TABLE OF CONTENTS

PAGE

TABLE OF CONTENTS .... **I**

LIST OF TABLES .... **IV**

LIST OF APPENDICES .... **V**

LIST OF FIGURES .... **VI**

ABBREVIATIONS .... **VII**

ACKNOWLEDGEMENTS .... **X**

ABSTRACT .... **XII**

1. INTRODUCTION **1**

2. LITERATURE REVIEW **5**

2.1 Aetiology **5**

2.1.1 Classification 5

2.1.2 Morphology 5

2.1.3 Staining 6

2.1.4 Structure and composition 6

2.1.5 Growth and cultural characteristics 7

2.2 Pathology **8**

2.2.1 Virulence 8

2.2.2 Routes of infection 9

2.2.3 Pathogenesis and lesion distribution 9

2.3 Immunology **10**

2.4 Epidemiology **11**

2.4.1 Distribution 11

2.4.2 Host range 12

2.4.3 Sources of infection and mode of transmission 13

2.4.4 Risk factors 15

2.4.4.1 Host Factors 15

2.4.4.2 Agent factors 16

2.4.4.3 Husbandry and environmental factors 16

2.5 Economic importance **17**

2.6 Zoonotic importance ... 18
2.7 Awareness of bovine tuberculosis and consumption habit ... 21
2.8 Diagnosis ... 22
2 8.1 Clinical examination ... 22
2.8.2 Post-mortem inspection ... 22
2.8.3 Histopathological techniques ... 23
2.8.4 Immunodiagnosis ... 23
2.8.4.1 Skin test ... 23
2.8.4.2 Blood-based laboratory tests ... 24
2.8.5 Isolation and identification of mycobacteria ... 26
2.8.5.1 Polymerase chain reaction (PCR) ... 26
2.8.5.2 Spoligotyping ... 28
2.9 Control ... 29
2.10 Bovine tuberculosis in Ethiopia ... 30
2.10.1 Magnitude and distribution ... 30
2.10.2 Economic importance ... 33
2.10.3 Isolation and identification of the agent ... 33
2.10.4 Awareness and habit of people ... 33
3. MATERIALS AND METHODS ... 36
3.1 Study area ... 36
3.2 Study subjects ... 37
3.3 Study design and sampling ... 38
3.4 Sample size determination ... 38
3.4.1 The comparative intradermal tuberculin test ... 38
3.4.2 Abattoir survey ... 39
3.5 Study methodology ... 40
3.5.1 The comparative intradermal tuberculin test ... 40
3.5.2 Abattoir survey ... 42
3.5.3 Isolation and identification of mycobacteria ... 42
3.5.3.1 Sample collection ... 42
3.5.3.2 Bacteriological culture ... 43

3.5.3.3 Multiplex-Polymerase chain reaction (m-PCR) 44
3.5.3.4 Deletion typing 44
3.5.3.5 Spoligotyping 45
3.5.4 The questionnaire survey 46
3.5.5 Retrospective case-record analysis 46
3.6 Data analysis 47
4. RESULT 48
4.1 Herd level characteristics 48
4.2 Animal characteristics 48
4.3 Abattoir findings 51
4.4 Isolation of mycobateria 53
4.5 Molecular analysis 54
4.6 The questionnaire survey 56
4.7 Retrospective case-record analysis 58
5. DISCUSSION 60
6. CONCLUSIONS AND RECOMMENDATIONS 64
7. REFERENCES 66
8. APPENDICES 82
9. CURRICULUM VITAE 92
10. SIGNED DECLARATION SHEET 95

LIST OF TABLES

Table 1. The growth requirements and cultural characteristics of pathogenic mycobacteria.....8
Table 2. The global distribution of bovine tuberculosis in animals...12
Table 3. The presence and absence of genomic regions of difference in MTBC members. 28
Table 4. The prevalence of bovine TB in different areas of Ethiopia using intradermal tuberculin test. 31
Table 5. The rate of bovine TB infection in abattoirs located in various parts of Ethiopia......32
Table 6. Mycobacteria isolated in samples collected from different parts of Ethiopia. 35
Table 7. Summary of the CIDT test result in cattle raised in and around Debre Birhan..........49
Table 8. The level of association of herd level characteristics with bovine tuberculosis.........49
Table 9. The level of association of animal characteristics with bovine tuberculosis.............50
Table 10. The distribution of tuberculous lesions in the tissues of infected animals. 52
Table 11. The level of association of sex and breeds of animals with tuberculous lesions......52
Table12. The outcome of primary culture of tuberculous tissues..53
Table 13. Summary of the result of m-PCR analysis of isolates from cattle tissues. 54
Table 14. The level of association of various factors with BTB awareness of respondents.....57
Table 15. Milk and meat consumption habit of the respondents in and around Debre Birhan.58
Table 16. TB patients on DOTS for the past five years in health institutions of Debre Birhan.59

LIST OF APPENDICES

Appendix 1. Comparative Intradermal Tuberculin Test (CIDT) data recording Format. 82

Appendix 2. Abattoir work recording format. 84

Appendix 3. Questionnaire presented to cattle owners. 85

Appendix 4. Preparation of Löwenstein-Jensen egg-based medium. 88

Appendix 5. Body condition scoring 90

LIST OF FIGURES

Figure 1. Electrophoretic separation of PCR products of m-PCR typing of the genomic DNA of mycobacteria isolated from tuberculous lesions of cattle……………………………………….55

Figure 2. The result of spoligotyping of isolates from tissue samples.………..… ……………55

ABBREVIATIONS

A	Adenine
AAU	Addis Ababa University
AFB	Acid fast bacilli
AIDS	Acquired immunodeficiency syndrome
ALIPB	Aklilu Lemma Institute of Pathobiology
AMP	Adenosine mono Phosphate
BCG	Bacillus of Calamette and Guerien
BCS	Body condition score
BTB	Bovine tuberculosis
C	Cytosine
^{0}C	Degree Celsius
CFP	Crude filtrate protein
CFT	Caudal fold test
CI	Confidence interval
CIDT	Comparative intradermal skin test
CMI	Cell mediated immunity
DDE	Dairy development enterprise
DNA	Deoxyribonucleic acid
DOTS	Direct observed treatment short course
DR	Direct repeat
DVM	Doctor of Veterinary Medicine
E	East
EDTA	Ethyl diamine tetra acetic acid
ELISA	Enzyme linked immunosorbent assay
*EMbs*1	Ethiopian *M. bovis* strain 1
ESAT	Early secretary target antigen
FAO	Food and Agriculture Organization
FPA	Fluorescence polarization assay
FVM	Faculty of Veterinary Medicine

G	Guanine
HBCs	High burden countries
HIV	Human immunodeficiency virus
iELISA	indirect enzyme linked immunosorbent assay
IFN-γ	Interfeeron-gamma
IL	Interleukin
IS	Insertion sequence
IU	International unit
MHC	Major histocompatibility complex
ml	Milli liter
MOH	Ministry of Health
m-PCR	Multiplex-Polymerase Chain Reaction
MSc	Master of Science
MTBC	*Mycobacterium tuberculosis* complex
N	North
NaOH	Sodium hydroxide
NE	Not educated
ng	Nano gram
NGL	No gross lesions
NI	No information
NVL	Non-visible lesions
OIE	Office International des Epizooties
OR	Odds ratios
PCR	Polymerase chain reaction
PGRS	polymorphic Guanine Cytosine rich sequences
PIM	phosphatidyl inositol mannoside
PPD A	pure protein derivative-Avian
PPD B	pure protein derivative-bovine
PPD	Purified protein derivative of tuberculin
RD	Regions of difference
RFLP	Restriction fragement length polymorphism

RNA	Ribonucleic acid
SIDT	single intradermal skin test
T	Thymine
TB	Tuberculosis
Th	T helper cell
TU	Tropical units
UK	United Kingdom
VNTR	Variable number tandem repeat
WHO	World Health Organization
µl	Micro liter

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ABSTRACT

A cross-sectional study to assess the epidemiology and zoonotic implication of bovine tuberculosis (BTB) was conducted on 1055 cattle in and around Debre Birhan between December 2006 and October 2007 using a comparative intradermal tuberculin (CIDT) test, abattoir surveillance, bacteriology and molecular typing. Besides, 140 livestock owners were interviewed for the evaluation of the zoonotic potential of BTB. On the basis of the CIDT test, animal and herd prevalence were 2.7% (14/524) and 9.3% (13/140), respectively, while in abattoir-based study the prevalence was 22% (117/531). Male animals were more likely (OR= 1.7; P=0.012; 95% CI: 1.12, 2.55) to exhibit tuberculous lesions as compared to female animals. The proportion of culture positivity was 32% (40/125) in tissue samples. But, because of the scarcity of molecular facilities, only nine isolates were typed in Veterinary Laboratory Agency (VLA), United Kingdom. Out of these, seven isolates were positive for the genus *Mycobacterium* of which two were identified as *M. tuberculosis.* The two *M. tuberculosis* species had the same spoligotype pattern. Awareness of cattle owners about BTB was poor (25.7%) and thus cattle owners were found to consume raw milk, soured milk product (yoghurt) and raw meat. A total of 3407 TB patients received short course therapy in health institutions found in Debre Birhan with in five years, of which 79.9% (2723/3407) were between 15 to 50 years. 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 would suggest the potential role of BTB as a zoonosis, while the isolation of *M. tuberculosis* from animal tissues warrants the existence of transmission of this agent from humans to animals. 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, CIDT test, Abattoir-based study, Epidemiology, Public health, Debre Birhan.

1. INTRODUCTION

Tuberculosis' (TB) is a clinical or pathological diagnosis that, by convention, refers to the clinical signs (or lesions) caused by infection with bacteria of the *Mycobacterium tuberculosis* complex (de la Rua-Domenech *et al.,* 2006). It is an important disease among many zoonoses (Une and Mori, 2007) that affects humans and many vertebrate animals and is characterized by the formation of granulomas in tissues and organs (Amanfu, 2006). Tuberculosis is an ancient scourge that has plagued humankind throughout known history and human prehistory (Daniel, 2006).

Tuberculosis remains a major global burden. Currently, one-third of the world population is estimated to have been infected with *Mycobacterium tuberculosis* (WHO 1998) and 8-9 million new cases of TB occur annually (Walker and Stevens, 2003). Dye *et al*. (1999) reported the actual global prevalence of *M. tuberculosis* infection to be 32%, corresponding approximately to 1.9 billion people. Very recently, it was described that more than 90 million TB patients were reported to WHO between 1980 and 2005 and the global prevalence and incidence of TB of all cases for the year 2005 was reported to be 217/100,000 population/year (14,052,000 TB patients) and 136/100,000 population/year (8,811,000 TB patients), respectively (WHO, 2007). Tuberculosis is among the top ten causes of death worldwide (Lopez *et al*., 2006). It results more adult death than any other single infectious disease and 98% of deaths occur in the developing world (Lawson *et al*., 2006). In 2005 alone it was estimated that a total of 1,577,000 (24/100,000 population/year) people died of TB worldwide. Of these, 195,000 (11%) TB patients were infected with HIV (WHO, 2007).

In Asia and sub-Saharan Africa, in 2005 there were an estimated 7.4 million new TB cases. In ranking countries by the estimated number of incident cases, 22 countries have been identified as high burden countries (HBCs). Among the top 15 countries with the highest estimated TB incidence rates, 12 are in Africa. The prevalence and incidence of TB of all cases in Africa for the year 2005 was reported to be 511/100,000 population/year (3,773,000 TB patients) and 343/100,000 population/year (2,529,000 TB patients), respectively. The high incidence rates of TB estimated for the African countries in 2005 are partly explained by the relatively high

rates of HIV coinfection. Among those TB patients, 544,000 (74/100,000 population/year) were reported dead (WHO, 2007).

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 stands 8th in the list of the world 22 high burden countries and rank 3rd in Africa (next to Nigeria and South Africa) with regard to the number of tuberculosis patients. The estimated prevalence, incidence and mortality of TB of all cases for 2005 was 546/100,000 population/year (422,529 TB patients), 344/100,000 population/year (266,288 TB patients) and 73/100,000 population/year (56,490 TB patients), respectively (WHO, 2007). Tuberculosis is the most frequent cause of hospital admission (9.4% of all cases admitted to hospital) and the leading cause of hospital deaths (accounting for 27% of all deaths) (MOH, 1997). In the year 2005, the HIV prevalence among adult TB patients was determined to be 11% (WHO, 2007).

Bovine tuberculosis (BTB) is an important disease caused by *Mycobacterium bovis*. It affects animal health, the economic value of cattle and food products derived from cattle. Moreover, this disease is transmissible to other species of animals and humans (Jolley *et al*., 2007). It is classified as a list B disease by the Office International des Epizooties (OIE) and has a potential significant impact on the international trade of animals and animal products (Wedlock *et al*., 2002). Tuberculosis, caused by *M. bovis*, is emerging as the most important disease affecting cattle (Romero *et al.,* 1999) and continues to be a problem both in countries with and without active control policies (Thom *et al*., 2006). It has been estimated that over 50 million cattle are infected worldwide, with resulting economic losses of approximately $3 billion (Hewinson, 2001).

Mycobacterium bovis is classified as a Risk 3 pathogen for public health (OIE, 2005). Its global relevance as the cause of human tuberculosis remains low compared to that of *M. tuberculosis* (Thoen *et al.,* 2006) and is responsible for 5–10% of human tuberculosis world wide (Haddad *et al.,* 2004). Bovine tuberculosis aggravates the ‘triple trouble’ of HIV/AIDS and TB infection and malnutrition (Ayele *et al*., 2004). Tuberculosis cases due to *M. bovis* in

HIV-positive persons also resemble disease caused by *M. tuberculosis*. Thus, they manifest as pulmonary disease, lymphadenopathy, or in a more profoundly immunosuppressed subjects as a disseminated disease (Cosivi *et al.*, 1998). However, there is often little awareness about the potential for zoonotic *Mycobacterium species*, such as *M. bovis*, to cause human tuberculosis and our understanding of the contribution of the bovine tubercle bacilli to the human epidemic worldwide is very limited (Cleaveland *et al.*, 2007).

The work on BTB in Ethiopia started in 1967 by FAO (Solomon, 1975). Since then several studies have been conducted to determine the rate of BTB infection in animals. These studies noted the wide presence of bovine tuberculosis in animals; cattle, sheep, goats and camels were the species reported to be infected (Solomon, 1975; Ameni and Roger, 1998; Kiros, 1998, Asseged *et al*., 2000; Nigussie, 2006; Tafesse, 2006; Kassaye, 2007). The introduction of exotic and crossbred cattle into the central highlands has created a favourable condition for the spread of BTB leaving cattle owners, and consumers of raw cattle products at risk for infection (Ameni *et al.*, 2003a). Furthermore, there are reports on the isolation and identification of *M. bovis* from clinical samples collected from human tuberculosis patients and reactor lactating cows (Kinfe and Eshetu, 1987; Kiros, 1998; Asseged *et al*., 2000; Kidane *et al*., 2002; Regassa *et al.*, 2007). Molecular typing of *M. bovis* has also indicated the existence of a unique strain in Ethiopia designated as *Embs*1 (Ethiopian *M. bovis* strain 1) that is not reported from other areas of the world (Lambert *et al.*, 2006).

