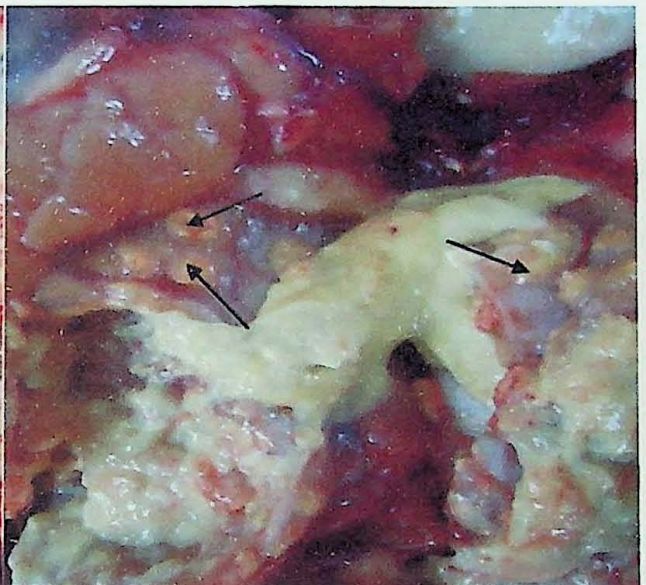
7. Tuberculous granulomas in bronchial lymph nodes (indicated by arrow)



8. Mediastinal lymph nodes with tuberculous granuloma (arrow)



9. Retropharyngeal lymph nodes with caseous abscess



10. Retropharyngeal lymph nodes with tubercle granuloma (arrow) and whitish abscess

Figure 5: Tuberculous lesions of bovine tuberculosis in different organ and tissues of four sacrificed CIDT test reactor cattle

4.2. Abattoir findings

For abattoir-based surveillance of bovine TB, 768 cattle slaughtered at Mekelle municipal abattoir were investigated. The vast majority were local (*Bos indicus*) breeds (97.26%) and male cattle (98.70%). Out of 768 cattle slaughtered, tuberculous lesion were detected in 49 cattle which gives a prevalence of 6.39% (95% CI: 4.65%-8.11%). Among the lesions observed, 75.51% were localized lesions involving frequently a single organ. Highly disseminated tuberculosis was seen in one animal whereby the lung and lymph nodes such as retropharyngeal, mediastinal, bronchial and prescapular were found studded with tuberculous granulomas. The frequency of lesion detection were slightly higher in bronchial lymph nodes (29.50%) followed by retropharyngeal lymph nodes (22.95%), mediastinal lymph nodes (21.32%) and lung (11.47%) (Table 12). More than half (53.06%) of the lesion were located in thoracic cavity. The rest lesions were found in cranial region (24.49%) and mesenteric lymph nodes (12.24%).

Table 12: Distribution and frequency of tuberculous lesions in the tissues of infected animals

Anatomic site	Frequency	Percent (%)
Retropharyngeal LN	14	22.95
Parotid LN	3	4.92
Bronchial LN	18	29.5
Lung	7	11.47
Mediastinal LN	13	21.32
Mesenteric LN	5	8.2
Prescapular LN	1	1.64
Total	61	100

LN=lymph nodes

Of the risk factors considered (Table 13) age was found significantly associated with BTB infection ($\chi^2=6.26$; $P=0.044$). As age increased the prevalence of BTB was increased. Those cattle greater than eight years old were found 3.2 fold more (OR=3.20; 95% CI: 0.74-13.80) likely to harbor tuberculous lesion than less than four years old cattle. However, prevalence of BTB was slightly higher in poor body condition than good and medium but not found

significantly associated with the presence of gross tuberculous lesions ($P>0.05$). However, due to incomparable proportion of cattle between sex and breed, it was difficult to speculate the effect of sex and breed on BTB prevalence.

Table 13: Association of host risk factors with tuberculous lesions in cattle slaughtered in Mekelle municipality abattoir

Variables	No. of Animals		χ^2 -Value	P-Value	OR(95%CI)
	Examined	Positive (%)			
Sex			9.46	0.002	
Male	758	46(6.07)			1
Female	10	3(30.0%)			6.63(1.66-26.49)
Breed			82.86	0.000	
Local	747	38(5.1%)			1
Cross	14	6(42.9%)			13.9(4.62-42.36)
Exotic	7	5(71.4%)			46.6(8.76-248.28)
Age			6.26	0.044*	
<4years	67	2(3.0)			1
4-8years	389	19(4.9)			1.67(0.38-7.34)
>8years	312	28(9.0)			3.20(0.74-13.80)
Body condition			4.66	0.097	
Good	122	4(3.3)			1
Medium	489	30(6.1)			1.92(0.66-5.58)
Local	157	15(9.5)			3.11(1.01-9.64)

*Statistically significant

4.3. Isolation of mycobacterium

Out of 61 tuberculous lesions that were cultured, culture positivity in primary culture media was confirmed in 44.2% (27/61). Smears were prepared on frosted microscopic slide from 27 culture isolates and stained by Ziehl-Neelsen technique, and all culture positive isolates were confirmed as acid fast bacilli. More than half (55.5%) of culture growth were observed from samples

obtained lung and its associated lymph nodes (Table 14). On the other hand, of 31 cultured milk sample collected from tuberculin-positive lactating cows, only 12.9 % (4/31) were positive for mycobacteria growth.

