Dissertation Ref. No. ____________

EPIDEMIOLOGY OF BOVINE TUBERCULOSIS AND ITS ZOONOTIC TRANSMISSION IN CONTACT HUMANS IN EAST SHEWA ZONE, CENTRAL ETHIOPIA

PhD Dissertation

BY

Tefera Woldemariam Agago

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Microbiology, Immunology and Veterinary Public Hhealth

PhD Program in Veterinary Public Health

FEBRUARY 2019

BISHOFTU, ETHIOPIA

EPIDEMIOLOGY OF BOVINE TUBERCULOSIS AND ITS ZOONOTIC TRANSMISSION IN CONTACT HUMANS IN EAST SHEWA, CENTRAL ETHIOPIA

A dissertation submitted to the Department of Microbiology, Immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture of Addis Ababa University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Veterinary Public Health

By

Tefera Woldemariam Agago

February 2019

Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Microbiology, Immunology and Veterinary Public Health

As members of the Examining Board of the final PhD open defense, we certify that we have read and evaluated the Dissertation prepared by Tefera Woldemariam titled: Epidemiology of bovine tuberculosis and its zoonotic transmission in humans in EastSshewa, central Ethiopia, and recommend that it is accepted as fulfilling the dissertation requirement for the degree of Philosophy in Veterinary Public Health.

Chairman (title and name)	Signature	Date
Internal Examiner (title and name)	Signature	Date
External Examiner (title and name)	Signature	Date

Final approval and acceptance of the dissertation is contingent upon the submission of its corrected copy to the CGC through the concerned departmental graduate committee.

I hereby certify that I have read the revised version of this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Dissertation advisor	Signature	Date
Dissertation advisor	Signature	Date
Department chair	Signature	Date

EPIDEMIOLOGY OF BOVINE TUBERCULOSIS AND ITS ZOONOTIC TRANSMISSION IN CONTACT HUMANS IN EAST SHEWA, CENTRAL ETHIOPIA

Tefera Woldemariam Agago
PhD Dissertation
Addis Ababa University (2019)

ABSTRACT

*Bovine tuberculosis (BTB) is a chronic disease characterized by progressive development of granulomatous lesions or tubercles in lung tissue, lymph nodes and or other organs. BTB is caused by Mycobacterium bovis (M. bovis) and has economic and public health significance. The available data indicated that BTB is endemic to Ethiopia with high prevalence in dairy farms although additional studies are required to establish the national prevalence and its zoonotic role in different areas of the country including East Shewa. Thus, the objective of this study was to investigate the epidemiology of BTB and its zoonotic transmission to humans in East Shewa, Central Ethiopia. Furthermore, the causative agents of TB in cattle and human were isolated and characterized using molecular tools. The study was conducted on 501 beef cattle, 1038 dairy catlle, 1896 cattle slaughtered at Adama and Bishoftu Abattoirs and 392 in contact humans. A cross-sectional study design, single intrademal cervical comparative tuberculin tests (*SICCTT*), mycobacteriological cultures, Ziel-Neelsen staining, and spoligotyping were used for undertaking this study. In addition, the SITVIT2 database and the online "Run TB-Lineage" database were used to identify the spoligotype international type (SIT) numbers and lineages or sub-lineages respectively. The herd prevalences of BTB in dairy cattle in Adama were 7.3% (95% CI: 4.29 - 11.98) and 5.8 %(95% CI: 3.05-9.95) at ≥ 2mm cut-off and ≥ 4mm cut-off points, respectively. While the individual animal prevalence were 2.1% (95% CI: 1.35 – 3.22) and 1.6% (CI: 0.9-2.6) at ≥ 2mm cut-off and ≥ 4mm cut-*off points, respectively. Furthermore, the individual *animal prevalence of BTB in the feedloat at Adama were 9.58 % at ≥ 2*mm cut-off and 4.39% *at ≥ 4mm cutoff while,* the TB like lesion based prevalence was 4.2% (80/1896) at Adama and Bishoftu Slaughter houses. Culture positivity was confirmed in 24.75% (25/101 tissues) of the 80 cattle with suspicious lesions. Zoonotic transmission was observed by the isolation of M. bovis in 1.13 % (1/88) possible culture positive sputum samples while the remaining 99% were idenified as Mycobacterium tuberculosis (MTB). Further identification of the MTB at the strain level showed that 20 shared types consisting of 67 isolates and 21 orphan types consisting of 21 isolates. In conclusion, the prevalence of BTB were low both in dairy and beef farms in East shewa. Moreover, the lesion prevalence was low in* *cattle slaughtered at Adama and Bishoftu abattoirs. Cosequently, the isolation of M.bovisfrom in contact humans was also low in the area while a significant number of MTB was isolated from lesions of cattle. Thus, in general, these findings warrant for launching of practically applicable control strategy before the magnitude of TB due to M. bovis both in cattle and humans.*