In Debre Birhan and the surrounding areas little is known about the situation with animal tuberculosis. There is no information on the detection of bovine tuberculosis either on live animals or in abattoir except the work of Dejenu (1998), who determined the prevalence of BTB in cattle using the single intradermal tuberculin test (SIDT). Hence, the degree of zoonotic transmission of tuberculosis from animals to humans is unknown. Various types of cattle husbandry methods and cultural practices that could facilitate transmission of BTB between cattle and humans exist. There had been extensive cross breeding program supported by Small-Scale Dairy Development Project (SDDP). 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. Small-scale dairy production serves as

a source of milk to dairy development enterprise (DDE). In addition, there are private dairy enterprises and dairy cooperatives that buy milk and sell dairy products to the community and the passer by. These have attracted many people towards the rearing of cross breed cattle. However, the intensification in cattle rearing, keeping exotic and cross breeds and mixing up of cattle herds favours the spread of BTB between cattle. People residing in the area consume raw and locally soured milk, and raw meat. Moreover, cattle are kept in close proximity to farmers' homes and are often slaughtered in nearby abattoirs or in the backyard, where the slaughter men and butchers wear minimal protective clothing and process offal from diseased carcasses with their bare hands. These activities offer ample opportunity for zoonotic transmission of infection. Finally, a number of TB patients are admitted to Debre Birhan Zonal hospital each year (personal communication) which depicts that tuberculosis is a major human health problem in the study area.

Therefore, the present study was conducted:-

- to determine the extent of bovine tuberculosis in cattle residing in the study area.
- to identify the risk factors associated to animal tuberculosis.
- to investigate food consumption habits predisposing cattle owners to zoonotic tuberculosis.
- to isolate and identify the agent responsible for bovine tuberculosis in the area.

2. LITERATURE REVIEW

2.1 Aetiology

2.1.1 Classification

Mycobacteria belong to the genus *Mycobacterium* of the family Mycobacteriacae (Seifert, 1996). The mycobacteria are a genus of more than 80 species, within a complex of related and poorly studied organisms (Rainey *et al.*, 1995). The majorities have not been formally classified and the taxonomy remains incomplete, partly because most cannot be cultured in the laboratory (Rook *et al.*, 2007). The genus *Mycobacterium* includes obligate parasites, saprophytes and intermediate forms differing in their nutritional requirement (Tauro *et al.*, 1996). The phylogenetic analysis of the genome of pathogenic mycobacteria has shown that all (except *M. avium*) belong to a single genetic species: the *Mycobacterium tuberculosis* complex (Haddad *et al.*, 2004). The cause of bovine tuberculosis, *Mycobacterium bovis*, belongs to 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* (Haddad *et al.*, 2004; Pavlik *et al.,* 2005).

2.1.2 Morphology

The mycobacteria are thin (slender) rods of varying lengths (0.2-0.6 by 1.0-10.0 μm) (Quinn *et al.*, 1994). Most of the members are slightly curved rods, sometimes branching filamentous or mycelium type growth may occur but usually gets fragmented into rods or coccoid cells. They are acid fast, non motile and do not form endospores, conidia or capsules (Tauro *et al.*, 1996). *M. tuberculosis* is straight or slightly curved rod, whereas *M. bovis* is usually straighter, stouter and shorter (Gupte, 2006).

2.1.3 Staining

Though mycobacteria are cytochemically gram positive, they often resist staining with gram stain (Biberstein and Hirsh, 1999). Thus, they cannot be classified as either gram-positive or gram-negative bacteria (Brooks *et al.*, 2004). 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). Mycobacterias' most noted staining property is their acid-fastness (Biberstein and Hirsh, 1999) that depends on the integrity of the waxy envelope and is related to the resistance of mycobacteria to the decolorizing effect of 95% ethyl alcohol containing 3% hydrochloric acid unlike other bacteria which quickly get decoulorized. Usually the Ziehl-Neelsen technique of staining is employed for identification of acid-fast bacteria including mycobacteria (Brooks *et al.*, 2004). Moreover, mycobacteria can also be stained with fluorescent dyes like auramine rhodamine (Biberstein and Hirsh, 1999).

2.1.4 Structure and composition

Mycobacteria are rich in lipids (mycolic acids, waxes and phosphatides) and are also composed of proteins and polysaccharides. The lipids are largely bound to proteins and polysaccharides (Brooks *et al.*, 2004). The cell wall of mycobacteria contains N-glycol muramic acid in place of N-acetyl muramic acid and has a high lipid content (60%) (Sharma and Adahlaka, 1996). Lipids account for acid-fastness and pathogenic and immunogenic properties. Various structural components that are found in mycobacteria includes surface mycosides (mostly glycolipids and peptidoglycolipids), mycolic acids and their esters, wax D, cord factor (dimycolil trehalose), sulfolipids (or sulfatides), phosphatidyl inositol mannoside (PIM), mycobactins, carotenoid pigments (in chromogens) and tuberculins (peptides liberated into culture media during growth) (Biberstein and Hirsh, 1999). 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 >99.95% identical to that of *M. tuberculosis*, but deletion of genetic information has led to a reduced genome size (Garnier *et al.,* 2003).

2.1.5 Growth and cultural characteristics

Examination of bacteriological culture provides definitive diagnosis of tuberculosis. However, the usual microbiological techniques of plating clinical material on selective or differential media and sub-culturing to obtain pure cultures cannot be applied to tuberculosis bacteriology. For that reason, many different media have been devised for cultivating tubercle bacilli and three main groups can be identified, viz egg-based media, agar-based media and liquid media (WHO, 1998). In addition, tubercle bacilli may also be grown on chick embryos and in tissue culture (Gupte, 2006). 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 (WHO, 1998). To date, the most frequently used media for isolation of *M. bovis* are Löwenstein-Jensen (L-J) and Ogawa-medium (both containing eggs phosphate, magnesium) and the former contains asparagine (Seifert, 1996).

The media are prepared as solid slants in screw capped bottles. *M. tuberculosis* and *M. avium* prefer the caps on the culture media to be loose while *M. bovis* grows best in airtight containers (Quinn *et al.*, 1994). 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.*, 1994). 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	*M. tuberculosis*	*M. bovis*	*M. avium* complex	*M. avium paratuberculosis*
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 coloured 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.* (1999).

2.2 Pathology

2.2.1 Virulence

The mycobacteria do not produce the classic exotoxins and endotoxins (Sharma and Adlakha, 1996). Most of the virulence factors of *M. bovis* are the same as those of *M. tuberculosis*, as both organisms can cause identical clinical disease in humans and are genetically very similar (Collins, 2001). 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 tuberculoproteins induce a delayed hypersensitivity reaction detected in the tuberculin test (Quinn *et al.*, 1994).

2.2.2 Routes of infection

The possible routes of infection with *M. bovis* are respiratory, alimentary, congenital, cutaneous, and venereal routes. Furthermore, infection can occur via the teat canal (Seifert, 1996). However, infection in animals is usually via the respiratory and intestinal tracts (Quinn *et al.*, 1994). On animals of the intensive dairy production systems, the infection is acquired aerogenously by the inhalation of infected droplets from a coughing or sneezing animal with open tuberculosis or infected dust particles from the floor of the paddock or cowshed; the primary focus of infection is localized in the lung. For animals in the extensive production system, the infection is acquired through the oral route; in this case, bacteria excreted with faeces, urine, milk, lochia (from cows with endometritis i.e. as high as 5%) and mycobacteria-laiden exudates from the lung are ingested with contaminated feed and water. The primary focus with this route of infection is in the lymph nodes of the intestinal system (Seifert, 1996).

2.2.3 Pathogenesis and lesion distribution

Mycobacterium bovis is an intracellular pathogen of macrophages and other cells of the monocytic type (de la Rua-Domenech *et al.,* 2006). In previously unexposed animals, following infection local multiplication of the mycobacteria occurs and the resistance to phagocytic killing allows continued intracellular and extra cellular multiplication. Before cell mediated immunity is established lymphatic spread occurs with general dissemination. At the site of a lesion aggregation of macrophages contributes to the formation of tubercle, and a fibrous layer may encompass the lesion. Caseous necrosis occurs at the centre of the lesion and this may proceed to calcification or liquefaction. After cell mediated immunity (CMI) is established spread occurs by contiguous extension or via the erosion of bronchi, blood vessels or viscera to new areas (Quinn *et al.*, 1994). The characteristic gross lesion seen in an animal infected with BTB is the presence of “tubercles” (a tubercle is a white nodule usually 1 mm to 2 cm in diameter) within the body. Tubercles commonly occur in the thoracic cavity (chest), though they may be found in other major organs such as the liver. In cattle, BTB most

commonly will cause lesions in the lymph nodes of an infected animal. Therefore, during necropsy, the lymph nodes, especially those associated with the head, thorax and abdomen are closely examined. It should be remembered that an animal may be infected with BTB despite the absence of tubercles (Patterson and Grooms, 2000).

2.3 Immunology

Immunity against mycobacteria is multifactorial and dependent on the balance between an inflammatory response that allows the host to develop a granuloma, which contains the microorganism and an anti-inflammatory response that restricts the extent of the granuloma and allows contact of effector T cells with the infected cells, which results in the killing of the infecting pathogen (Villarreal-Ramos *et al*., 2003). Immunity to mycobacteria is characterized by a CMI response where infected macrophages become surrounded by a zone of lymphocytes to form a granuloma (Wedlock *et al.*, 2002). In cattle, both humoral and cell-mediated responses can be induced following *M. bovis* infection. Although the importance of the latter in the pathogenesis of the illness is very clear, the role played by antibodies in immunity still remains unclear (Silva, 2001). The immune response to *M. bovis* is a T helper type-1 response as evidenced by IFN-γ, interleukin (IL)-12, and tumour necrosis factor (TNF)-α production to pathogen-associated antigen (Flynn, 2004). All of the main T-cell subsets (γδ T-cells, CD4 and CD8 αβ T-cells) have been shown to be involved in the anti-mycobacterial immune response in cattle (Pollock *et al.*, 2005). The humoral immune response to *M. bovis* infection in cattle is characterized by highly heterogeneous antigen recognition. Six proteins, ESAT-6, 14-kDa protein, MPT63, MPT70, MPT51, and MPT32, were identified as major sero-reactive antigens in bovine tuberculosis (Lyashchenko *et al.,* 1998).

2.4 Epidemiology

Tuberculosis caused by *M. bovis,* is present in animals in most developing countries. However, many epidemiologic and public health aspects of infection remain largely unknown (Cosivi *et al.*, 1998). Similarly, the epidemiology and public health significance of bovine TB in Africa remains largely unknown (Ayele *et al.*, 2004).

2.4.1 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).

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 Belgium the prevalence of BTB caused by *M. bovis* is very low (<0.02% infected herds) (Walravens *et al*., 2002). On the contrary, in Brazil, it is an important disease in various regions of the country, especially in those where the dairy farms are numerous. It has been estimated that approximately 10% of the Brazilian herd of 140 million cattle may be infected. Moreover, it was reported that 12.7% (n=1,632) animals from 13 dairy farms gave a positive reaction with the intradermal tuberculin test in Brazil (Lilenbaum *et al*., 1999). In México from 1,201 carcasses of dairy animals inspected at slaughter, 17% presented gross TB lesions and 79% of the lesions (n=102) were positive to isolation of *M. bovis* (Milián *et al*., 2000).

Bovine tuberculosis is widespread throughout Africa (Cleaveland *et al*., 2007). There are several studies which showed its importance in the continent. Using the single comparative intradermal tuberculin test the prevalence of bovine tuberculosis was found to be 0.2% (n=8190) in cattle in the Lake Victoria zone of Tanzania (Jiwa *et al*., 1997), 14.5% (n=1813) in dairy cattle in Asmara (Omer *et al*., 2001), 7.4% (n=2226) in cattle in the Monze district of Zambia (Cook *et al*., 1996) and 1.5% (n=1861) among zebu cattle in the transhumance regions of Karamoja and Nakasongola district of Uganda (Oloya *et al*., 2006). However, 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	**Number of countries reported**			
	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%)**

Americas: North and south America and the Caribbean Islands
Source: Adapted from Amanfu (2006)

2.4.2 Host range

Mycobacterium bovis has a broad host range, the largest of any member of the *Mycobacterium tuberculosis* complex (Biet *et al*., 2005). All terrestrial mammals are susceptible to *M. bovis* infection to a variable degree, as determined by the exposure level, innate resistance, predominant immunological pathways and type of husbandry (Buick, 2006). The host range includes free ranging wildlife, captive wildlife, domestic livestock, non-human primates and humans (Biet *et al*., 2005). Susceptible domestic species include cattle, goats, cats, dogs, pigs,

sheep, equine, camel and llama (O'Reilly and Daborn, 1995; Jahans and Worth, 2006). 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). In addition to domestic animals, bovine tuberculosis was diagnosed in free-ranging wildlife (de Lisle *et al.*, 2002; Pavlik *et al.*, 2005; Delahay *et al.*, 2007) and in animals that were found in zoological gardens (Aranaz *et al.*, 2004) in different parts of the world. In Africa, bovines, wild ruminants and carnivores are also affected and are the natural reservoirs of the infectious agent in the wild (Ayele *et al.*, 2004). In South Africa *M. bovis* infection has been confirmed in several wildlife species (Michel *et al.*, 2006).

2.4.3 Sources of infection and mode of transmission

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). *M. bovis* is usually excreted in respiratory discharges, faeces, urine, semen (Quinn *et al.*, 1994), genital discharges (lochia in cows with tuberculous endometritis) and milk from infected mammary glands (Biberstein and Hirsh, 1999).

Natural transmission of *M. bovis* can occur between domestic and wild animals of the same or different species, from animals to humans and, more rarely, from humans to animals or between humans (Collins, 2000). *M. bovis* infection in cattle can be transmitted by a number of routes and transmission of infection is possible both in the field and indoors (Phillips *et al.*, 2003). The possible routes of infection include the respiratory, alimentary, congenital, cutaneous, venereal, percutaneous routes and via the teat canal. 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 (Biberstein and Hirsh, 1999).

Inhalation is the most probable and principal route to bovine infection and is facilitated by close, prolonged contact between infected and healthy animals. 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, auction stations, market places, during transportation, during concentration under trees or other shaded areas and salt supplementing sites favouring 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).

Tubercle bacilli shed in the semen can be a potential hazard for unlimited number of cows through artificial insemination. Many antibiotics included in semen extenders and cryoprotectant media are less effective against *M. tuberculosis* complex, because of drug resistance. This makes infected semen a potential source of infection even in the form of cryopreserved germ plasm through artificial insemination (Niyaz Ahmed *et al*., 1999).

In wild animals tuberculosis is typically a disease of captivity and domestication. Game farms, animal parks, and zoos remain foci of *M. bovis* in technically advanced countries (Biberstein and Hirsh, 1999). Wild animal TB represents a permanent reservoir of infection and poses a serious threat to control and elimination programs (Cosivi *et al*., 1998). The transmission of the disease from wildlife to cattle is through direct cattle contact with wildlife in the field, their excreta, and wildlife contamination of buildings and cattle foods (Phillips *et al.*, 2003).