4.4. Molecular characterization of mycobacterial isolates

Further *Mycobacterium* genus typing was conducted on the 29 culture positive (26 from tissue and 3 from milk) isolates. Based on multiplex PCR analysis of those twenty nine isolate, 13.8% (4/29) isolates gave signal to the genus *Mycobacterium* (Figure 6). Fifty percent (2/4) of these isolates were confirmed to be members of the *M. tuberculosis* complex. Of these two isolates, one of isolate was from mesentric lymph nodes and the other was from milk. None of the isolates were *M. avium* complex. Figure 8 presents the gel electrophoresis results of PCR products.



Figure 6: Gel electrophoresis separations of PCR products of multiplex PCR genus typing of mycobacteria isolated from cattle. Lane 26 and 37 = 100bp DNA Ladder; Lane 25 and 36 = *M. avium* complex (positive control), Lane 24 and 35 = Qiagen H₂O (negative control), Lane 23 and 34 = *M. tuberculosis* complex (positive control), Lanes 1–22 and 27–33 were isolates from individual cattle with

tuberculous lesions. Lane A-O were others sample. Lane 12, 15, 20 and 28 were positive for genus *Mycobacterium*. Lane 15 and 28 were positive for *M. tuberculosis* complex and Lane 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 19, 21, 22, 27 29 30 31 32 and 33 were negative for genus *Mycobacterium*.

Table 14: Summary of the culture positivity and m-PCR typed of mycobacterial isolates

Sampled type	Growth in LJ media	Result of m-PCR typed	
		Genus <i>Mycobacterium</i>	<i>M. tuberculosis</i> complex
Retropharyngeal LN	6	1	-
Bronchial LN	7	-	-
Lung	4	-	-
Mediastinal LN	7	1	-
Mesenteric LN	3	1	1
Milk	4	1	1
Total	31	4	2

LN=Lymph nodes

4.5. Questionnaire survey

In this survey a total of 120 household owners or members of these households were interviewed. Of these, 37 (30.8%; 95% CI: 22.45%-39.22%) respondents reported they knew or had heard about TB of cattle, but only 15% (18/120) of them were aware of zoonotic importance of BTB (Table 15). Out of the total 120 households interviewed during the study period, 13 (10.8%) had TB case in their families or farm worker. In these 13 households, 10 (76.9%) were pulmonary tuberculosis (PTB) and 3 (23.1%) were EPTB. In five households had both PPD-positive cattle and human tuberculosis case in their family but not statistically significant ($P>0.05$) (Table 5).

Regarding the risk factors for transmission of tuberculosis (Table 16), out of 120 interviewed farmers, 11(9.2%) drank raw milk, 66 (55.0%) consume both raw and boiled milk and, 113 (94.2%) consume soured milk. Only twenty four (20.0%) of the respondents consume both

cocked and raw or poorly heat treated meat but the rest 96 (80.0%) consume heat treated meat. In 65 (54.2%) of the farms, farm workers or owner shared living room with animals.

Table 15: Knowledge of cattle owners about BTB and its transmission to humans

Statement	Number interviewed	of Owners who know (%)
Know BTB can affect animals	120	37 (30.83%)
Know BTB is Zoonotic	120	18 (15%)
Know milk is vehicle for <i>M. bovis</i>	120	18 (15.0%)
Know meat is vehicle for <i>M. bovis</i>	120	14 (11.7%)
Know BTB can transmit by inhalation of cough spray	120	18 (15%)
Know BTB can transmit by direct contact	120	4 (3.3%)

Table 16: Summary on habit of the respondents

Habit of respondents	Number of respondents	Present (%)
Milk drinking		
Raw milk	11	9.2
Boiled milk	43	35.8
Both raw and boiled milk	66	55
Yoghurt milk consumption		
Consume	113	94.2
Do not consume	7	5.8
Meat eating habit		
Cooked meat	96	80.0
Both cooked and row meat	24	20.0
House sharing		
Sharing	65	54.2
Not sharing	55	45.8

Several demographic characteristics and other factors were considered (Table 17) to investigate their possible association with BTB recognition of the respondents. The consciousness of the households about cattle infected by BTB ($\chi^2=64.9$; $P=0.000$) and the transmission of BTB from cattle to man ($\chi^2=21.9$; $P=0.000$) improved as the educational background of the household increased. Similarly, sex difference were observed on knowledge about cattle infected by bovine tuberculosis ($\chi^2=10.3$; $P=0.001$) and transmission of BTB from cattle to man ($\chi^2=3.92$; $P=0.048$).