Key words: Abattoir, *Beef farm, , Dairy farm, East Shewa, Prevalence, Tuberculosis, Zoonosis*

STATEMENT OF AUTHOR

First, I declare that this dissertation is my bonafide work and that all sources of material used for this dissertation have been duly acknowledged. This dissertation has been submitted in partial fulfillment of the requirements for a PhD degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College Library to be made available to borrowers under rules of the Library. I solemnly declare that this dissertation is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Brief quotations from this dissertation are allowable without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however permission must be obtained from the author.

Name: Tefera Woldemariam Agago Signature: _______________

Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia

Date of Submission: ___________________________

ACKNOWLEDGEMENTS

I am highly indebted to my advisors, Prof. Gobena Ameni and Dr Gezahegne Mamo as without their encouragement, insight, guidance and professional expertise the completion of this work would not have been possible. My special appreciation also goes to Prof. Mahendra Pal who had advised me on matters pertinent to the dissertation. A special word of thanks goes to Prof. Etana Debela from the very beginning; he facilitated to join this PhD research. I thank all other staffs for their support, encouragement and understanding in sharing my teaching responsibility during the study period. I would like to thank Drs Zerhun Asefa, Teshale Sori and Girmay Medhin for offering me Stata software for data analysis. The PhD research work was partially supported by thematic Research Grand obtained from ALIPB and CVMA Addis Ababa University and Hawassa University.

In addition, I thank laboratory staffs of ALIPB for all supports and encouragement during field and laboratory work. Especially I am deeply grateful to Aboma Zewude, Samuel Bekele, Mahlet Chanyalew, Frehiwot Ayele, Yemisrach Girma, Adane Worku, Tadesse Regassa, Nega Nigussie, Hailu Getu and Dr Temesgen Mohammed for their excellent support in field research work in the challenging condition with cattle and in laboratory work at ALIPB. My great appreciation to Dr Zufan Bedawe for her support in searching some molecular databases. I thank all farm owners and East Shewa Zone Agricultural Office staffs that participated in this study, particularly Kedir Gena and Gizaw Sidda. My unreserved gratitude also goes to my sincere wife Dr. Olga Karanfil for her love, dedication, care, patience, responsibilities and continuous encouragement during those study years of long time. Special thanks go to my daughter Katarina Tefera and my son Tony Tefera for their understanding and everlasting love. Finally but not least I would like to thank all my friends certainly not forgotten for their support and encouragement during the study period.

DEDICATION

This dissertation manuscript is dedicated to my father, Woldemariam (Eligo) Agago and my mother Kurre Boto, for their love and excellent thought in education and dedicated willing in the success of my life. You will be always in my heart and never forgotten.

LIST OF TABLES

LIST OF FIGURES

Figure | Page

LIST OF APPENDICES

ABBREVIATIONS

AAU	Addis Ababa University
AFB	Acid-fast bacillus
AIDS	Acquired immune deficiency syndrome
ALIPB	Aklilu Lemma Institute of Pathobiology
Bp	base pair
BTB	Bovine tuberculosis
CAS	Central-Asian
CBN	Conformal Bayesian network
CDC	Center for disease control
CFSPH	Center for Food Security and Public Health
CFU	Colony forming unit
CI	Confidence interval
CIDT	Comparative intradermal tuberculin test
CM/AS	Common mycobacteria/additional species
CSA	Central statistical agency
DNA	Deoxyribonucleic acid
DOTS	Direct observation treatment short course
DR	Direct repeat
DTH	Delayed type hypersensitivity
DVR	Direct variant repeat
EA	Euro-American
EAI	East African-Indian
ELISA	Enzyme-linked immuno-sorbent assay
EPTB	Extra-pulmonary tuberculosis
ETR	Exact tandem repeats
FAO	Food and agriculture organization
FMOH	Federal Ministry of Health
GDP	Gross domestic product
HBCs	High burden countries
HIV	Human immunodeficiency virus
IFN-γ	Interferon gamma