The detection of mixed infection with the mycoabcteria pathogenic due in humans and bovines denotes the prospect of potential transmission of these pathogens from humans to cattle (zoonosis) and vice versa (reverse zoonosis) (Prasada *et al.*, 2005). A high incidence of HIV infection in a pastoralist community could lead to many more cases of overt tuberculosis occurring in those exposed to *M. bovis.* There could, for the same reason, be more disease resulting from human-to-human transmission and there could also be more transmission back to cattle by the aerogenous route. Thus, a full cycle of transmission from cattle to human

beings and back to cattle could become established, with serious public health and economic consequences (Grange, 2001).

2.4.4 Risk factors

Risk factor assessment and identification of *M. bovis* in both humans and animals will be the first step towards adopting dependable preventive, therapeutic and control measures (Ayele *et al.*, 2004). The epidemiology of *M. bovis* and other mycobacterial species infection in animals is complex, with a dynamic interaction between ‘‘host–agent–environment’’ determinants (Ryan *et al.*, 2006).

2.4.4.1 Host Factors

Many individual animal and herd level characteristics are reported to be associated with BTB infection. Breed susceptibilities to BTB infection differ: zebu cattle are more resistant than European breeds. 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). Kazwala *et al.* (2001) identified increased skin test reactivity in older animals than yearlings and calves, in animals with exotic blood than short horn zebu, in bulls often used for drought power than the entire bulls mainly used for breeding, in lactating than not lactating cows and in males than females. 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).

2.4.4.2 Agent factors

The tubercle bacilli of various types are quite sensitive to heat and are killed at 60°C in 15 minutes. They are also susceptible to ionic detergents (Sharma and Adlakha, 1996) and are killed by sunlight, ultraviolet irradiation, and pasteurization (Biberstein and Hirsh, 1999). However, tubercle bacilli are usually resistant to drying, to most disinfectants, acids and alkalies. This resistance is due to hydrophobic lipid surface. They also remain viable for long period in the dark, putrefying material, faeces, sputum and soil (Sharma and Adlakha, 1996; Biberstein and Hirsh, 1999).

Mycobacterium bovis is an obligate pathogen and is highly susceptible to sunlight (UV radiation, but 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). It 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 65°C, and cream is heated at 85°C for 2 minutes. The organism can also survive for over 2 years in frozen carcasses (Seifert, 1996). Natural exposure to *M. bovis* or environmental mycobacteria may assist in the development of specific immunity (Foster *et al*., 2002).

2.4.4.3 Husbandry and environmental factors

Several husbandry practices and environmental factors were reported to influence the occurrence of BTB in cattle. Factors that play role in predisposing animals to infection include type of production (dairy cattle are at risk because husbandry methods allow close contact between animals at milking and when housed during winter months), suckling (calves become infected by ingesting contaminated milk), and presence of wild reservoirs of *M. bovis* infection for grazing cattle (Quinn *et al.*, 1999). The prevalence of BTB infection was higher in the intensive than pastoral production systems and in the small-scale dairy sector than in other dairy-production systems in the eastern Zone of Tanzania (Shirima *et al.*, 2003). 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. There is evidence that the transmission of the disease between cattle following movement accounts for 10–15% of

outbreaks in the British Isles and that transmission can occur across farm boundaries (Phillips *et al.*, 2003). 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).

Circumstantial evidence suggests that an adequate intake of mineral, vitamin and protein reduces the susceptibility of cattle to BTB infection. 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). The existence of a widespread intermediate host is also reported as the greatest contributor to infection in cattle (Phillips *et al.*, 2003).

2.5 Economic importance

The tuberculosis epidemic has a number of implications, for which the full effect of some might only be seen in the long term (Michel *et al.*, 2006). Bovine tuberculosis affects the individual animal or human not only at the private household's level but also, as a contagious disease, on the level of the whole livestock industry and the public health sector, as well as the national economy. It also affects international trade, as many countries ban the import of livestock from countries where it is endemic. Effects may occur also within wildlife ecosystems, with unknown indirect consequences for the whole ecosystem and for economic activities (tourism, agriculture) (Zinsstag *et al.*, 2006). The full economic implications of zoonotic TB are, however, overlooked in many developing nations where the overall impact of the disease on human health and animal production needs to be assessed (Cosivi *et al.*, 1998).

In spite of its difficult and non-specific diagnosis *M. bovis* has been declared to be one of the etiologic agents causing significant economic loss in the cattle industry (Romero *et al.,* 1999). Direct losses due to infection become evident by a decrease in productivity, a decrease in beef production, and additional processing costs for tuberculous animals and condemnation at

slaughterhouses (Dabron *et al.*, 1996). The economic losses incurred by the disease are represented by a reduction of 10 to 20 per cent in milk production and weight, in addition to infertility and condemnation of the meat. Without considering the deaths, the loss is estimated to be 10 to 25% of the productive efficiency (Lilenbaum *et al.*, 1999). Among dairy cattle, there was also a decrease in milk (10-18%) and in meat production (ranges 15%). The culling loss is estimated to be 30-50% of the difference between the value of a dairy or beef breeding cow and its value at slaughter (Dabron *et al.*, 1996). Studies done on the economics in Spain indicated that the loss in meat production was 10% in calves born from infected cows, 12% in milk production, 5% due to sterility and about 1.4% due to carcass condemnation (Bernues *et al*., 1997). In Argentina, the loss of milk in tuberculous cows was found to be 18% as a result of delay in the first lactation and a decrease in the number and duration of lactation, as compared to healthy animals (de Kantor and Ritacco, 1994).

Potential negative long-term effects on the population dynamics of certain social animal species and the direct threat for the survival of endangered species pose particular problems for wildlife conservationists. From an economic point of view, wildlife tuberculosis has resulted in national and international trade restrictions for affected species (Michel *et al.*, 2006). In Michigan, the $ 25 million lost to BTB annually is about 2% of the regions overall tourism market which result from tourists that wouldn't visit an infected area at all, would visit less frequently, modify their travel behaviour or stopped their hunting behaviour (Holecek and Bristor, 2003).

2.6 Zoonotic importance

Mycobacterium bovis and closely associated acid-fast bacilli cause disease in humans (Theon *et al.,* 2006). Nowadays, the human immunodeficiency virus (HIV) is associated with a greatly increased risk of overt disease in humans infected with *M. tuberculosis*. It is believed, this increased risk also occurs in the case of *M. bovis* infections in humans (O'Reilly and Daborn, 1995).

There are three potential routes of transmission or modes of infection to the disease caused by *M. bovis*: ingestion of contaminated dairy products (animal-to-human), inhalation of infectious droplet (animal-to human or human-to animal), and direct inoculation of the skin (animal-to-huamn) (LoBue, 2006). Close physical contact between humans and potentially infected animals is present in some communities. Therefore, agricultural workers may acquire the disease by inhaling cough spray from infected cattle (Cosivi *et al.*, 1998). Furthermore, airborne infection continues to occur among meat industry and slaughterhouse workers, in regions where the infection is still prevalent in cattle (Thoen *et al.*, 2006). Occupational exposure to infectious aerosols from tuberculous animals and their carcasses were identified as risks for *M. bovis* infection in certain segments of the UK population (de la Rua-Domenech, 2006). Infections in humans may result in asymptomatic infections, pulmonary tuberculosis, or disseminated infections. Those occupational groups working with *M. bovis* infected cattle or deer, on the farm or in the slaughterhouse, are more likely to develop pulmonary disease than alimentary disease (O'Reilly and Daborn, 1995). The symptoms of pulmonary infection can include fever, cough, chest pain, cavitation, hemoptysis, and fibrosis. Untreated infections may be fatal (OIE, 2004).

Consumption of unpasteurized milk, poorly heat-treated meat and close contact with infected animals represent the main sources of infection to human. Extra pulmonary lesions may occur associated to the consumption of infected milk (Theon *et al.,* 2006). Furthermore, vegetable contamination represents a potential source of infection to humans, as *M. bovis* survives in soil (Ayele *et al.,* 2004). Continuing on-farm consumption of unpasteurized cows' milk, retail sales by approved establishments of unpasteurized milk and dairy products were identified as risks for *M. bovis* infection in certain segments of the UK population (de la Rua-Domenech, 2006).

A third, and much less common, route of infection is by traumatic inoculation into the skin. This was once an occupational hazard to those handling contaminated meat and the local lesion was termed the 'Butcher's Wart'. In common with the analogous 'Prosector's Wart' caused by *M. tuberculosis* in pathologists and anatomists, the lesion was usually benign and self-limiting. In general, it would be very difficult to prove an association when, as is usually

the case, many years elapse between infection and development of bacteriologically positive disease (Grange, 2001).

In general, factors that contribute to acquisition of TB infection by humans are demography (income, education, age, number of families per dwelling, number of individuals per m^2 in a dwelling, sanitation), eating habits (consumption of unpasteurized milk and milk products and consumption of raw or inadequately cooked meat), occupation (abattoir workers, veterinarians, laboratory technicians, animal care takers in the zoo, workers in animal reservations and national parks), living status of families (family ownership of cattle, previous livestock ownership, history of working with animals and living with a relative who owns cattle), culture and customs (the stigma of tuberculosis) (Ayele *et al*., 2004).

Information on human disease due to *M. bovis* in developed and developing countries is scarce. *M. bovis* is thought to account for up to 10% of cases of human TB (Cosivi *et al.*, 1998). Bovine tuberculosis is now quite rare in humans living in industrialized countries, as a result of TB control in cattle, increased hygiene, pasteurization of milk, and improved husbandry practices (Ayele *et al.,* 2004). In regions where bovine TB has been largely eliminated, a few residual cases occur among elderly persons as a result of the reactivation of dormant lesions. These are fewer than 1% of all TB cases (Cosivi, *et al*., 1998). In England, Wales, and Northern Ireland, 296 cases of human *M. bovis* tuberculosis were reported from 1994 to 2004, with an average of 27 cases per year (Evans *et al*., 2007). Furthermore, *M. bovis* was the cause of 0.5% of cases of pulmonary tuberculosis in London and South England, 1% in Wales, 1.5% in Northern England and between 5% and 8.5% in rural Scotland (Grange, 2001).

In countries where bovine TB is uncontrolled, most human cases occur in young persons and result from drinking or handling contaminated milk; cervical lymphadenopathy, intestinal lesions, chronic skin TB (lupus vulgaris), and other nonpulmonary forms are particularly common (Cosivi *et al.*, 1998). In Latin America, an estimated average of 2% of cases of pulmonary, and 8% of non pulmonary, tuberculosis are caused by *M. bovis*, with the percentages being higher in regions where there is intensive dairy cattle industry (Grange,

2001). On the basis of information from Argentina, *M. bovis* is estimated to cause 2% of all human cases of tuberculosis in the Region. Slaughterhouse and dairy farms workers are most frequently infected, with infection occurring via the respiratory tract (de Kantor and Ritacco, 2006). In humans, 8.7% of the extrapulmonary samples were classified as *M. tuberculosis and M. bovis* mixed infection in India (Prasada *et al.*, 2005). Cleaveland *et al.* (2007) reported that *M. bovis* was confirmed in 10.8% (n=65) human cervical adenitis cases in Tanzania, of which only one come from a household owning infected cattle. In Ibadan, southwestern Nigeria, approximately 13% of the disease in humans was caused by strains of the RD9-deleted lineage (*M. africanum* type I and *M. bovis*) rather than *M. tuberculosis*. On spoligotyping of 60 isolates from human samples (55 sputum and 5 fine-needle cervical aspirates), 3 strains of *M. bovis* were identified (Cadmus *et al*., 2006).

2.7 Awareness of bovine tuberculosis and consumption habit

There is often little awareness about *M. bovis*, to cause human tuberculosis (Cleaveland *et al.*, 2007). For instance awareness of bovine TB is relatively low (7.3%) across the general population in Michigan, adjacent states and Ontario. On the other hand, slightly more than a quarter (26.8%) of those who travel in Michigan are aware that bovine TB is present in northeast Michigan, and have been influenced to modify their travel behaviour because of the presence of the disease (Holecek and Bristor, 2003). A study made in the Dangme-West district of Ghana has indicated that out of 30 herdsmen interviewed, 28 did not know about tuberculosis and all failed to recognize its transmission from cattle to man. Moreover, 25 herdsmen didn't boil their milk before use, and the remainders boil the milk simply because they have been told to do so. On the other hand, most cattle owners interviewed (n=15) told they knew or had heard about tuberculosis and eight of them knew its transmission from cattle to man. Despite this none of them, however, boiled milk before use (Bonsu *et al.*, 2000).

2.8 Diagnosis

Various types of diagnostic techniques have been developed for the detection of BTB. However, because of the dynamics of *M. bovis* transmission, the microscopic size of early lesions and the time it takes for an animal to mount a detectable immune response, no single ante-mortem or post-mortem test for TB can be expected, on its own, to detect every infected herd and every infected animal in such herds (de la Rua-Domenech *et al.*, 2006). The various diagnostic methods used may be well suited for one stage of the disease progression but not necessarily others. Each diagnostic method or point of surveillance offers some advantage but has associated disadvantages (Jolley *et al.,* 2007). The diagnosis of bovine tuberculosis in live animals mainly depends on clinical manifestations of the disease, skin testing, and subsequent identification of the pathogen by biochemical testing (Vitale *et al.,* 1998). After death, it is diagnosed by post-mortem examination and then confirmed in the laboratory by histopathological and bacteriological techniques (Jahans and Worth, 2006).

2 8.1 Clinical examination

Bovine tuberculosis may be diagnosed by its clinical symptoms (Seifert, 1996). Clinical signs such as chronic debilitation, moist cough, low-grade fever and enlargement of local lymph nodes may suggest infection with tuberculosis (OIE, 2004). Nevertheless, other techniques are required to ensure the diagnosis.

2.8.2 Post-mortem inspection

A tentative/presumptive diagnosis of bovine tuberculosis can be made following the macroscopic detection at necropsy of typical lesions (Corner, 1994; Liebana *et al*., 2007). Therefore, during necropsy, the lymph nodes, especially those associated with the head, thorax and abdomen are closely examined (Patterson and Grooms, 2000). 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 reactors may be due to early infection, poor necropsy technique or infection with mycobacteria other than *M. bovis* (Corner, 1994). Above all, it is not always possible to differentiate TB lesions by gross examination from those of other infections (Liebana *et al*., 2007).

2.8.3 Histopathological techniques

Another important diagnostic method for detection of tuberculosis is histopathologic examination conducted following post-mortem (Jolley *et al*., 2007). For histopathology a fixed samples (in 10 percent formalin) of lesion are required (Quinn *et al.*, 1994) 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.*, 1999). The presumptive diagnosis of mycobacteriosis can be made if the tissue has characteristic histological lesions such as caseous necrosis, mineralization, epitheloid cells, multinucleated giant cells and macrophages. The presence of acid-fast organisms in histological sections may not be detected (OIE, 2004).

Histopathological examination requires considerable technical skill, an equipped histopathology laboratory and substantial time to perform. For these reasons, this method is not well suited to large volume surveillance testing and is limited in that definitive identification of *M. bovis* is not possible. Consequently, animals and herds may be identified incorrectly as infected with *M. bovis* if other tests are not used for diagnostic confirmation. This false diagnostic result could lead to economic losses for producers (Jolley *et al*., 2007).