Table 17: Summary on association of various factors with BTB awareness of respondents

Variable	Knowledge of owner				
	TB affect cattle		χ^2 value	TB transmit from	χ^2 -value
	Yes (%)	No (%)	(P value)	sick cattle to human	(P-value)
				Yes (%)	No(%)
Residence			3.82 (0.051)		0.68 (0.409)
Urban	18 (41.9)	25 (58.1)		8 (18.6)	35 (81.4)
Peri-urban	19 (24.7)	58 (75.3)		10 (12.9)	67 (87.1)
Age			13.0 (0.005)*		6.25 (0.10)
10-30	11 (55.0)	9 (45.0)		6 (30.0)	14 (70.0)
31-50	18 (37.5)	30 (62.5)		7 (14.6)	41 (85.4)
51-70	7(18.9)	30 (81.1)		5 (13.5)	32 (86.5)
>70	1 (6.7)	14 (93.3)		0 (0)	15 (100)
Sex			10.3 (0.001)*		3.92 (0.048)*
Male	31 (41.3)	44 (58.7)		15 (20.0)	60 (80.0)
Female	6 (13.3)	39 (86.7)		3 (6.7)	42 (93.3)
Education			64.9 (0.000)*		21.9 (0.000)*
Illiterate	0 (0)	40 (100)		0 (0)	40 (100)
1-4 grade	5 (17.9)	23 (82.1)		2 (7.1)	26 (92.9)
5-7 grade	6 (27.3)	16 (72.7)		2 (9.1)	20 (90.9)
8-12 grade	18 (81.8)	4 (18.2)		2 (9.1)	20 (90.9)
Certificate and above	8 (100)	0 (0)		4 (50.0)	4 (50.0)

* Statistically significant

4.7. Retrospective case record analysis

Analysis of secondary data from Mekelle hospital has showed that a total of 1272 TB patients were admitted and diagnosed as TB infected in a five year time period (September 2005 to August 2010) (Table 18). Those 1272 TB patients were received the direct observed short course (DOTS) treatment regimen. Of these 62.03% (789/1,272) were pulmonary TB and 37.97% (483/1,272) were extra-pulmonary TB patients. Out of those TB patients on short course therapy, 75.3% (957/1,272) were between 15 and 45 years, at their more productive age. Between February 2009 and October 2010, 259 TB patients were tested for HIV in the hospital and 105 (40.5%; 95% CI: 34.52%-46.56%) were found infected with HIV.

Table 18: TB patients received DOTS for the past five years in Mekelle hospital

Year	Pulmonary tuberculosis	Extra-pulmonary tuberculosis
2005/06	234(62.7%)	139(37.3%)
2006/07	183(63.3%)	106(36.7%)
2007/08	140(60.1%)	93(39.9%)
2008/09	141(60%)	94(40%)
2009/10	91(64.1%)	51(35.9%)
Total	789(62.03%)	483(37.97%)

Source: Mekelle Hospital

5. DISCUSSION

Infection of cattle with BTB constitutes a human health hazard as well as an animal welfare problem. Furthermore, the economic implications in terms of trade restrictions and productivity losses have direct and indirect implications for human health and the food supply (Villarreal-Ramos *et al.*, 2003). Bovine tuberculosis has been known to be endemic to Ethiopia since 1967 (FAO, 1967; Hailemariam, 1975). However, there is a lack of adequate data on the epidemiology and public health implication of this disease. Most of the studies conducted in Ethiopia were localized around the central Ethiopia. Therefore, one of the contributions of this study was to give preliminary information on the occurrence BTB in Mekelle in particular and Tigray regional state in general.

In this study, an overall animal and herd prevalence of 11.25% and 20% of BTB was recorded using CIDT test, respectively. This was in agreement with the finding of Ameni and Erkihun, (2007) in Adama town, who report 11% and 15% prevalence of BTB individual animal and herd level, respectively. This agreement could be the similarity in study subjects, herd size and management condition. Majority of the herds tested in both study were smallholders found in and around urban area with smaller in herd size. However, both the herd and animal prevalence recorded in this study were lower than that the previous study carried out by Tsegaye *et al.* (2010) who reported a herd and individual BTB prevalence rate of 53.6% and 34.1%, respectively. Similarly, Elias *et al.* (2008) also reported that higher prevalence of BTB than this study, 23.7% and 43.4% animal and herd level prevalence, respectively. Moreover, the individual prevalence in the present study was much lower than previous studies conducted by Ameni *et al.* (2003b) and, Ameni and Roger, (1998) who report 46.8% and 50%, respectively. This difference may be due to the fact that the present study was conducted on herds of small sizes in contrast to previous studies, which were conducted on relatively large herd-sized farms. O'Reilly and Daborn (1995) have indicated that the transmission of BTB from cattle to cattle is largely influenced by herd size, i.e. the larger the herd size the greater the chance of transmission. Moreover, this variation is ascribed to the difference in the study animals used and type of farming system. The disease is said to be more prevalent in dairy cattle kept under

intensive management than others due to closer confinement, longer life spans and greater productivity stress (Biberstein and Hirsh, 1999; Ayele *et al.*, 2004).