IGAD	Intergovernmental authority on development
IS	Insertion sequence
ITIS	Integrated Taxonomic Information System
KB	Kilo-base
LJ	Lowenstein-Jensen
MDGs	Millennium development goals
MDR	Multi drug resistant
MEDC	Ministry of economic development and cooperation
MIQD	Meat inspection and quarantine division
MIRU	Mycobacterium interspersed repeated unit
MLVA	Multiple-locus variable analysis
MMWR	Morbidity and Mortality Weekly Report
MoARD	Ministry of agriculture and rural development
MPCR	Multiplex polymerase chain reaction
MTB	Mycobacterium tuberculosis
MTC	Mycobacterium tuberculosis complex
NCBI	National center for biotechnology information
NMSA	National meteorological service agency
NTM	Non-tuberculosis mycobacteria
OIE	Office international des epizooties
OR	Odds ratio
PCR	Polymerase chain reaction
PPD	Purified protein derivatives
PPD-A	Purified protein derivatives-avian
PPD-B	Purified protein derivatives -bovis
PTB	Pulmonary Tuberculosis
RD	Region of difference
RDAf1	Region of difference Africa 1
RDAf2	Region of difference Africa 2
RDEu1	Region of difference European 1
RFLP	Restriction fragment length polymorphism
SICCT	Single Intra-dermal Comparative Cervical Tuberculin
SIT	Shared international type

SOP	Standard operating procedure
SNP	single nucleotid polymorphism
TB	Tuberculosis
TST	Tuberculin skin test
VNTR	Variable nucleotide tandem repeat
WHO	World health organization
χ^2	Chi-square
ZN	Ziehl Neelsen

TABLE OF CONTENTS

1. INTRODUCTION

Mycobacterium bovis (M. bovis) is the causative agent of bovine tuberculosis (BTB) and belongs to the *Mycobacterium tuberculosis* complex (MTC) of bacterial strains (Smith *et al.,* 2006; Wirth *et al.*, 2008). The MTC is a group of genetically very closely related pathogens, which can cause tuberculosis (TB) disease with similar pathology in a variety of mammalian species (Smith *et al.,* 2006). The most prominent member of the MTC is *Mycobacterium tuberculosis (*MTB*),* the principle causative agent of TB in humans, causing each year more than 1.5 million deaths and having experienced a recent re-emergence through the advent of extended forms and the appearance of multi drug resistant strains (Young *et al.,* 2008). Other members of MTC are *M .tuberculosis, M. bovis, M. africanum, M. canettii, M. microti, M. caprae- M. orygis, M. suricattae, and lastly recognized M. mungi (Alexander et al., 2010).*

BTB is a chronic, generally respiratory disease, which is clinically difficult to diagnose although emaciation, loss of appetite, chronic cough and other signs of pneumonia could be symptoms developing at relatively late stages of the infection in cattle (Ayele *et al.,* 2004). Especially in developing countries, clinical forms of many other chronic, emaciating diseases, like African trypanosomiasis, chronic contagious bovine pleuro-pneumonia (CBPP) or chronic multiparasitism, are difficult to be distinguished from BTB. BTB pathology is characterized by the formation of granulomatous lesions, which can within the course of the disease regress or exhibit extensive necrosis, calcify or liquefy and subsequently lead to cavity formation (Cassidy, 2006).

During meat inspection, procedures on cattle carcasses in slaughter houses, TB lesions are primarily found in the upper and lower respiratory tract and associated lymph nodes (Cassidy, 2006). However, the bacteria can also develop a systemic infection, disseminate within its host and affect other organs (Coetzer, 2004). *M. bovis* is a slow growing, facultative intracellular, aerobic and gram-positive bacterium with a dysgonic colony shape when cultured on Löwenstein-Jensen (LJ) medium (Kubica *et al.,* 2006). As all *Mycobacterium* spp., *M. bovis* has an unusual cell wall surface structure characterized by the dominant presence of mycolic acids and a wide array of lipids (Glickman and Jacobs, 2001). This waxy lipid envelope confers an extreme hydrophobicity, which renders the bacteria acid- and alcohol-fast, a feature that can be exploited to identify mycobacteria via the Ziehl-Neelsen staining technique (Staingart *et al.*, 2006).