2.8.4 Immunodiagnosis

2.8.4.1 Skin test

The skin tests are the international standard for ante-mortem diagnosis of bovine TB in cattle herds and individual animals. They are based on eliciting a delayed-type hypersensitivity

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). It provides a measure of CMI dependent delayed-type hypersensitivity (DTH) reaction in response to tuberculin (Pollock *et al.*, 2006). Tuberculin test may be performed using bovine tuberculin (PPD-B) alone or as a comparative test (OIE, 2004). 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 delayed type hypersensitivity (DTH) response to purified protein derivative (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). Tuberculin skin testing lacks sensitivity, can be confounded by exposure to non tuberculous mycobacteria, 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) the reliability of the test may be questioned and confirmatory tests may be required. The tuberculin PPDs are complex antigens and the presence of cross-reactive antigens between other mycobacterial species has driven research to identify *M. bovis*-specific epitopes (Pollock *et al.*, 2006).

2.8.4.2 Blood-based laboratory tests

A number of new diagnostic blood tests have become available. However, they are usually used as ancillary tests to confirm or negate the results of the intradermal test. Of this tests, the lymphocyte proliferation assay and the gamma-interferon assay correspond to cellular immunity, while the enzyme linked immunosorbent assay (ELISA) corresponds to humoral immunity (OIE, 2004). Serological assays provide an important and needed tool for large

volume testing for exposure to *M. bovis*. They offer the important advantages of ease of use, assay speed and relatively low cost (Jolley *et al*., 2007).

To overcome difficulties associated tuberculin skin-testing, an effective whole blood cellular immunoassay for bovine gamma interferon (IFN-γ) has been developed. The test is commonly used in conjunction with tuberculin skin testing as a confirmatory test following a positive response to the caudal fold test (CFT) (Palmer *et al*., 2006). IFN-γ diagnostic test is a rapid whole blood assay. The assay is based on the release of gamma-interferon from sensitized lymphocytes during an overnight incubation with a specific antigen. The detection of plasma gamma-interferon is carried out by means of a sandwich ELISA using specific monoclonal antibodies (Walravens *et al*., 2002). This test in its current form will not generally be acceptable as a direct substitution for skin testing, but could be applied rather as an ancillary test. Benefits of this test include accelerated elimination of tuberculosis from infected herds and the possibility of the test to be performed as soon as 10 days after the application of a tuberculin skin test. Other applications of the IFN- γ test include confirmation of the immunological status of skin test reactors and the investigation of fraudulent intervention into the skin test (Vordermeier *et al*., 2001). Combinations of specific antigens/microbial proteins such as CFP-10, ESAT-6, TB27.4, TB16.2, TB15.8, and TB10.4 induce strong gamma interferon responses in cattle and have potential as diagnostic reagents which could be used in a whole blood assay for diagnosis of tuberculosis (Aagaard *et al*., 2003).

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 (Fra´guas *et al*., 2006). A study demonstrated that iELISA (indirect enzyme linked immunosorbent assay) using rM70-83-E6 antigen is simple, sensitive and easy to perform and can be used for the analysis of a large number of samples for serodiagnosis of bovine tuberculosis (Liu *et al.*, 2007). However, due to its low sensitivity ELISA should not be considered as a reliable confirmatory test for bovine tuberculosis diagnosis at the slaughter (Fra´guas *et al*., 2006).

2.8.5 Isolation and identification of mycobacteria

The presence of *M. bovis* in clinical samples and post-mortem specimens may be demonstrated by examination of stained smears and confirmed by cultivation of the organism on primary isolation medium (OIE, 2004). Recently, molecular genetic methods have been introduced in studies of the epidemiology of tuberculosis (Szewzyk *et al.*, 1995). These tools can help to determine the origin of outbreaks, to understand the link between different outbreaks, to show the relationships between domestic and wild TB, and identify the source of human infection (Haddad *et al.*, 2004).

In the genome of mycobacteria polymorphism is limited compared to its genome size. But some regions are highly polymorphic, either by a variation in number and/or position or by a variation in primary structure. These areas of higher polymorphism appear to correspond essentially to segments of genes encoding proteins where variability provides a selective advantage to the bacteria, e.g., antibiotic resistance, antigens involved in escaping the immune response, or to non-coding sequences probably involved in inducing variability of neighbour genetic areas, such as insertion sequences or repeated sequences. These are the basis for molecular typing techniques (Haddad *et al.*, 2004). The various molecular techniques used in tuberculosis bacteriology includes polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) using various genetic markers and spoligotyping.

2.8.5.1 Polymerase chain reaction (PCR)

DNA amplification by PCR provides a rapid and sensitive method for the detection of *M. tuberculosis* complex (Clarridge *et al.*, 1993). The development of any species-specific PCR assay requires that the target gene or DNA fragment be present in all the isolates from the particular species of interest and be absent from all other unrelated species (Bakshi *et al.,* 2005). The PCR technique is much faster than culture, provides detection of *M. bovis* when rapidly growing *Mycobacterium species* are present in the sample and may be able to detect the presence of *M. bovis* in samples even when organisms have become nonviable. It could be used as a rapid screening technique which would be complementary to culture of tissue

specimens for the routine diagnosis of bovine tuberculosis (Liebana *et al.*, 2007). Generally, a sample found to be positive by either culture or PCR is regarded as a true positive. However, a negative result by either method means that *M. bovis* was not detected in the sample of tissue that was tested. Other samples of tissue from the same animal could harbour *M. bovis*. Therefore, a negative test result from PCR does not provide conclusive proof that an animal is not infected with *M. bovis*, especially if the animal originated from an infected or suspect herd. PCR requires sophisticated laboratory equipment, facilities, and advanced technical skills (Jolley *et al.,* 2007).

A conventional PCR generally only uses one set of primers (Jahans and Worth, 2006). 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). PCR amplification of genomes such as 16S rRNA (Wilton and Cousins, 1992), mtp40 gene (Parra *et al*., 1991), mpb70 gene (Radford *et al.,* 1988), pncA gene (Shah *et al*., 2002), the oxyR gene (Espinosa de los Monteros *et al*., 1998) and the regions of difference (RD) (Haddad *et al.,* 2004) were used to differentiate the genus *Mycobacterium* or, *M. tuberculosis* from *M. bovis* isolates. The genomic regions of difference that are either present or absent in different members of the *Mycobacterium tuberculosis* complex are presented in (Table 3).

Multiplex Polymerase chain reaction (m-PCR) is used to test acid-fast organisms which are neither recognized by spoligotyping nor show growth characteristics typical to *M. bovis* (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).

Table 3. The presence and absence of genomic regions of difference in MTBC members.

No.	RD locus	*M. tuberculosis*	*M. canettii*	*M. africanum*	*M. microti*	*M. pinnipedii*	*M. caprae*	*M. bovis*
1	TbD1	Present/absent	absent	absent	absent	absent	absent	absent
2	RD9	present	present	absent	absent	absent	absent	absent
3	RD10	present	absent	absent	absent	absent	absent	absent
4	$RD1^{Mic}$			present	absent	present		
5	RD12	present	absent	present	present	present	absent	absent
6	RD4	present	present	present	present	present	present	absent
7	RD1	Present	present	present	present	present	present	present
8	$RD2^{Seal}$			absent	absent	absent		
9	RD^{Can}	absent	present	absent	absent	absent	absent	absent

Source: Adapted from Hewinson *et al.* (2006) and Warren *et al.* (2006).

2.8.5.2 Spoligotyping

Spoligotyping (''spacer oligotyping'' or ''reverse line blot hybridization technique'' method (Zuma´rraga *et al*., 1999) is the principal molecular typing method that is based on DNA polymorphism present at one particular chromosome locus, the 'Direct Repeat' region, which is unique to the *Mycobacterium tuberculosis* complex bacteria of which *M. bovis* is a member (Jahans and Worth, 2006). It relies on determination of the presence or absence of 43 spacer DNA sequences in the in vitro-amplified DNA by hybridization to multiple synthetic spacer oligonucleotides covalently bound to a filter. A study made in order to compare the discriminative power showed differentiation of *M. bovis* by spoligotyping was less discriminatory than differentiation by Restriction Fragment Length Polymorphism (RFLP) analysis with the DR (Direct Repeat) and Polymorphic Guanine Cytosine Rich sequence (PGRS) probes, however spoligotyping is easier to perform and its results are easier to interpret (Kamerbeek *et al*., 1997; Zuma´rraga *et al*., 1999).

2.9 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 many countries, the test-and-slaughter policies based on tuberculin skin testing have made a significant impact on the control of bovine tuberculosis (caused by infection with *M. bovis*) (Pollock *et al.*, 2006). Alternatively, vaccination of domestic livestock or wildlife (Wedlock *et al.*, 2002) and wildlife population control help for control of infection (Ryan *et al.,* 2006). 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).

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 (FSAI, 2003) 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).

2.10 Bovine tuberculosis in Ethiopia

2.10.1 Magnitude and distribution

Ethiopia is one of the African countries where tuberculosis is wide spread in both humans and cattle and the endemic nature of tuberculosis in humans and cattle has long been documented (Shitaye *et al.*, 2007). There are reports on the occurrence of the disease in camels (Kassaye, 2007), sheep (Nigussie, 2006), goats (Nigussie, 2006; Tafesse, 2006) and pigs (Shitaye *et al.*, 2006), an evidence to the spillover of infection to hosts other than the natural host. Table 4 and 5 summarizes the results of studies on BTB made in various parts of Ethiopia.

An enormous number of studies have been conducted using the intradermal tuberculin test and abattoir surveillance to determine the extent of bovine tuberculosis in different parts of Ethiopia. A prevalence rate as high as 50% (n=486) has been reported in dairy cattle in eastern Shoa (Ameni and Roger, 1998) and 38.5% (n=441) in goats in Adami Tulu district (Tafesse, 2006) using the SIDT test. Even, studies made using the recommended CIDT test showed a higher individual animal prevalence of BTB. Ameni *et al.* (2003b) reported a prevalence rate of 46.8% (n=1171) in cattle from 12 selected dairy farms of Ethiopia. The study showed that except one private farm with small number of animals the rest 11 dairy farms contained the infection (i.e. a herd prevalence of 91.7%) ranging from 10.81% (n=37) in Tegen farm (Sebeta) to 87.1% (n=140) in Debre Zeit state farm. Abattoir inspection of carcasses (the gold standard method) also showed the widespread presence of bovine tuberculosis in Ethiopia. A prevalence rate as high as 44.27% (n=1441) in cattle slaughtered at Bahirdar municipality abattoir was recorded (Negash, 2006). In addition, bovine tuberculosis was reported in 4.34% (n=1,152) of goats and 2.86% (n=384) of sheep slaughtered at Helmix abattoir, in Debre Zeit (Nigussie, 2006), and 5.07% (n=276) of camels slaughtered at Dire Dawa municipality abattoir (Kassaye, 2007). Moreover, a ten year (1996-2005) retrospective analysis of inspection of 2,455,289 slaughtered animals at Addis Ababa municipality abattoir showed that 0.052% (n=1,336,266) of cattle, 0.001% (n=534,436) of sheep, 0.001% (n=573,767) of goats and 0.009% (n=10,820) of pigs were found harbouring tuberculous lesions in parenchymatous organs (Shitaye, *et al*., 2006).

Table 4. The prevalence of bovine TB in different areas of Ethiopia using intradermal tuberculin test.

No.	Location	Test type	Species	Sample size	Positive	Source
1	Eastern Shoa	SIDT	Cattle	486	243(50%)	Ameni and Roger, 1998
2	N. Shoa & S.Wollo	SIDT	Cattle	538	132(24.5%)	Dejenu, 1998
3	Eastern shoa	CIDT	Cattle	788	234(29.7%)	Kiros, 1998
4	Wolaita Soddo	SIDT	Cattle	416	149(35.8%)	Regassa, 1999
5	Addis Ababa	CIDT	Cattle	1,241	128(10.31)	Asseged *et al*., 2000
6	Fitche town	SIDT	Cattle	735	31(4.25%)	Belay, 2000
7	Dairy Farms of Eth.	CIDT	Cattle	1,171	548(46.8%)	Ameni *et al*., 2003b
8	Wuchale Jidda	CIDT	Cattle	763	60(7.9%)	Ameni *et al*., 2003a
9	Assela	CIDT	Cattle	514	18(3.5%)	Redi, 2003
10	Bodi District	CIDT	Cattle	320	12(3.8%)	Laval and Ameni, 2004
11	Dire Dawa	CIDT	Cattle	301	93(31%)	Adugna, 2005
12	Adama	CIDT	Cattle	524	59(11.3%)	Aklilu, 2005
13	Addis Ababa	CIDT	Cattle	1,071	197(18.4%)	Kebede, 2005
14	North Gonder	CIDT	Cattle	1,025	215(21%)	Ali, 2006a
15	Holeta	CIDT	Cattle	705	41(5.82%)	Dabese, 2006
16	Sululta	CIDT	Cattle	786	73 (9.29%)	Firdessa, 2006
17	Addis Ababa	CIDT	Cattle	798	246(32.2%)	Hussien, 2006a
18	Holeta DF	CIDT	Cattle	243	54(22%)	Lambert *et al*., 2006
19	Addis Ababa	CIDT	Cattle	2,098	392(19%)	Shitaye *et al*., 2006
20	ATRAC	SIDT	Goats	191	50(26.18%)	Tafesse, 2006
21	Adami Tulu	SIDT	Goats	441	170(38.5%)	Tafesse, 2006
22	North West Shoa	CIDT	Cattle	1,041	259(24.9%)	Regassa *et al.,* 2007

ATRAC: Adami Tulu Agricultural Research Center; DF: Dairy farm; Eth.: Ethiopia.

N. Shoa: North Shoa: S. Wollo: South Wollo

Table 5. The rate of bovine TB infection in abattoirs located in various parts of Ethiopia.

No.	Location	Species	Sample Size	Infected	Source
1	Nazereth	Cattle	1,125	58(5.15%)	Ameni and Wudie, 2003
2	Addis Ababa	Cattle	1,350	21(1.5%)	Asseged *et al.,* 2004
3	Hossana	Cattle	751	34(4.5%)	Tekelu *et al.*, 2004
4	Modjo	Goats	1,536	65(4.2%)	Hiko, 2005
5	Awassa	Cattle	469	11(2.34%)	Jemale, 2005
6	Nazereth	Cattle	1,601	112(7.0%)	Molla, 2005
7	Gonder	Cattle	402	64(15.9%)	Worku, 2005
8	Woldiya	Cattle	605	33(5.5%)	Ali, 2006b
9	Adama	Cattle	1,018	48(4.7%)	Anebo, 2006
10	Dessie	Cattle	805	28(3.4%)	Ayanaw, 2006
11	Butajira	Cattle	321	40(12.5%)	Hussien, 2006b
12	Ghimbi	Cattle	420	60(14.3%)	Mulu, 2006
13	Bahirdar	Cattle	1,441	638(44.27%)	Negash, 2006
14	HEA, Debre Zeit	Goats	1,152	50(4.34%)	Nigussie, 2006
15	HEA, Debre Zeit	Sheep	384	11(2.86%)	Nigussie, 2006
16	Addis Ababa	Cattle	984	34(3.5%)	Shitaye *et al.*, 2006
17	Jinka	Cattle	337	52(15.4%)	Taddle, 2006
18	Yabello	Cattle	433	18(4.2%)	Adane, 2007
19	Woldiya	Cattle	500	30(6.0%)	Hassen, 2007
20	Dire Dawa	Camel	276	14(5.07%)	Kassaye, 2007
21	Adama	Cattle	522	129(24.7%)	Mamo, 2007
22	Butajira	Cattle	353	74(20.96%)	Mekonnen, 2007
23	Jinka	Cattle	317	47(14.8%)	Milkessa, 2007
24	Addis Ababa	Cattle	600	92(15.36%)	Seid, 2007
25	North Gonder	Cattle	350	54(15.4%)	Terefe, 2007
26	Ghimbi	Cattle	758	116(15.3%)	Woyessa, 2007

HEA: Helmix abattoir

2.10.2 Economic importance

In Ethiopia, there are few reports on the economic loss associated with BTB. Even the existing reports describe only losses associated to condemnation of carcasses. The condemnation rate of carcasses and organs due tuberculosis infection in cattle slaughtered in Nazareth municipality abattoir was showed to be increasing from 0.38% to 1.9% from 1999 to 2003 (Ameni and Wudie, 2003). In addition, Asseged *et al.* (2004) reported that the annual rate of whole-carcass condemnation due to generalized tuberculosis in Addis Ababa abattoir was 0.024% and it has increased annually by 0.34% over the past decade.