However, the present animal prevalence was much higher than the prevalence reported by Tschopp *et al.* (2010), South Omo (Southern Ethiopia) and Tschopp *et al.* (2009) in various region of Ethiopia who reported a prevalence of 0.8% and 3%, respectively. This may be due to difference in study subject and management system. The previous studies conducted exclusively in rural traditionally managed zebu cattle but the present study conducted in both extensive and intensive production type comprising higher number of cross breeds and exotic breeds (considered to be more susceptible to BTB). Moreover, when larger proportion of the study animals was grazing in the field, the level of confinement is reduced to a certain degree, which in turn minimizes the rate of infection in the herd (OIE, 1996).

Radostits *et al.* (2007) indicated that, BTB is a diseases of overcrowding. In accordance with this the current finding, both the herd and individual animal BTB prevalence showed a statistically significant association with herd size; also, the proportion of reactors increased parallel to an increasing herd size. This finding was consistent with previous reports (Barwinek and Taylor, 1996; Cook *et al.*, 1996; Ameni and Erkihun, 2007; Elias *et al.*, 2008) and this might be due to the fact that risk of an individual animal introducing tuberculosis infection into a negative herd may increase with herd size, and the lateral spread of infection within the herd may also be favored. On the other hand, this study revealed that intensification was strongly significant with prevalence of bovine TB which was consistent with previous reports (Elias *et al.*, 2008). Intensive farming systems that promote close contact between animals, thereby favoring the spread of *M. bovis*. This would make extensive farming systems safer than zero grazing and more effective in preventing transmission of BTB (Ayele *et al.*, 2004). In addition, cattle herd managed under poor management conditions were more likely to become infected with BTB than cattle herd kept under good management conditions. This result agrees with previous studies in Ethiopia (Kiros, 1998; Bogale *et al.*, 2000). There are numerous reports documenting that poor housing and other poor managerial inputs predispose to tubercular infection (Barwinek and Taylor, 1996; Griffin *et al.*, 1993). Moreover, animal resistance to tuberculosis is reduced by a

shortage of feed and/or an imbalanced diet (Griffin *et al.*, 1993). The lack of hygiene predisposes to the proliferation of pathogens in general (Humblet *et al.*, 2009).

In this study the prevalence of bovine TB was significantly high in females (OR=4.81) as compared to males, which was in agreement with the finding at Wolita Sodo, Ethiopia (Ameni *et al.*, 2001) and Uganda (Inangolet *et al.*, 2008). This is because cows are confined in a barn and kept long for production which may facilitate infection and acquiring the disease. Moreover, dairy cows experience more production stress and gathering of cattle during milking increases the risk of bovine TB transmission as shown by bovine TB transmission modeling in New Zealand in 1997 (Barlow, 1997). In contrary, this finding was inconsistent with finding from Tanzania revealed that male cattle were significantly more affected by bovine TB than female animals (Kazwala *et al.*, 2001).

In the present study, high prevalence of BTB was recorded in crosses (15.8%) and pure Holstein (14.8%) as compared to Zebu breeds (2.7%), which was consistent with previous reports in Ethiopia (Ameni *et al.*, 2001) and Uganda (Kazwala *et al.*, 2001). Barwinek and Taylor (1996) reported that genetically improved cattle might suffer more severely from malnutrition, production stress and poor housing systems in tropical countries, and consequently become more susceptible to BTB infection.

In accord to an earlier report from Addis Ababa (Elias *et al.*, 2008), in this finding body condition score showed a statistically significant association with tuberculin reactivity. The present result showed that animals under poor body condition reacted four times (OR=4.0) more to tuberculin test than those of with good body condition (Table 11). This conforms well to the established fact that an animal's resistance to tuberculosis is reduced by a shortage of feed and/or unbalanced diet, attributable to a deficiency of proteins, minerals and vitamins in the diet (Griffin *et al.*, 1993). Previous workers (Cook *et al.*, 1996; Kazwala *et al.*, 2001; Asseged *et al.*, 2004) similarly reported higher BTB prevalence in animals with poor body condition compared to those with good body condition scores. However, it was inconsistent with the previous studies in Ethiopia (Ameni, 1996; Kiros, 1998) and in Turkey (Barwinek and Taylor, 1996).

In this study, association between reports of human cases of tuberculosis in the households and reactor cattle in the household's herd was not significant. This result was consistent with the report from Wuchale-Jida district central Ethiopia (Ameni *et al.*, 2003a). But statistically significant associations between human tuberculosis cases and reactor herds were reported in some studies (Cook *et al.*, 1996; Ameni *et al.*, 2001; Ameni and Erkihun, 2007).

In postmortem examination of the four tuberculin reactor cattle, gross pathological lesions typical of bovine TB were detected in all scarified cattle 100% (4/4), which was higher than the previous report by Tsegaye *et al.* (2010), who detected in 72.7% (8/11) of the euthanized animals. In this study, lesions were predominantly detected in thoracic cavity mainly mediastinal lymph nodes and bronchial lymph nodes which was harmonize with the reports by Ameni *et al.* (2006) and Tsegaye *et al.* (2010). Reports from developed countries indicate that tuberculosis lesions are found most frequently in the lymphatic tissues of the thoracic cavity (Tsegaye *et al.*, 2010). In intensive husbandry system, 90% of the lesions occur in the respiratory tract (Corner, 1994). Contrary to this finding, in the recent report by Ameni *et al.* (2007), 94.5 % of the lesions were detected in mesenteric lymph nodes in animals that kept on pasture.

Abattoir inspection of carcasses revealed a prevalence of 6.39% in cattle slaughtered at Mekelle municipality abattoir. This finding was much lower than from that obtained at Adama municipality abattoir (Mamo, 2007) who reported a prevalence rate of 24.7%. But higher than that reported by Jemale (2005) i.e. a prevalence rate of 2.34% in Hawassa municipality abattoir. However, comparable results with the present study were obtained in Adama municipality abattoir, where the prevalence rate was 5.2% (Ameni and Wudie, 2003). The present abattoir based study revealed that as age increased the chance of harboring tuberculous lesion was increased. This may be due to chronic nature of the disease and the chance of infection with *M. bovis* increases as the age increases (Ameni,1996; Kazwala *et al.*, 2001).

In this study the tuberculous lesions and culture positivity were observed predominantly in the lung and associated lymph nodes, which was consistent with the previous findings (Ameni and wudie, 2003; Asseged *et al.*, 2004; Teklul *et al.*, 2004). A number of studies revealed that the majority of tuberculous lesions are located in the thoracic cavity suggesting the inhalation route being the principal route of BTB transmission (Corner, 1994; Ameni and wudie, 2003; Phillips *et*

al., 2003; Teklul *et al.*, 2004). In contrary, Cleaveland *et al.* (2007) reported that 61.3% (791/1290) of carcasses in Tanzania had lesions in the gastrointestinal tract. Lesions of the alimentary tract are more common in temperate climates where conditions favor dissemination and survival of *M. bovis* on forage, while respiratory tract lesions are more common in arid climates where conditions favor aerosolization of *M. bovis* (Lepper and Pearson, 1973).

The high frequency of respiratory tract lesions in the four sacrificed cattle and abattoir survey may result inhalation of cough spray from infected animals or human might be the source of infection. As most herds in the intensive type of management system confined in poorly ventilated house cough spray from a single infected herd has the potential to infect several herds. Radostits *et al.* (2007) believed that, inhalation is the almost invariable portal of entry in housed cattle, and even in those at pasture it is considered to be the principal mode of transmission.

Out of 61 tuberculous lesions that were cultured, culture positivity in primary culture media was confirmed in 44.2% (27/61), which is consistent with recent report by (Ameni *et al.*, 2010) who report 47% culture positivity. This low isolation rate of mycobacteria may have resulted from reduced sensitivity of culture arising from prolonged storage at field sites, and the freeze-thaw cycles that occurred during transportation and contamination of tissue samples and overgrowth of *M. bovis* with environmental mycobacteria (Cleaveland *et al.*, 2007). Besides, *M. bovis* grows poorly on standard Löwenstein-Jensen medium (Amanfu, 2006). On the other hand, of 31 cultured milk sample collected from tuberculin-positive lactating cows, four were positive for mycobacteria growth. However, only one milk sample were confirmed as *M. tuberculosis* complex by multiplex PCR analysis. Genus typing of the isolates using multiplex PCR revealed that out of 4 isolates which showed signals for the genus *Mycobacterium*. Half of these isolates were confirmed to be members of the *M. tuberculosis* complex. One of the isolate was from milk which may signify the hazard of unpasteurized milk for public health in the area.

Bovine TB is a significant human pathogen that aggravates the ‘triple trouble’ of HIV/AIDS and TB infection and malnutrition (Cosivi *et al.*, 1998). On the other hand, there is widespread ignorance of how bovine TB is spread from animal to human and vice versa. Community awareness is important to control bovine TB. The fact that impact of *M. bovis* on humans is

underemphasized may affect control of the disease at the source. To this effect, in present study less than a third (30.83%) of the respondents knew about bovine TB, and only 15.0% of the respondents were conscious of its transmission from cattle to humans. These finding disagree with that of Bonsu *et al.* (2000), who reported 28 (n=30) of the herdsmen didn't know BTB and all did not know that tuberculosis could be transmitted from cattle to man in Ghana. However, this result was consistent with the previous reports (Ameni *et al.*, 2003a) in Ethiopia.

Among the interviewed households, 10.8% had experienced at least one human tuberculosis case in the family. Of these families, 20% had reactor cattle. Nevertheless, no statistically significant association was observed between reactor cattle and human tuberculosis cases in households. Similarly, Ameni *et al.* (2003a) also reported no statistical association between reactor cattle and human tuberculosis cases in Wuchale Jida, central Ethiopia. However, contradict finding was reported in Wolaita Soddo, southern Ethiopia by Ameni *et al.* (2001) and in Fitch town, central Ethiopia by Ameni *et al.* (2003c). According to Seifert (1996) tubercle bacilli can survives in acid milk for 15 days and in milk products such as cheese and butter for weeks. In this survey, more than half (55%) of the respondent consume both raw (buttermilk) and boiled milk, and 94.2% of the interviewed persons had the habit of yoghurt consumption. Therefore, this habit might expose the public to risk of acquiring of tubercle bacilli from animal.