2.10.3 Isolation and identification of the agent

There are several reports indicating the isolation of the human and bovine tubercle bacilli from clinical and tissue samples (in animals only) obtained from humans and animals in different parts of Ethiopia. Isolation of the agents was made through culture on the Lowenstein-Jensen media and confirmed using acid-fast staining, colony morphology, biochemical tests, PCR and even by spoligotyping. Table 6 summarizes the results of isolation and identification of *M. bovis*, *M. tuberculosis* and other mycobacteria in Ethiopia. Moreover, Ameni *et al.* (2007) 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 1100000101111110111111100010000000000100000, 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 *EMbs*1 (Ethiopian *M. bovis* strain 1). The variable tandem repeat (VNTR) profile of the strain was 5254*33.1, which differed from the VNTR profile of strains reported from the UK.

2.10.4 Awareness and habit of people

Several studies were conducted in Ethiopia to investigate the knowledge of residents on zoonotic tuberculosis. Ameni *et al.* (2003a) reported that 38.3% (n=94) of the respondents knew that cattle can have tuberculosis, and 30.8% (n=94) recognized BTB is zoonotic.

Regarding the habit of milk and meat consumption, 52.1%, 9% and 90% of heads of the households consume raw milk, raw meat and mixed (cooked and raw) meat, respectively in Wuchale-Jida District, North Ethiopia. The knowledge of residents on BTB was found to be 46.2% (n=173) in Butajira. Moreover, it was reported that 39.3% and 21% knew BTB is zoonotic and meat is a vehicle of transmission, respectively. However, 34.15% of the respondents consume raw milk while 53.8% and 12.1% consume both boiled and fresh milk and soured milk, respectively (Mekonnen, 2007). These evidences demonstrate the endemicity of the disease in Ethiopia and depicted that the public is at high risk of contracting the disease due to the habit of consuming unpasteurized/unboiled dairy products and raw meat.

Table 6. Mycobacteria isolated in samples collected from different parts of Ethiopia.

					Samples found positive								
							Biochemical test			PCR			
No.	**Area**	**Species**	**Sample type**	**Quantity**	**Culture**	**AFB**	***M. bovis***	***M. tuberculosis***	**Others**	***M. bovis***	***M. tuberculosis***	**Others**	**Source**
1	South Ethiopia	Human	FNA	40	-	-	-	-	-	6	29		Kidane *et al.*, 2002
2	DFE	Cattle	Milk	30	4	-	-	-	-				Ameni *et al.*, 2003b
3	Nazareth	Cattle	Tissue	30	7	7	7	-	-	-	-	-	Ameni and Wudie, 2003
4	Gonder	Cattle	Tissue	64	10	4	-	-	-	-	-	-	Worku, 2005
5	Holeta	Cattle	Milk	23	9	9	-	-	-	-	-	-	Lambert *et al.*, 2006
		Cattle	Nasal	19	7	7	-	-	-	-	-	-	
6	Gonder	Human	Sputum	55	26	-	1	23	2	-	-	-	Ali, 2006a
		Human	FNA	13	2	-	1	-	1	-	-	-	
		Human	PF	7	1	-	1	-	-	-	-	-	
		Cattle	Milk	99	7	-	4	1	2	-	-	-	
7	Woldiya	Cattle	Tissue	41	8	-	-	-	-	1	2	-	Hassen, 2007
8	Dire Dawa	Camel	Tissue	24	5	4	-	-	-	-	-	1	Kassaye, 2007
9	Adama	Cattle	Tissue	70	20	9	-	-	-	3	2	-	Mammo, 2007
10	Butajira	Cattle	Tissue	74	10	10	-		-	1	-	3	Mekonnen, 2007
11	Central	Human	Sputum	36	-	-	4	29	3				Regassa *et al.,* 2007
	Ethiopia	Human	FNA	6	-	-	3	2	1				
		Cattle	Milk	11	-	-	2	5	4				
12	Addis Ababa	Cattle	Tissue	54	-	12	-	-	-	12	-	-	Seid, 2007
13	Gonder	Cattle	Tissue	54	14	-	-	-	-	-	-	-	Terefe, 2007
14	Ghimbi	Cattle	Tissue	116	17	17	-	-	-	2	1	-	Woyessa, 2007

DFE: Dairy farms of Ethiopia; FNA: Fine needle aspiration; PF: Peritonial fluid

3. MATERIALS AND METHODS

3.1 Study area

This study was conducted in Debre Birhan town and the surrounding Basonawerana district. Debre Birhan is located in the central highlands of Ethiopia, 130 km northeast of Addis Ababa, at an altitude of 2780 m above sea level and at 9°36'N and 39°38'E. The town has got its own urban administration and is subdivided in to 9 kebeles (comprising 9 urban and rural kebeles) and the area surrounding Debre Birhan is under the administration of Basonawerana ditrct and is subdivided in to 29 Peasant Associations.

The climate of the study area is characterized by a biannual rainfall, a long dry season, and relatively cool temperature. The mean annual rainfall recorded is about 950 ml and is obtained from July to September (72%) and from March to April (28%) but the occurrence of this rain is variable. The long dry season extends from October to February. The average monthly minimum temperature ranges from 2.5^{o}C in November to 8.4^{o}C in July, whereas the average monthly maximum temperature ranges from 17.6^{o}C in August to 22.5^{o}C in June. The mean relative humidity recorded from 1985 to 1995 was 68.2% (Rege, *et al.*, 1996).

In the study area out of 135,020 agricultural population (i.e. at least one member of the household is involved in agricultural activities), 5,897(4.37%) are involved in crop production, 3,685(2.73%) in livestock, 24,854(18.40%) in mixed crop and livestock production and 100,584(74.50%) being non holders (CACC, 2003a). The livestock population of the study area was estimated to be 92,241 cattle, 6,317 horses, 26,048 asses, 486 mules, 123,993 sheep, 42,090 goats and 814,892 poultry. Out of 92,241 cattle, 2,984(3.24%) are found in urban area where as the remainder live in rural areas. Of those cattle that are found in urban area, 849(28.45%), 41(1.37%) and 2,080(69.7%) are indigenous breeds, hybrids and exotic breeds, respectively. On the other hand, among 89,257 cattle that are found in rural areas 77,593(87%) and 11,423(13%) are indigenous breeds and hybrids, respectively (CACC, 2003b).

In the study area the existing farming system is a mixed crop livestock farming system. The agriculture is cereal-based and entirely depends on oxen draught power to till the land. The major animal species kept include cattle, sheep, horses and donkeys. Cattle usually provide draught power for cultivation and milk for home use and sale. In addition, cattle are slaughtered during public and religious holidays, for funerals or other ceremonies and as emergency in case of accidents. Besides, cattle provide manure for fuel and fertilization and cattle ownership is a sign of wealth.

The abattoir work was conducted at Debre Birhan municipality abattoir. Usually cattle and very recently small ruminants are slaughtered each day with the exception of Tuesday and Thursday. On an average around 15 animals are slaughtered per day, many being slaughtered on the eve of the market day Saturday and the Christian religion holydays. Though the abattoir has two slaughter halls, only one of these halls is used to slaughter animals. All the procedures including stunning, skinning, evisceration, removal of rumen contents, washing of offals etc. are usually performed in one hall which favours contamination of carcasses.

With regard to the laboratory work, isolation of the agent was done at Addis Ababa University, Aklilu Lemma Institute of Pathobiology (ALIPB) in Immunology section that is also involved in Mycobacteriology. In cultivating mycobacteria we processed not more than 20 samples per day. Molecular typing of the isolates was made at Veterinary Laboratory Agency (VLA), UK.

3.2 Study subjects

This study was made on live and slaughtered animals and on people dwelling in the area. Comparative intradermal tuberculin test and abattoir-based surveillance was conducted on 524 and 531 cattle, respectively. Among those animals tested, 331 (63.2%) were from Basonawerana district and the remaining 193 (36.8%) were from Debre Birhan town. Moreover, the heads of 140 households whose animals were tested or their family members were interviewed. Of those members of 140 cattle owning family interviewed, 58 (41.4%) were females and 82 (58.6%) were males.

3.3 Study design and sampling

The type of study conducted using the comparative intradermal tuberculin test and questionnaire was cross-sectional and the method involved was a two stage sampling method. The primary stages were urban kebeles and rural peasant associations and the secondory stages were households. Out of 13 rural peasant associations and 9 urban kebeles that are known to keep indigenous and hybrid and/or exotic breeds of cattle, a total of 14 primary sample units (7 rural peasant associations and 7 urban kebeles) were selected randomly. In the second stage households were selected based on their willingness to participate in the study and from each primary unit 10 households were selected. One hundred and fourty herds containing 524 cattle belonging to the selected 140 households were tested using the comparative intradermal tuberculin test and any person who is a member of the respective selected household that was found during the visit was interviewed.

Likewise, in the abattoir-based survey the study type involved was cross-sectional, however the sampling method used was systematic random sampling. Detailed inspection of animal carcasses was done on Monday, Wednesday and Friday. This was adopted because sufficient numbers of animals were slaughtered on these days.

3.4 Sample size determination

3.4.1 The comparative intradermal tuberculin test

The sample size required for the CIDT test was calculated according to the formula given by Thrusfield (1995) for cluster sampling.

$$g = \frac{1.96^2\,[nVc + Pexp(1 - Pexp)]}{nd^2}$$

Where,

g = number of clusters to be sampled

n = predicted average number of animals per cluster

Pexp = expected prevalence

Vc = between cluster variance

D = absolute precision

Using the SIDT test, Dejenu (1998) reported that 5.9% (3/51) of cattle from small scale dairy farms and 14.5% (11/76) of cattle from traditionally managed dairy farms were reactors to bovine tuberculosis in Debre Birhan. Therefore, the sample size (household) required for the present study at 14.5% prevalence rate of BTB, 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 is taken), the desired precision is 0.05 (5%) and the predicted average number of animals per cluster (household or herd) being 4 is 63 households (herds). If in 10% of the households their cattle were absent on the day of measuring skin fold thickness after 72 hours following PPD administration, the total sample size for the study will be 70 herds or clusters. If we double this in order to cover a large area, the sample size will be 140 herds or households.

3.4.2 Abattoir survey

For the determination of the magnitude of BTB in the slaughterhouse, the sample size required was obtained using the formula given by Thrusfield (1995) for simple random sampling.

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

Where,

n = required sample size

Pexp = expected prevalence

d = desired absolute precision

Therefore, the sample size required at 14.5% prevalence rate with a desired precision of 0.05 and 95% confidence interval is 191 animals. If we fail to find all the organs needed to be examined in 10% of the slaughtered animals due to a hasty working condition prevailing in the slaughterhouse, the required sample size will be 211 animals. However, in order to increase our chances of finding several isolates for further molecular characterization, the carcasses of 531 animals were inspected.

3.5 Study methodology

3.5.1 The comparative intradermal tuberculin test

Cattle were tested by house-to-house visits as early in the day as possible. All cattle belonging to the selected households were individually identified by names which were previously given by their owners or assigned on the day of tuberculin inoculation based on their coat colour, age and sex. Their age, body condition score (BCS), sex, lactational status, reported pregnancy status (sometimes checked) and skin test measurements were recorded on preformed format (Appendix 1). 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. Appendix 5 presents the method of body condition scoring of animals.

In order to evaluate the effect of management, information on the feed and water source, method of feeding and water provision, and mixing of cattle was assessed. In the extensive management system the herd obtained feed from communal grazing lands and drinks river water frequently being mixed with other herds, whereas in the semi-intensive management system the cattle herd was provided with purchased feed and drink pipe water with minimal contact with other animals when they are released for some hours during the day to communal grazing fields. Finally, households were asked for the presence of human TB patient in their home or family.

Test procedure

The type of skin test performed was CIDT test and applied on the middle thirds of the neck. The bovine tuberculin was inoculated at the border of the anterior and middle thirds of the neck and that of the avian tuberculin administered 12-14 cm caudal to the former site. The same side of the neck was selected except in young animals in which their neck length was found insufficient to separate the sites for avian and bovine tuberculin. The skin inoculated was without any pathological changes and equally thick. The injection site was shaved and cleansed. Then, a cutaneous fold was formed with the thumb and the point finger within each clipped area and the skin fold thickness was measured using TB caliper in millimeters and recorded. A dosage of 0.1 ml of bovine tuberculin (*M. bovis* strain AN 5, Bovitubal 28, 000 IU/ml) (Bioveta, a. s. Komenskeho 212, ivanovice na Hane, Czech Republic) and 0.1 ml of Avian tuberculin (*M. avium* strain D 4 ER, Avitubal 28, 000 IU/ml) (Bioveta, Ivanovice na Hane, Czech Republic) were inoculated and their correct administration was checked if papula (pea like swelling) was formed and detected by palpation in the place of allergen inoculation. When the tuberculin was not administered intradermally, the administration was repeated in the place in the prescribed dosage. Whenever skin changes were determined before tuberculin administration; inoculation was made on another place of the same neck side. The reaction was evaluated within 72$\pm$(4) hours after tuberculin administration by inspection, skin palpation and by measuring of the skin fold thickness with TB caliper. The same person measured the skin fold thickness before and after tuberculin injection.

Interpretation

A reaction was considered to be positive if the difference in skin fold thickness at the bovine site of injection (anterior) was 4 mm or more higher than the reaction shown at the site of the avian injection (posterior). When the difference in the skin fold thickness at the bovine site of inoculation was greater than 2 mm but lower than 4 mm it was considered as doubtful but if lower than or equal to 2 mm it was taken as negative (OIE, 2004). Even though the difference in skin fold thickness between the two sites was lower than or equal to 2 mm, when the difference in skin fold thickness before and after tuberculin inoculation of each site was found higher than or equal to 4 mm it was taken as a non-specified infection.

ΔPPD-B = Skin fold thickness after inoculation of PPD-B – Skin fold thickness before inoculation PPD-B.

ΔPPD-A = Skin fold thickness after inoculation of PPD-A – Skin fold thickness before inoculation PPD-A.

ΔPPD-B – ΔPPD-A = $\geq$ 4 mm: Positive

ΔPPD-B – ΔPPD-A = > 2 mm and < 4 mm: Doubtful

ΔPPD-B – ΔPPD-A = $\leq$ 2mm: Negative

3.5.2 Abattoir survey

In the slaughterhouse animals and their carcasses and offals were identified using the butcher number. Epidemiological informations such as breed, sex and organs affected were obtained following stunning and during carcass inspection. 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 harbour tuberculous lesions. Similarly, the cranial and caudal mediastinal, left and right bronchial, medial retropharyngeal and mesenteric lymph nodes were inspected and then cut into about 2 cm slices for identification. The animal was classified as lesioned (infected) when any tuberculous lesion is found, and if not as non lesioned (not infected). Appendix 2 shows abattoir work recording format.

3.5.3 Isolation and identification of mycobacteria

3.5.3.1 Sample collection

A total of 125 tissues containing tuberculous lesions were collected. They were carefully removed from the carcass and placed in a 50 ml capacity tubes with screw caps containing 5 ml of sterile 0.85% saline water in a cooler with ice, and kept at -20°C (frozen) at Basonawerana Agricultural and Rural Development Office animal health clinic until they are transported. Then, they were transported to Aklilu Lemma Institute of Pathobiology (ALIPB) for bacteriological examination being kept at +4°C.

3.5.3.2 Bacteriological culture

Löwenstein-Jensen media was used for culturing and prepared according to the procedures of WHO (1998). The procedure for the preparation of Löwenstein-Jensen is presented in (Appendix 4). In processing samples of lesions from cattle in the laboratory, first the fat was removed from tissues containing the tuberculous lesion and then sectioned into pieces using sterile scissors and forceps. Thereafter, 0.85% saline water and sterile sand were added and grinding with pestle and mortar was done. Using a sterile, 15 ml centrifuge tube with a screw cap, equal amounts of specimen and 4% NaOH of about 5 ml each were added. The centrifuge tube was capped and mixed on a vortex mixer until the specimen was liquefied. Thereafter, they were centrifuged at 3000 rpm for 15 minutes. The supernatant was carefully decanted, and 2 ml of 0.85% saline was added to resuspend the sediment. The samples were neutralized by adding 2N HCl drop by drop until the colour of the indicator (0.1% phenol red) turned yellow. The suspension was inoculated onto two Löwenstein-Jensen slopes, one with pyruvate and the other with glycerol and incubated at 37°C for between 8 and 12 weeks (OIE, 2004; Ameni *et al.*, 2006; Cadmus *et al*., 2006; Lambert *et al.*, 2006). Cultures were checked once a week for the growth of mycobacteria.

Slow growth and colony characteristics were considered as an evidence of growth of mycobacteria and further subculturing to obtain sufficient number of colonies for further molecular analysis was done from the positive cultures. The observation of isolates with euogenic growth on Löwenstein–Jensen media supplemented with glycerol were tentatively identified as *M. tuberculosis* while those with euogenic growth on the pyruvate-containing media were regarded as suggestive of *M. bovis*. Finally, isolates were harvested for molecular typing analysis by scrapping the growth from a slope into 200 µl of sterile distilled water and heating at 80°C for 1 hour (Cadmus *et al*., 2006). Then, the harvest was kept at -20°C until the day of transportation to the Veterinary Laboratory Agency (VLA) in UK for molecular analysis. In the Veterinary Laboratories Agency (VLA), the current methods of choice for the molecular typing of *M. bovis* isolates are spacer-oligonucleotide typing (spoligotyping) and variable number of tandem repeat (VNTR) typing (Hewinson *et al.,* 2006).

3.5.3.3 Multiplex Polymerase chain reaction (m-PCR)

For m-PCR, the procedure described by Wilton and Cousins (1992) was followed. This analysis differentiates *M. tuberculosis* complex from *M. avium*, *M. intracellularae* and other mycobacterial species. The PCR targets the sequence of the genus *Mycobacterium* within the 16S rRNA gene (G1, G2), sequences within the hyper-variable region of 16S rRNA that is known to be specific to *M. intracellularae* (MYCINT-F) and *M. avium* (MYCAV-R), and the MTB70 gene specific for *M. tuberculosis* complex (TB-1A, TB-1B). The primers used were MYCGEN-F, 5'AGA GTT TGA TCC TGG CTC GA 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 reaction was carried out using Thermal Cycler (Applied Biosystems, GeneAMP 9700) for 10 minutes at 95°C; 35 cycles of 1 minute at 95°C, 1 minute at 61°C, and 1.5 minutes at 72°C; and 10 minutes at 72°C. Each PCR tube consisted of 5.2 µl H_2O Qiagen, 8 µl HotStarTaqMasterMix, 0.3 µl of each of the six primers (concentration given above), 5 µl of DNA templates of samples or controls making the total volume 20µl. *M. avium*, *M. intracellularae*, H37Rv and *M. bovis* strain 2122/97 were used as positive controls while H_2O Qiagen, was as a negative control. The product was electrophoresed in 2% agarose gel in TAE running buffer 10X SYBR safe at a ratio of 1:10 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.

3.5.3.4 Deletion typing

In this analysis the procedure described by Cadmus *et al*. (2006) was followed. Each sample was done in a separate PCR tube. Primers directed against the RD4, RD9 and RD10 loci were used to generate a deletion profile that would allow species identification of the isolate. Primers that were used include RD4intF ACA CGC TGG CGA AGT ATA GC, RD4flankF CTC GTC GAA GGC

CAC TAA AG and RD4flankR AAG GCG AAC AGA TTC AGC AT to check for the presence of RD4 locus; RD9intR CTG GAC CTC GAT GAC CAC TC, RD9flankF GTG TAG GTC AGC CCC ATC C and RD9flankR GCC CAA CAG CTC GAC ATC to check for the presence of RD9 locus; RD10intR GAA GTC GTA ACT CAC CGG GA, RD10flankF CTG CAA CCA TCC GGT ACA C and RD10flankR AAG CGC TAC ATC GCC AAG to for the presence of RD10 locus. The HotStarTaq Master Mix system from Qiagen was used for PCR, with primers described previously. The reaction mixture was 10 µl of HotStarTaq Master Mix, 0.3 µl ×3 of each primer (flank R, F and int), 2 µl DNA template and distilled water to a final volume of 50 µl. The PCR cycle was performed on a Perkin-Elmer GeneAmp machine using an initial hot start of 95°C for 15 minutes, followed by 35 cycles of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute; a final extension step of 72°C for 10 minutes completed the cycle. Products were visualized by electrophoresis through 1% agarose gel.

The presence of RD4 (i.e. *M. tuberculosis*, *M. africanum*), RD9 (i.e. *M. tuberculosis*) and RD10 (i.e. *M. tuberculosis*), is shown by the amplification of a product size of 335bp (RD4intF + RD4flankR), 1.421kb (RD9flankF + RD9intR) and 308bp (RD10flankF + RD10flankR), respectively, whereas the occurrence of an amplification product size of 446 bp (RD4flankF + RD4flankR), 472bp (RD9flankF + RD9flankR) and 202bp (RD10falnkF + RD10flankR) indicates deletion of RD4 (*M. bovis*), RD9 (*M. africanum and M. bovis*) and RD10 (*M. africanum and M. bovis*), respectively.

3.5.3.5 Spoligotyping

Spoligotyping was performed as following Cadmus *et al*. (2006). The direct repeat (DR) region was amplified by PCR with oligonucleotide primers derived from the DR sequence. Twenty-five microliters of the following reaction mixture was used for the PCR: 12.5 µl of HotStarTaq Master Mix (Qiagen; this solution provides a final concentration of 1.5 mM $MgCl_2$ and 200 µM each deoxynucleoside triphosphate.), 2 µl of each primer (20 pmol each), 5 µl of the suspension of heat-killed cells (approximately 10 to 50 ng), and 3.5 µl of distilled water. The mixture was heated for 15 minutes at 96°C and subjected to 30 cycles of 1 minutes at 96°C, 1 minute at 55°C, and 30 seconds at 72°C. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequences within the DR

locus. After hybridization, the membrane was washed twice for 10 minutes in 2× SSPE (1×SSPE is 0.18 M NaCl, 10 mM NaH_2PO4, and 1 mM EDTA [pH 7.7])–0.5% sodium dodecyl sulfate at 60°C and then incubated in 1:4,000-diluted streptavidin-peroxidase conjugate (Boehringer)for 45 to 60 minutes at 42°C. The membrane was washed twice for 10 minutes in 2× SSPE–0.5% sodium dodecyl sulfate at 42°C and rinsed with 2× SSPE for 5 minutes at room temperature. Hybridizing DNA was detected by the enhanced chemiluminescence method (Amersham Plc. Lt, UK) and by exposure to X-ray film (Hyper-film ECL; Amersham Plc. Lt, UK) as specified by the manufacturer.

3.5.4 The questionnaire survey

A total of 140 heads of cattle owning households whose animals were tested or their family members were interviewed with a preformed questionnaire. Their recognition of bovine TB, awareness on BTB transmission onto human beings and dairy product consumption and meat eating habits were investigated. Appendix 3 shows the preformed questionnaire presented to cattle owners

3.5.5 Retrospective case-record analysis

Annual activity report of Debre Birhan zonal hospital and the records of TB patients that were admitted to Debre Birhan zonal hospital and Debre Birhan health center from July 2002 G.C to June 2007 G.C. were analyzed. The total number of TB patients admitted, their sex and age, the TB category identified, HIV status and the outcome of treatment were carefully documented to assess the extent and importance of tuberculosis in the area.

3.6 Data analysis

All the data obtained from the study were entered into MS Excel data sheets. Then, coded and were analyzed using STATA 8 intercoded statistical software programme. The infection rate 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 is inspected multiplied by 100. Likewise, awareness of cattle owners was determined by dividing the proportion of people who knew or heard about BTB by the total number of respondents. The risk factors associated with bovine infection were determined using percent values and using Pearson's Chi-square (χ^2). Odds Ratio (OR) was obtained to investigate the strength of association. 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 1.0. For the analysis of the effect of different risk factors on tuberculosis status of animals, doubtful reactors were not considered positive.

4. RESULT

4.1 Herd level characteristics

Out of 140 herds tested, 27 (19.3%) and 13(9.3%) were found TB infected with and with out considering doubtful reactors as positives, respectively. Therefore, the herd prevalence was 19.3% (95% CI: 13.1%, 26.8%) with and 9.3% (95% CI: 5.0%, 15.4%) without considering doubtful reactors as bovine TB infected herds. However, only positive reactors were considered in investigating the possible risk factors. The herd level characteristics (Table 8) such as type of management, herd size and previous or present infection of owners with TB were not found associated with BTB infection of the respective herds (P>0.05). But the prevalence rate was slightly higher in cattle under extensive-management system (11.1%) than those under semi-intensive management (6.8%) and in those herds containing more than 4 animals (11.4%) than in those with 4 and lower number of animals (7.6%).

4.2 Animal characteristics

Out of 524 animals tested, 14 (2.7%) were found positive and 14 (2.7%) were determined as doubtful reactors. Therefore, the individual animal prevalence of bovine TB obtained was 2.7% (95% CI: 1.5, 4.4) if doubtful animals were considered negative. On the other hand, if doubtful reactors were considered as positive the prevalence of tuberculosis would be 5.3% (95% CI: 3.6, 7.6). Besides, 3.5% (18/524) of the tested animals reacted to both bovine and avian type tuberculin and classified as non-specific reactors, which resulted from infection with other mycobacteria. The prevalence of BTB was found to be 2.6% in Debre Birhan town and 2.7% in Basonawerana district (Table 7). Many animal characteristics were considered (Table 9) to determine the risk factors predisposing animals to BTB infection. However, all the considered animal level characteristics were found not associated with tuberculosis (P>0.05).

Table 7. Summary of the CIDT test result in cattle raised in and around Debre Birhan.

No.	Study area	Number of animals			Prevalence
		Tested	Positive reactors	Doubtful reactors	
1	Debre Birhan town	193	5	3	2.6%
2	Basonawerana District	331	9	11	2.7%
	Total	**524**	**14**	**14**	**2.7%**

Table 8. The level of association of herd level characteristics with bovine tuberculosis.

No.	Herd characteristics	Number of herds			χ^2 Value	P-value
		Positive	Negative	Total		
1	Management					
	Semi-intensive	4(6.8%)	55(93.2%)	59(100.0%)	0.7603	0.383
	Extensive	9(11.1%)	72(88.9%)	81(100.0%)		
2	Herd size					
	$\leq$ 4 cattle	6(7.6%)	73(92.4%)	79(100.0%)	0.6177	0.734
	$\geq$ 5 and $\leq$ 8 cattle	5(11.4%)	39(88.6%)	44(100.0%)		
	$\geq$ 9 cattle	2(11.8%)	15(88.2%)	17(100.0%)		
3	Owners' health					
	Sick with TB	-	10(100%)	10(100.0%)	1.1024	0.294
	Not sick	13(10.0%)	117(90%)	130(100.0%)		

Table 9. The level of association of animal characteristics with bovine tuberculosis.

No.	Individual animal characteristics	No of animals			χ^2 Value	P-value
		Positive	Negative	Total		
1	Study area					
	Basonawerana	9(2.7%)	322(97.3%)	331(100.0%)	0.0077	0.930
	Debre Birhan	5(2.6%)	188(97.4%)	193(100.0%)		
2	Sex					
	Female	10(2.8%)	350(97.2%)	360(100.0%)	0.0497	0.824
	Male	4(2.4%)	160(97.6%)	164(100.0%)		
3	Age					
	<1 year	-	66(100.0%)	66(100.0%)	4.6037	0.203
	≥ 1 and ≤ 3 years	5(2.9%)	166(97.1%)	171(100.0%)		
	≥ 4 and ≤ 7 years	3(1.8%)	161(98.2%)	164(100.0%)		
	≥ 8 years	6(4.9%)	117(95.1%)	123(100.0%)		
4	Breed					
	Local	3(2.4%)	127(97.6%)	130(100.0%)	0.4052	0.817
	Cross	7(2.5%)	273(97.5%)	280(100.0%)		
	Exotic	4(3.5%)	110(96.5%)	114(100.0%)		
5	Body condition					
	Poor	3(2.2%)	131(97.8%)	134(100.0%)	0.1520	0.927
	Medium	7(2.9%)	233(97.1%)	240(100.0%)		
	Good	4(2.7%)	146(97.3%)	150(100.0%)		
6	Lactation					
	Not lactating	-	61(100.0%)	61(100.0%)	3.2801	0.070
	Lactating	7(5.2%)	128(94.8%)	135(100.0%)		
7	Pregnancy					
	Not Pregnant	7(3.6%)	132(96.4%)	139(100.0%)	3.2350	0.072
	Pregnant	-	62(100.0%)	62(100.0%)		

4.3 Abattoir findings

The prevalence of BTB was investigated to be 22.0% (95% CI: 18.6%, 25.8%) in abattoir-based surveillance. The distribution of tuberculous lesions in tissues of positive animals is presented in (Table 10). Among the lesions observed, 89.6% were localized lesions involving frequently a single organ and the mean number of tissues affected by tuberculous lesion was 1.05. The majority (73.8%) of the lesions were located on the mesenteric lymph nodes meanwhile 19.1% and 7.1% of the lesions were found in the thoracic cavity and cranial regions, respectively. Highly disseminated tuberculosis was seen in one animal whereby the mediastinal, bronchial, retropharyngeal and mandibular lymph nodes were found studded with tuberculous granulomas.