The consciousness of the households about cattle infected by bovine tuberculosis and the transmission of BTB from cattle to man improved as the educational background of the household increased. This finding was consistent with the work by Ameni *et al.* (2003c) in Fitch town, central Ethiopia. On the other hand male were aware of BTB in cattle and zoonotic importance. The probable explanation is that 40% of interviewed women were illiterate in which all illiterate were not know the disease whereas 70% interviewed male had at least primary school grade. In general, BTB awareness among cattle owners was found to be insufficient and this might have impact on epidemiological studies, prevention and control of the disease.

Secondary data from Mekelle Hospital indicated that a total of 1272 TB patients were received the direct observed short course (DOTS) treatment regimen. Of these 62.03% (789/1,272) were pulmonary TB and 37.97% (483/1,272) were extra-pulmonary TB patients, which were

harmonized with the nationwide report, that 36% of incident TB cases in Ethiopia are extrapulmonary (WHO, 2008). This is exceptionally high, considering that, 15% of incident TB is extrapulmonary in most high burden countries (WHO, 2008). This rise in the extrapulmonary TB could be associated with *M. bovis* infection and/or associated with the rise in the incidence of HIV in the country although the exact cause has not yet been identified so far. But the prevailing socio-economic conditions such as the low level of awareness in the community about the transmission of tuberculosis between animals and humans, the habit of consumption of raw animal product and the HIV pandemic could promote the transmission of mycobacteria between animals and humans. In general, bovine TB was found as a neglected disease in the study area that needs many more resources. There is a need for molecular characterization of clinical isolates from human cases to ensure that correct estimates are made of the true burden of infection due to *M. bovis*. Moreover, the identification of the role of *M. bovis* as a cause of human TB in the area will have great aid to the existing TB control campaign.

6. CONCLUSION AND RECOMMENDATIONS

This study demonstrates a widespread occurrence of BTB infection in animals. A higher infection rate was found in animal managed under intensive production with increased herd size and poor husbandry practices. Similarly higher prevalence was recorded in female, exotic and their crosses. Most of the lesion detected in postmortem examination were found in thoracic cavity which may indicate inhalation is the prevailing mode of transmission of BTB from cattle to cattle in the area. The majority of cattle owning family in the study area were not aware of the existence BTB and its public health significance. Moreover, a large portion of the public had habit of consuming raw animal product predisposing them to zoonotic tuberculosis. Though analysis of data from Mekelle hospital revealed TB is major human health problem and high incidence of extrapulmonary TB patients, nothing is known about the role of *M. bovis* in human tuberculosis of the study area. So the extent of bovine tubercle bacilli as a contributor to the tuberculosis causative agent in humans remains undetermined. The widespread presence of BTB in cattle, increasing incidence of tuberculosis/HIV/AIDS co-infection, the high incidence of extra pulmonary TB, the public consumption habit of raw food of animal origin and sharing the same house with their livestock, together with the isolation of *M. tuberculosis* complex from milk and meat may warrant the public health implication of BTB in the area. Therefore, emphasize the need for paying attention towards the control of bovine tuberculosis. Test and slaughter program is economically unaffordable in Ethiopia. Therefore, Alternative control measures, which are feasible and acceptable under local condition, have to be designed urgently.

In light with the abovementioned findings, the following recommendations are forwarded:

- Alternative control strategies such as test and segregation, improved farm management by limiting the stocking densities, isolation of calves from reactor cow and rearing in a BTB free replacement stock need to be applied,
- Public education should be done in order to raise the awareness of public on BTB and its zoonotic importance with due consideration on the danger of consuming unboiled or unpasteurized dairy products and uncooked meat,

- Strictly meat inspection procedures must be implemented at Mekelle municipality abattoir,
- Pasteurization of milk and milk products should be initiated and adapted in Mekelle,
- Furthermore, more detailed studies are needed to assess and evaluate the scale of the problem, to assess extent of *M. bovis* as a contributor to human tuberculosis and, to design feasible and cost-effective control methods through close collaboration between the medical and veterinary professions.

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8. ANNEXES

Annex 1: Questionnaire presented to cattle owners

Date _____

Code No _____

I. Personal and Socio economic data

1. Socio-demographic information

- a. Owner's Name _____
- b. Address: local administration _____ House No _____
- c. Age in Years: _____
- d. Sex: a) Male b) Female
- e. Marital status a. Married b. unmarried c. divorced d. other _____
- f. Occupation: _____
- g. Education status:
 - i) No formal education
 - ii) 1-4 grade
 - iii) 5-7 grade
 - iv) 8-12 grade
 - v) Certificate or above (specify) _____

2. Which species of animal you own? a) Cattle b) Horse c) Mule d) Donkey e) Sheep f) Goat g) Cat h) dog i) Poultry

3. Which breeds of cattle you own? a) Exotic b) Cross c) Local

4. For what purpose do you rear cattle? a) Drought Power b) Milk for home use c) Milk for sale d) Source of meat e) Others _____

5. How many cattle do you have? a. Less than 5 b) 6-10 c) More than 10

II. Questions related to husbandry practices

1. Source of feed for your cattle a) Communal pasture b) Privately hold pasture
c) Purchased feed _____
2. Do you provide feed supplement to your cattle? a) Yes _____ b) No
3. Is feeding of cattle separate? a) Yes b) No
4. Is the feeding trough raised? a) Yes b) No
5. Do you clean the feed trough clean regularly? a) Yes b) No
6. Source of water for your cattle a) Communal watering Point b) Pipe c) Other _____
7. What type of drinking utensil do you use?
a) Permanently constructed pond
b) Household utensil
c) Other (specify) _____
8. Is drinking utensil separate each animals? a) Yes b) No
If no, when do you replace the water and clean the utensil/pond? a) For every animal b)
Twice per day c) Once per day d) Every week e) Every month f) Do not replace
9. Is the drinking utensil raised? a) Yes b) No
10. What type housing system do you use for your cattle?
a) free roaming b) separate shed c) indoor with people
11. Do you regularly clean the barn? a) Yes b) No
12. Is there sufficient ventilation in the barn? a) Yes b) No
13. Do you mix your cattle with cattle of others? a) Yes b) No
14. Where do your cattle get mixed? a) Communal grazing area b) Watering points c) Market
places d) Veterinary clinic e) Vaccination posts f) Other _____
15. Are there wild animals in your area where your animals graze or drink water? A) Yes b) No
14. How your animal breed?
a) Natural mating by your own bull
b) Natural mating by bull from neighbors
c) Artificial insemination

III. Questions related to risk factors associated with infection and transmission of bovine tuberculosis in human and animals

1. Do you have close contact with animals? A) Yes b) No

If yes, specify the contacts _____

2. Do you use the same watering point with animals? a) Yes b) No

3. Do you share the same house with your animals? a) Yes b) No

4. Do you drink milk? A) Yes b) No

If yes, your choice is a) raw milk b) boiled milk c) both raw and boiled milk

d) Other (specify) _____

5. Do you think that drinking raw milk has a risk to your health? a) Yes b) No

If yes, mention the possible risk _____

6. Do you drink milk by sharing the same cup with other individuals? a) Yes b) No

7. Do you boil milk? a) Yes b) No

If yes, reason for boiling a) Fear of milk borne diseases b) due to culture

8. Do you sell milk? A) Yes b) No

If yes, where a) Local market (local people within the kebele) b) Milk collection unit
(specify the site) _____ c) other (specify) _____

9. Meat eating habit a) raw meat b) cooked c) both raw and cooked d) do not eat meat

10. Where do you slaughter your animals?

a. Municipal abattoir

b. In open field within the village

c. In separate slaughter site where animals have no access to the site

d. Near watering point where animals drink

f. Other (specify) _____

IV. Questions related to farmer recognition level

1. Have you heard about the disease tuberculosis? (*Cross check the signs*) a) Yes b) No

If yes, do you or any of your family member diagnosed for TB? a) Yes b) No

2. Does tuberculosis affect cattle? a) Yes b) No c) I don't know

If yes, What are the clinical signs? _____

3. How bovine tuberculosis is transmitted between animals? _____

4. Is TB of cattle communicable to man? a) Yes b) No c) I don't know

If yes, how it is transmitted from animal to man?

a) By consumption of raw milk

b) By consumption of raw or poorly heat treated meat

c) By inhalation of cough spray from TB infected animal

d) By direct contact (through break skin)

5. Can TB transmit from human to animal? a) yes b) No

If yes, how it is transmitted from man to animal? _____

Annex 2: Comparative intradermal tuberculin test (CIDT) data recording format

No	Date	Name of owner	Animal identification	Sex	Age	Breed	BCS	Pregnancy	State of pregnancy	State of lactation	Farming type	Herd size	Skin fold thickness measurement (mm)					
													Before		After		Difference	
													PPD-A	PPD-B	PPD-A	PPD-B	ΔA	ΔB

*BCS=Body condition score

Annex 3: Abattoir survey record sheet format

N o.	Date	Butcher No.	Breed	Sex	Age	BCS*	TB status	Type of Lesion	Tissues of tuberculous lesion

*BCS=Body condition score

Annex 4: Preparation of Löwenstein-Jensen egg-based medium

1. Mineral salt solution

1.1 Ingredients

Potassium dihydrogen phosphate anhydrous (KH ₂ PO ₄)	2.4 g
Magnesium sulphate (Mg SO ₄ . 7H ₂ O)	0.24 g
Magnesium citrate	0.6 g
Asparagine	3.6 g
Glycerol (reagent grade)	12 ml
Distilled water	600 ml

1.2 Preparation

Dissolve the ingredients *in order* in the distilled water by heating. Autoclave at 121 °C for 30 minutes to sterilize. Cool to room temperature. This solution keeps indefinitely and maybe stored in suitable amounts in the refrigerator.