Of the two risk factors considered (Table 11), only sex was found significantly associated with BTB infection ($P<0.05$). Male animals were found harbouring tuberculous lesions 1.7 times more than that of females (OR= 1.7; P=0.012; 95% CI: 1.1, 2.5). However, breed was not found significantly associated with the presence of gross tuberculous lesions ($P>0.05$).

A retrospective analysis of a one year (January to December 2006 G.C.) abattoir data revealed the slaughter of 2855 cattle and the subsequent condemnation of 6 lungs due to abscesses which might or might not be tuberculous lesions. However, except that piece of information there was no single official document that indicates the finding of tuberculous lesions or condemnation of carcasses due to BTB.

Table 10. The distribution of tuberculous lesions in the tissues of infected animals.

No.	Anatomic site	Number of infected tissue	Percent (%)
1	Medial retropharyngeal lymph node	7	5.6
2	Mandibular lymph node	2	1.6
3	Mediastinal lymphnode	4	3.2
4	Tracheobronchial lymph node	5	4.0
5	Lung	15	12.0
6	Mesentric lymph nodes	92	73.6
	Total	**125**	**100.0**

Table 11. The level of association of sex and breeds of animals with tuberculous lesions.

No.	Variable	No. of animals			χ^2 Value	P-Value
		Positive	Negative	Total		
1	Sex					
	Male	66(27.2%)	177(72.8%)	243(100.0%)	6.8544	0.009*
	Female	51(17.7%)	237(82.3%)	288(100.0%)		
2	Breed					
	Local	100(21.4%)	367(78.6%)	467(100.0%)	2.2331	0.327
	Cross	15(25.0%)	45(75.0%)	60(100.0%)		
	Exotic	2(50.0%)	2(50.0%)	4(100.0%)		

Significant*

4.4 Isolation of mycobateria

Out of 125 tuberculous lesions that were cultured, 32% (40/125) showed growth on the primary culture media. The outcome of primary culturing activity is presented in (Table 12). Among those cultures which showed visible growth on primary cultures, 82.5% (33/40) of the isolates were subcultured. However, due to the scarcity of resources only nine isolates undergone molecular analysis.

Table12. The outcome of primary culture of tuberculous tissues from slaughtered cattle.

No.	Sample type	Outcome of primary culture			
		L-J media containing Glycerol		L-J media containing Pyruvate	
		Total	Positive	Total	Positive
1	Mesenteric LN	92	20(21.7%)	92	18(19.6%)
2	Bronchial LN	5	-	5	3(60.0%)
3	Mandibular LN	2	1(50.0%)	2	-
4	Mediastinal LN	4	2(50.0%)	4	2(50.0%)
5	Retropharyngeal LN	7	2(28.6%)	7	1(14.3%)
6	Lung	15	2(13.3%)	15	1(6.7%)
	Total	**125**	**27(21.6%)**	**125**	**25(20.0%)**

LN: Lymph node

4.5 Molecular analysis

The PCR analysis of those nine isolates obtained from animal tissues (Table 13) indicated the presence of mycobacteria in 77.8% (7/9) of the samples. However, only 28.6% (2/7) of them showed the presence of *Mycobacterium tuberculosis* complex and 14.3% (1/7) was positive for the presence of *M. avium* complex. The remaining 22.2% (2/9) isolates didn't show the amplification products characteristic to mycobacteria. Figure 1 presents the electrophoretic separation of PCR products. Upon deletion typing of those two samples (that showed amplification product for *M. tuberculosis* complex in m- PCR), neither of them showed amplification products for RD4, RD9 and RD10 primers.

Spoligotyping of those two isolates that showed amplification products characteristic to both the genus *Mycobacterium* and to *Mycobacterium tuberculosis* complex resulted in identical strains of *M. tuberculosis.* Figure 2 shows the spoligotypes obtained from the present and other studies.

Table 13. Summary of the result of m-PCR analysis of isolates from cattle tissues.

			Result of m-PCR		
No.	**Sample No.**	**Sample type**	**Genus** *Mycobaterium*	*M. tuberculosis* **complex**	*M. avium* **complex**
1	SS65Med	Mediastinal LN	Positive	Negative	
2	SS26BR	Bronchial LN	Positive	Positive	
3	SS10Mes	Mesenteric LN	Positive	Negative	
4	SS16Mes	Mesenteric LN	Negative	Negative	
5	SS14Med	Mediastinal LN	Positive	Negative	
6	SS34Br	Bronchial LN	Positive	Negative	Positive
7	SS12Br	Bronchial LN	Positive	Positive	
8	SS13Mes	Mesenteric LN	Positive	Negative	
9	SS15Mes	Mesenteric LN	Negative	Negative	

LN: Lymph node

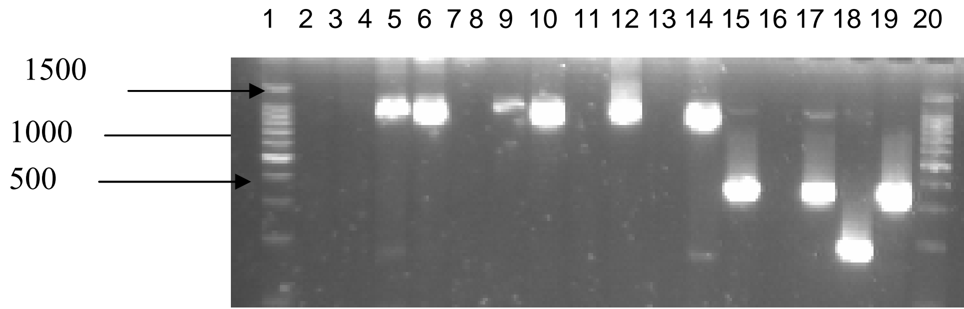

Figure 1. Electrophoretic separation of PCR products of multiplex PCR typing of the genomic DNA of mycobacteria isolated from tuberculous lesions of cattle. Lanes 20 and 1, 100bp DNA ladder; Lane 19, *Mycobacterium tuberculosis* complex positive control; Lane 18, *M. avium* positive control; Lane 16, water negative control. Lane 5, 6, 9, 10, 12, and 14 are positive only for Genus *Mycobacterium* and lane 15 and 17 for genus *Mycobacterium* and *Mycobacterium tuberculosis* complex. Lane 2, 3, 4, 7, 8, 11, 13 are negative.

Spacers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	Source
H37Rv*	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	Jahans and Worth, 2006
SS30HSP/99*	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■		present study
SS54HSP/99*	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				■					■	■	■	■	■	■	■	present study
NH1*	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				■	■	■	■	■	■	■					■	■	■	■	■	■	■	Cadmus *et al*., 2006
BCG***	■	■		■	■	■	■	■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■						Jahans and Worth, 2006
A12BrLN**	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	present study
A26BrLN**	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	present study
AN5***	■	■		■		■	■	■		■			■	■	■		■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■						Jahans and Worth, 2006
9***	■	■		■	■		■						■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■						Jahans and Worth, 2006
17***	■	■		■	■		■						■	■	■		■	■	■	■	■	■	■	■	■							■	■	■	■	■	■	■						Jahans and Worth, 2006
Embs1***	■	■						■		■	■	■	■	■	■		■	■	■	■	■	■	■				■											■						Ameni *et al*., 2007
SB0994***	■	■		■	■	■	■	■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■						Cadmus *et al*., 2006

M. tuberculosis strain*s**; *M. tuberculosis* isolates from animal samples**; *M. bovis* strains***

Figure 2. The result of spoligotyping of isolates from tissue samples.

4.6 The questionnaire survey

A total of 140 heads of cattle owning households or members of these households were interviewed. Of these, 36 (25.7%; 95% CI: 18.7%, 33.3%) respondents reported they knew or had heard about TB of cattle. Among these 36 respondents who knew or heard about BTB, 72.2% didn't recognize the symptoms of the disease and all most all of them didn't distinguish the post-mortem lesions. Besides, only 27.8% (10/36) of them were aware of BTB transmission between the cattle population, whereas 36.6% (13/36) described possible transmission of the disease from sick cattle to humans through inhalation and ingestion of contaminated milk and meat.

Several demographic characteristics and other factors were considered (Table 14) to investigate their possible association with BTB recognition of the respondents. But all the factors considered i.e. residential area, sex, age, occupation and level of education were not found associated with the knowledge of residents on BTB ($P>0.05$).

Concerning dairy product consumption habits, 1.4% (2/140), 22.9% (32/140) and 82.9% (116/140) of the respondents drink raw milk, both raw and boiled milk and consume locally soured milk (Yoghurt) respectively. Moreover, 45% of the respondents have the habit of eating mixed (raw and cooked) meat. Table 15 summarizes the milk and meat consumption habits of the households.

Table 14. The level of association of various factors with BTB awareness of respondents.

No	Variables	Knowledge of BTB			χ^2 Value	P-Value
		Yes	No	Total		
1	Residence					
	Debre Birhan	14(20.0%)	56(80.0%)	70(100.0%)	2.3932	0.122
	Basonawerana	22(31.4%)	48(68.6%)	70(100.0%		
2	Sex					
	Female	11(20.0%)	47(80.0%)	58(100.0%)	2.361	0.124
	Male	25(30.5%)	57(69.5%)	82(100.0%)		
3	Age					
	≥ 11 and ≤ 30 years	15(30.0%)	35(70.0%)	50(100.0%)	0.9206	0.820
	≥ 31 and ≤ 50 years	12(21.8%)	43(78.9%)	55(100.0%)		
	≥ 51 and ≤ 70 years	7(25.9%)	20(74.1%)	27(100.0 %)		
	≥ 71 years	2(25.0%)	6(75.0%)	8(100.0%)		
4	Occupation					
	Farmer	17(27.4%)	45(72.6%)	62(100.0%)	5.2376	0.264
	Housewife	5(13.9%)	31(86.1%)	36(100.0%)		
	Student	5(31.3%)	11(68.7%)	16(100.0%)		
	Civil servant	5(45.5%)	6(54.5%)	11(100.0%)		
	Others	4(26.7%)	11(73.3%)	15(100.0%)		
5	Education					
	Not educated	2(11.1%)	16(88.9%)	18(100.0%)	2.3315	0.312
	Informal	13(27.1%)	35(72.9%)	48(100.0%)		
	Formal	21(28.4%)	53(71.6%)	74(100.0%)		

Table 15. Milk and meat consumption habit of the respondents in and Debre Birhan.

No.	Habit of respondents	Number of respondents	Percent (%)
1	**Milk drinking**	**140**	**100.0**
	Raw milk	2	1.4
	Boiled milk	95	67.9
	Both raw and boiled milk	32	22.9
	Do not drink	11	7.8
2	**Soured milk consumption (yoghurt)**	**140**	**100.0**
	Consume	116	82.9
	Do not consume	24	17.1
3	**Meat eating habit**	**140**	**100.0**
	Cooked meat	77	55.0
	Both raw and cooked meat	63	45.0

4.7 Retrospective case-record analysis

Reviewing the annual activity report of Debre Birhan zonal hospital has showed that a total of 9,502 patients were admitted and diagnosed as TB infected in a five year time period (from July 2002 G.C. to June 2007 G.C.). Furthermore, a 5 year (from July 2002 G.C. to June 2007 G.C.) retrospective analysis of TB unit registry of Debre Birhan zonal hospital and Debre Birhan health center have indicated (Table 16) that 3,407 TB patients received the direct observed short course (DOTS) treatment regimen. Of these 86.9% (2,961/3,407) were pulmonary TB and 12.9% (438/3,407) were extra-pulmonary TB patients. Among these TB patients, 264 (7.7%) of were reported dead. Strikingly, out of those TB patients on short course therapy, 79.9% (2,723/3,407) were between 15 and 50 years, at their more productive age. Between May 2006 and August 2007 G.C. 364 TB patients were HIV tested in Debre Birhan zonal hospital and 43.4% (95% CI: 38.2%, 48.7%) were found infected with HIV.

Table 16. TB patients on DOTS for the past five years in health institutions of Debre Birhan.

		Type of tuberculosis					
		Debre Birhan zonal hospital			Debre Birhan health center		
No.	**Year**	**Pul. TB**	**EP. TB**	**Missing**	**Pul. TB**	**EP. TB**	**Missing**
1	2002/2003 G.C.	615	98	3	115	20	1
2	2003/2004 G.C.	461	45	0	114	25	0
3	2004/2005 G.C.	475	59	1	137	20	0
4	2005/2006 G.C.	431	81	0	116	17	0
5	2006/2007 G.C.	401	57	1	96	16	2
	Total	**2383**	**340**	**5**	**578**	**98**	**3**

Pul. TB: Pulmonary TB; EP.TB: Extra-pulmonary TB

Source: Debre Birhan zonal hospital and Debre Birhan health center.

5. DISCUSSION

Infection of cattle with *M. bovis* 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). The present study using the comparative intradermal tuberculin test (CIDT) showed that the prevalence of BTB in and around Debre Birhan is 2.7%. This infection rate is low as the prevalence of tuberculosis in cattle is said to be so when it is 5% or less (Bonsu *et al.*, 2000). The prevalence obtained from the present study is found to be lower than that of Kiros (1998) and Adugna (2005) who reported prevalence rates of 29.7% (234/788) in dairy cattle in eastern Shoa and 31% (93/301) in dairy cattle in Dire Dawa, respectively. 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). Comparable results with the present study were reported by Redi (2003) and Laval and Ameni (2004) who described prevalence rates of 3.5% (18/514) in Assela and 3.8% (12/320) in Bodji district, respectively. This similarity may be related the resemblance in the type of management of cattle (extensive management) adopted and identical environmental conditions.

The lower prevalence rate obtained with the CIDT test in the present study may be due to extensive management system (Shirima *et al.*, 2003), small herd size of cattle (Cleaveland *et al.*, 2007), high prevalence of fasciolosis in cattle (Ameni and Medhin, 2000; Flynn *et al.,* 2007) or increased resistance due to non-specific response to environmental mycobacteria (Oloya *et al*., 2006). Though the prevalence obtained in this study is said to be lower, enormous numbers of tubercle bacilli can be excreted by a cow with tuberculous mastitis. One cow can excrete enough viable bacilli to contaminate the milk of 100 cows, when their milk is mixed (Kleeberg, 1984). This might occur especially in dairy cooperatives and dairy enterprises which sell unpasteurized dairy products to the residents and the passer by.

Abattoir inspection of carcasses revealed a high infection rate of BTB, i.e. 22% (117/531), in animals slaughtered at Debre Birhan municipality abattoir. This finding is lower than from that obtained at Bahirdar municipality abattoir, which is reported to be 44.27% (638/1441) (Negash, 2006) but higher than that reported by Jemale (2005) i.e. a prevalence rate of 2.34% in Awassa municipality abattoir. However, comparable results with the present study were obtained by Mamo (2007) who reported a prevalence rate of 24.7% in Adama municipality abattoir and by Cleaveland *et al.* (2007) who determined 19.8% (1502/7589) prevalence in cattle carcasses examined by meat inspectors in Tanzania. Majority of the animals slaughtered in Debre Birhan municipality abattoir were obtained from the surrounding areas. However, the origin of animals slaughtered in the abattoir couldn't exactly be traced back.

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; Tekelu *et al.*, 2004). However, in this study tuberculous lesions were recorded mainly in the mesenteric lymph nodes. This distribution of tuberculous lesions (Table 2) strongly suggested that these animals were mainly infected through ingestion. Lesions of the alimentary tract are more common in temperate climates (characteristic to the study area) where conditions favour dissemination and survival of *M. bovis* on forage, while respiratory tract lesions are more common in arid climates where conditions favour aerosolization of *M. bovis* (Lepper and Pearson, 1973). Cleaveland *et al.* (2007) reported that 61.3% (791/1290) of carcasses in Tanzania had lesions in the gastrointestinal tract. The high frequency of gastrointestinal tract lesions may result in faecal excretion and widespread environmental contamination. As most herds in the extensive type of management system share common grazing and watering points, environmental contamination from a single infected herd has the potential to infect several herds.

Several herd and animal characteristics have been described as risk factors predisposing cattle to BTB infection. However, in the present study none of the factors were found significantly associated with positive skin reactivity of animals. Similarly, Redi (2003) in Assela showed the prevalence of BTB was lower and individual animal characterstics such as age, sex, breed, pregnancy, lactation and body condition were not significantly related with positive skin test

reactivity of cattle. In abattoir-based surveillance, of the two factors considered breed was not found associated with the presence of gross lesions. This result is consistent with the findings of Mamo (2007). Meanwhile, male animals were found harbouring tuberculous lesions more frequently than their counterparts indicating sex was significantly associated with bovine tuberculosis but this finding is inconsistent with the findings of Hassen (2007) and Woyessa (2007). In the present study area, male animals are sold for slaughter when they retire from serving as a source of drought power and they are castrates, while local breed females are sold for slaughter at early ages in their productive life and are slaughtered against the meat inspection regulation of the country. So males are kept for a long period of time as compared to females this long life span might have predisposed them to BTB infection. Kazwala *et al*. (2001) reported that female cattle have less contact with cattle from other herds which decreased their exposure to infection and male animals had a higher chance of being positive to skin test than female animals and related this variation to the occupation of the male cattle. Male cattle in particular castrates, are mostly used as oxen, who are kept in the herd for longer and there for have more chance of being exposed to infection compared to female cattle.

Out of 125 tissue samples cultured, only 32% (40/125) showed growth on primary culture media (Table 15). A 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).

Although molecular typing of the isolates using m-PCR revealed that seven isolates belong to the genus *Mycobacterium*, only two of them were found to be members of the *Mycobacterium tuberculosis* complex. Strain typing of these isolates indicated that both belong to a single species of *M. tuberculosis* and found to be identical strains. These *M. tuberculosis* strains identified in this study were previously reported by Bruchfeld *et al*. (2002). Out of 32 isolates from human sputum samples taken from patients at Black Lion University Hospital, 12 strains were with a one band cluster. Isolation of *M. tuberculosis* from animal samples has been reported by many authors. For instance, Cadmus *et al*. (2006) reported the finding of two *M. tuberculosis* strains on

spoligotyping among 17 isolates from cattle in Nigeria. Moreover, in India as assessed by nested-PCR (N-PCR) and culture, 15%–28% (n=52) of the animals were discovered to be infected with *M. tuberculosis* (Prasad *et al*., 2005). The finding of *M. tuberculosis* in animals might indicate the potential of transmission the agent between cattle and man in the study area.

In the present study it was investigated that only 25.7% of cattle owning family knew or have heard about bovine tuberculosis. 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 Dangme-West district of Ghana.. However, our result is consistent with the previous reports (Ameni *et al.*, 2003a; Mekonnen, 2007). The knowledge of cattle owning family was not found to vary among the various demographic characterstics and other factors considered. 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.

A total of 3407 patients were received short course treatment in Debre Birhan zonal hospital and Debre Birhan health center. Out of these, 2,961 (86.9%) were pulmonary TB patients and 264 (7.7%) died. Similarly, in México, from a 5-year retrospective study (1992 G.C. to 1996 G.C.), Milian *et al.* (2000) reported a similar observation in that 80% of the cases were pulmonary and 6% of TB patients died. Reviewing records of TB patients in Debre Birhan zonal hospital showed that 43.4% of them were HIV coinfected. Similarly, Demissie *et al.* (2000) also reported 45.3% (n=236) smear positive tuberculosis patients were HIV positive. The HIV-TB coinfection was highest in the age group 20-49 in Addis Ababa. Infection with HIV is the strongest yet identified risk factor for progression from tuberculosis infection to tuberculosis (Rieder, 2002). 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 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. CONCLUSIONS AND RECOMMENDATIONS

This study demonstrates a widespread presence of BTB infection in cattle residing in and around Debre Birhan. A higher infection rate was found in male animals than females and the latter were found slaughtered at their productive age irrespective of the meat inspection regulation of the country. The hygienic condition of the abattoir was poor that favours contamination of carcasses. 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 drinking and eating habits predisposing them to zoonotic tuberculosis. The attempt of molecular typing *M. bovis* (agent of BTB) was not successful because of its poor adaptation in subcultures. On the other hand, the finding of *M. tuberculosis* isolates from animal samples warrants that cattle could serve as a source of the disease agent to humans. At last, the extent of human TB in the area was huge and HIV infection among TB patients constituted a significant figure. Though analysis of datas from health institutions revealed TB is major human health problem, nothing is known about the extent to which human tuberculosis is caused by *M. bovis* in the study area. So the extent of bovine tubercle bacilli as a contributor to the tuberculosis epidemic in humans remains undetermined.

Thus, the presence of the BTB in cattle determined by the CIDT test and abattoir-based study together with lack of awareness of cattle owners and their family members on the presence of TB in cattle, existence of habits predisposing to BTB and the finding of *M. tuberculosis*, from animal tissues highlights the risks of infection from the consumption of unboiled/unpasteurized dairy products and uncooked meat. In addition, if the cattle which tested positive had the pulmonary form of BTB, then the risk of passing this to humans by aerosol contamination might also be significant. This is of particular importance in this study area since animals are kept in very close proximity to human dwellings and people have contact with animals while feeding, herding, milking and ploughing.

In light with the aforementioned findings, the following recommendations are forwarded

- ☞ Public health education to raise the awareness of cattle owners on the dangers of consuming unpasteurized /unboiled/ dairy products and uncooked meat must be done.

- ☞ Meat inspection procedures must be strictly implemented at Debre Birhan municipality abattoir.

- ☞ The epidemiology of BTB, the extent of *M. bovis* as a contributor to human tuberculosis and the role of cattle as a source of *M. tuberculosis* to man need to be further studied.

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8. APPENDICES

Appendix 1. Comparative Intradermal Tuberculin Test (CIDT) data recording Format.

<u>Comparative Intradermal Tuberculin Test (CIDT) data recording Format</u>

No.	Date	Name of the owner	Identification	Sex	Age	Breed	Body condition scoring	Management	Parity

No.	Name of the owner	Source of animal	State of lactation	State of pregnancy	Skin fold thickness (mm)						Remark
					Before		After		Difference		
					PPD-A	PPD-B	PPD-A	PPD-B	PPD-A	PPD-B	

Appendix 2. Abattoir work recording format.

Abattoir Work Recording Format

No.	Date	Butcer No.	Sex	Breed	TB status	Tissue with tuberculous lesion					
						Lung	Bronchial LN	Mediastinal LN	Retropharyngeal LN	Mesenteric LN	Mandibular LN

LN: Lymph node

Appendix 3. Questionnaire presented to cattle owners.

A QUESTIONNAIRE PRESENTED TO CATTLE OWNERS

Number---------------------- Date of Interview----------------------------

Personal data

1. Name ----------------------------- 2. Address District ----------Kebele/PA ---------Village -------
House No---------

3. Sex--------------------- 4. Age-------------------- 5.Occupation----------------

6 Level of education a) Formal---------------- b) Informal --------------- c) Not educated

7. Marital status a) Single b) Married c) Divorced d) Widowed

Socio economic data

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

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

10. 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 ------------------------

Husbandry practices

11. Source of feed for your cattle
a) Communal pasture b) Privately hold pasture c) Purchased feed -----------------------

12. Do you provide feed supplement to your cattle? a) Yes------------------- b) No

13. Is feeding of cattle separate? a) Yes b) No

14. Is the feeding trough raised? a) Yes b) No

15. Source of water for your cattle a) Communal watering Point---------- b) Pipe c) Other

16. Is drinking utensil separate? a) Yes b) No

17. Is the drinking utensil raised? a) Yes b) No

18. Do you mix your cattle with cattle of others? a) Yes b) No

19. Where do your cattle get mixed? a) Communal grazing area b) Watering points
    c) Market places d) Veterinary clinic e) Vaccination posts f) Other--------------------
20. Do you regularly clean the barn? a) Yes b) No
21. Is there sufficient ventilation in the barn? a) Yes b) No
22. Do you regularly get your cattle vaccinated? a) Yes b) No
    c) market places d) veterinary clinics e) vaccination posts f) others---------------------

Farmer recognition level

23. Do you know any communicable disease from animals to man? a) Yes b) No

If yes, mention ---
----

24. Does tuberculosis affect cattle? a) Yes b) No c) I don't know
25. What are the clinical signs?---

    ----
26. How bovine tuberculosis is transmitted between animals? --
    ----
27. Which sex groups of cattle are affected more? a) Males b) Female c) both
    d) I don't know
28. Which age group is affected more? a) Calves b) Young c) Adult animals
    d) All are equally affected e) I don't know
29. Which production group is affected more? a) Dry cow b) Pregnant c) Lactating
    d) Heifers e) All are affected equally f) I don't know
30. Is TB of cattle communicable to man? a) Yes b) No c) I don't know
31. If yes, how it is transmitted from animal to man? --
    ----

32 Does bovine tuberculosis have treatment? a) Yes b) No c) I don't know

33. Where did you get the treatment? a) Government veterinary clinic b) Traditional healer c) Market, d) Other source----------------------
34. Mention methods of prevention you know? ---

Personal habits

35. Your habit of drinking milk a) Raw b) Boiled c) Both boiled and raw c) Don't drink
36. Your reason of boiling milk before drinking ---
37. Which milk products you consume? a) Yoghurt b) Cheese c) Whey d) Butter
    e) Other f) Don't consume
38. Do you eat meat? a) Yes b) No
39. If yes, which one you eat? a) Raw meat b) Cooked meat c) Mixed
40. If you don't consume raw meat, why? --
41. Is there or was there any TB patient in your family with in the past 2 years? a) Yes b) No

For TB patients only

42. Have you had any previous contact with human TB patient? a) Yes b) No
43. If yes, mention the date or period of contact ---
44. For how long have you been sick with (TB)? ---
45. Which type of (TB) did you suffer from? a) Pulmonary TB b) Extra pulmonary TB
    c) I don't know
46. Did you get treatment? a) Yes b) No
47. Where did you get treatment a) Modern clinics b) Hospital c) Traditional healers
48. Did you recover fully? a) Yes b) No
49. Where did you get the disease? ---
50. Do you have any contact with cattle? a) Yes b) No
51. If yes, which way? a) Feeding b) Milking c) Herding d) slaughtering
    e) Sharing the house

Appendix 4. Preparation of Löwenstein-Jensen egg-based medium.

1. Mineral salt solution

1.1 Ingredients

Potassium dihydrogen phosphate anhydrous (KH_2PO_4)	2.4 g
Magnesium sulphate ($Mg\ SO_4.\ 7H_2O$)	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 ^{o}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 insipissator 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)

Appendix 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 can not 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 can not 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).

9. CURRICULUM VITAE

1. PERSONAL DATA

Full name	Shihun Shimeles
Sex	Male
Date of birth	March 2, 1969 G.C.
Place of birth	Lideta subcity, Addis Ababa
Nationality	Ethiopian
Language proficiency	Amharic and English (speak, write, listen)
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Contact address	Tel.: 0911 70 35 48 and 0913 23 18 26
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2. EDUCATIONAL BACK GROUND

- Addis Ababa University, Faculty of Veterinary Medicine.
  From 2005/2006 G.C.-2007/2008 G.C.
  MSc degree in Veterinary Epidemiology
- Addis Ababa University, Faculty of Veterinary Medicine.
  From 1986/87 G.C. to 1993/94 G.C.
  DVM degree
- Debresina comperehensive secondary school
  From 1981/82 G.C. to 1985/86 G.C.
  ESLCE certificate
- Abeye elementary school
  From 1975/76 G.C. to 1980/81 G.C.

3. ADDITIONAL TRAINING

From August 15 to September 2, 2005 refresher training entitled Vetrinary Public Health, Faculty of Veterinary Medicine, Addis Ababa University.

4. WORK EXPERIENCE

- Alage Agricultural Technical and Vocational Education Training (ATVET) College
  From August 2002 G.C. upto now
  Senior instructor
- Kewot District Agricultural Office
  From May 2001 to July 2002 G.C.
  District Veterinary Officer
- Berehet District Agricultural Office
  From March 2000 to April 2001 G.C.
  District Veterinary Officer
- Wag Hemera Adminstrative Zone Department of Agriculture
  From August 1997 to February 2000 G.C.
  Zone Veterinary Officer
- Sekota District Agricultural Office
  From March 1995 to July 1997 G.C.
  District veterinary Officer

5. EXTRA-EXPERIENCES

- Amhara National Regional State-Bureau of Agriculture (ANRS-BOA)
  From April to June 1998 G.C.
  Participated on Mange mite survey
- Former Kombolcha Agricultural Training Center (KATC)
  From April to June 2001 G.C.
  Participated on training of animal health technicians (thaught paracitology cource)
- Alage ATVET College
  In January 2005 G.C.
  Participated on training of Community Animal Health Workers (CAHWs)

6. RESEARCH AND PUBLICATIONS

1. In 2008 G.C. Bovine Tuberculosis: Epidemiologic Aspects and Public Health Implication in and Around Debre Birhan, Ethiopia. (unpublished MSc thesis, AAU, FVM).
2. In 2006 G.C. Arthropod Parasites and their effect on the production and productivity of small ruminants. (unpublished MSc seminar paper, AAU, FVM).
3. In 2000 G.C. Mange a disease of growing threat for the production of small ruminants in the Amhara regional state. A. Demeissie *et al*. (In the opportunities and challenges of enhancing goat production in East Africa edited by Roger C. Meskel, Girma Abebe and A.C. Gotetsch).
4. In 1994 G.C. The Prevalence of Canine Gastro-Intestinal Helmenthiasis in Debre Zeit. (unpublished DVM thesis, AAU, FVM).
5. In 1991 G.C. The Prevalence of Mastitis in Ethiopia and The Effect of Mastitis on Milk and Milk Products. (unpublished DVM seminar paper, AAU, FVM).

7. REFERENCES

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10. SIGNED DECLARATION SHEET

I, the undersigned, declare that the thesis is my original work and has not been presented for a degree in another university, and that all sources of material used for the thesis have been duly acknowledged.

Name Shihun Shimeles Beyene

Signature ____________________

Date of submission_________________

The thesis has been submitted for examination with our approval as university advisors

Dr. Gezahegne Mamo_____________________

Dr. Gobena Ameni________________________