2. Malachite green solution, 2%

2.1 Ingredient

Malachite green dye	2.0g
Sterile distilled water	100 ml

2.2 Preparation

Using aseptic techniques dissolve the dye in sterile distilled water by placing the solution in the incubator for 1-2 hours.

3. Homogenized whole eggs

Homogenised eggs (20-25 eggs, depending on size) 1000 ml.

Fresh hens' eggs, not more than seven days old, are cleaned by scrubbing thoroughly with a band brush in warm water and plain alkaline soap. Let the eggs soak for 30 minutes in the soap solution. Rinse eggs thoroughly in running water and soak them in 70% ethanol for 15 minutes. Before handling the clean dry eggs scrub the hands and wash them. Crack the eggs with a sterile knife into a sterile flask and beat with sterile egg whisk or in a sterile blender.

The ingredients are aseptically pooled in a large, sterile flask and mixed well.

Mineral salt solution	600ml
Malachite green solution	20ml
Homogenized whole eggs	1000ml

The complete egg medium is distributed in 6-8ml volumes in sterile 14ml or 28ml McCartney bottles or in 20ml volumes in 20 × 150ml screw-capped test tubes and the tops are securely fastened.

Before loading, heat the inspissator to 80°C to quicken the build-up of the temperature. Place the bottles in a slanted position in the inspissator and coagulate the medium for 45 minutes at 80°C-85°C. Inspissate the medium within 15 minutes of distribution to prevent sedimentation of heavier ingredients.

Source: WHO (1998)

Annex 5: Body condition scoring

a) Body condition scoring for exotic and cross-breed animals

Score	Body condition
0	Animals are emaciated with spinous processes, hipbones, tail head and ribs projected prominently. No fatty tissue can be detected, neural spines and transverse processes fit sharp.
1	Individual spinous process are still fairly sharp to the touch and there is no fat around tail, head, hip bones, tail head and ribs still prominent, but appear less obvious.
2	Spinous processes can be identified individually when touched, but feel rounded rather than sharp. There is some tissue cover rounded tail, over hip bones and flank individual ribs are no longer visually obvious.
3	Spinous processes can only be felt with firm pressure. Areas on either side on tail head now have a degree of fat cover which can be easily felt.
4	Fat cover around tail head is evident as slight rounds soft to touch spinous processes cannot be felt even with firm pressure and folds of fat are beginning to develop over and thigh of animal.
5	Bone structure is no longer noticeable and animal presents a blocky appearance. Tail head and hip bone are almost completely buried in fatty tissue and folds of fat are aspparent over ribs and thighs. Spinous processes are completely covered by fat and animal's mobility is impaired by large amounts of fat carried.

b) Body condition scoring for indigenous breeds of cattle (zebu)

Score	Body condition
1 (L-)	Marked emaciation the animal could be condemned ante-mortem.
2 (L)	Transverse processes project prominently, spines appear sharply.
3 (L+)	Individual dorsal spines are pointed to the touch, hips, tail-head and ribs are prominent.
4 (M-)	Ribs, hips and pins are clearly visible, muscle mass between hooks and pins are slightly concave.
5 (M)	Ribs usually visible, little fat cover, dorsal spines are barely visible.
6 (M+)	The animal is smooth, dorsal spines cannot be seen, but are easily fat.
7 (F-)	The animal is smooth and well covered but fat deposits are not marked.
8 (F)	Fat cover in critical areas can easily be seen and felt; transverse processes cannot be seen or felt.
9 (F+)	Heavy deposits of fat is clearly visible on tail-head, brisket, dorsal spines, ribs and hooks.

Source: Nicholson and Butterworth (1986).

Annex 6: Age determination based on dental table

Age (Years)	Characteristic change
1 ¹ / ₂ - 2	I ₁ erupts
2-2 ¹ / ₂	I ₂ erupts
3	I ₃ erupts
3 ¹ / ₂ - 4	*I ₄ erupts
5	All incisors and canine are in wear
6	I ₁ is level and the neck is visible
7	I ₂ is level and the neck is visible
8	I ₃ is level and the neck is visible, I ₄ may be visible
9	I ₄ is level and the neck is visible
10	The dental star is square in I ₁ and in all teeth by 12 years
15	The teeth that have not fallen out are reduced to small round pegs

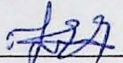
*Canine of ruminant is usually considered as fourth incisor

Source: De Launta and Habel, (1986)

9. SIGNED STATEMENT OF DECLARATION

"I the undersigned, declare that this thesis is my original work, and has not been presented for a degree in any other University and that all sources of materials used for the thesis have been duly acknowledged"

Name: Fikre Zeru Kflewahd

Signature 

Date of submission _____

This thesis has been submitted for examination with our approval as University advisors

Advisors

Signature

Tesfaye Sisay (DVM, MSc, PhD, Assistant Professor)

Gobena Ameni (DVM, PhD, Associate Professor)

Gezahegn Mamo (DVM, MSc, Assistant Professor)