The mycobacterial surface lipids also have a potent biologic activity and are thought to play a crucial role in pathogenesis (Glickman and Jacobes, 2001). *M. bovis* can be identified based on specific biochemical and metabolic properties. e.g., *M. bovis* requires pyruvate as a growth supplement, is negative for niacin accumulation and nitrate reduction, shows microaerophilic growth on Lebek medium and is generally resistant to pyrazinamide (Kubica *et al.*, 2006 and Cole, 2002). In contrast, MTB does not require pyruvate as a growth supplement, is positive for niacin accumulation and nitrate reduction, shows aerophilic growth on Lebek medium, and is usually not mono-resistant to pyrazinamide (Kubica *et al.*, 2006 ; Cole, 2002).

M. bovis isolates are resistant to pyrazinamide because the organism does not produce the enzyme pyrazinamidase, which is needed to convert pyrazinamide into pyrazinic acid, the active form of the antimicrobial agent (Barouni *et al.*2004). This resistance is one of the basic features, which can be used to distinguish isolates of M. bovis (universally resistant to pyrazinamide) from M. tuberculosis (commonly susceptible).

Although still present in some industrialized countries, BTB today mostly affects developing countries lacking the resources to apply expensive test and slaughter schemes. In Africa, BTB is present virtually on the whole continent; however, little accurate information on its distribution and prevalence is available. Identification of *M. bovis* by cultureand molecular techniques is important for definitive diagnosis. The MTC is a genetically related group of mycobacterial species that can cause TB in humans or other living things. MTC comprises of MTB, *M. bovis, M. africanum, M. canettii, M. microti, M. orygis, M. caprae, M. pinnipedii, M. suricattae* and recently recognized *M. mungi* (Alexander *et al.,* 2010).

The existence of BTB in Ethiopia first reported in 1960s based on detection of tuberculous lesions in cattle at slaughterhouse (Haile-Mariam, 1975).Until very recently, that much of the information regarding the status of BTB in different regions of Ethiopia emanates from recording of lesions at abattoirs and skin test result in different part of the country.Since then pathological findings including BTB lesions, have compiled from major abattoirs and reports sent monthly and annually to the meat inspection and quarantine division of the Ministry of Agriculture.

Zoonotic TB is an important public health concern worldwide, especially in developing countries, because of deficiencies in preventive and/or control measures (Etter *et al.,* 2006). In developed countries, the disease has almost been eradicated after the implementation of preventive and control measures such as testing, culling or pasteurization of milk. Since BTB remains a worldwide problem, it is imperative to intensify control and preventive measures aimed at its eradication. The incidence of *M. bovis* in humans probably remains underestimated, as a distinction between the mycobacterial species, i.e. *M. bovis* and MTB, is not systematically performed. Since the real incidence of *M. bovis* in human health is still unknown, it is essential to advance the eradication of BTB worldwide by means of adequate programmes, especially in developing countries (Grange, 2001).

TB in human caused by MTB and *M. bovis* is indistinguishable clinically, radiologically and pathologically (Wedlock *et al.,* 2002). Humans often acquire infections through inhalation of aerosols or consumption of contaminated milk and possibly meat. The World Health Organization's (WHO) "END-TB" goal is to eliminate all forms of human TB as a public health problem by 2035. However, the contribution of zoonotic TB to human TB is poorly described, particularly in sub-Saharan Africa, where a combination of endemic bovine BTB evolving human-animal interfaces (e.g. expanding dairy production and increasing global animal-based protein consumption).

Moreover, the disease causes significant animal health-induced economic loss, and its impacts include reduction in productivity, movement restrictions, screening costs, culling of affected animals, and trade restrictions (OIE, 2016).

Different molecular markers and techniques have been discovered and developed in the past that allow the unambiguous identification and differentiation of *Mycobacterium* species and the members of the MTC (Boddinghaus *et al.,* 1990; Durr *et al.,* 2000).

1.1 The Rationale of the study

East Shewa Zone is one of the zones known for its dairy and beef cattle production. Particularly, beef production is widely practiced in the Zone. In addition, there are several butchers in the area and these butchers have been engaged in selling predominantly of raw meat to the consumers including people who travel to Sodere Resort from the different regions of Ethiopia. There are around 100 butchers, 97 feedlot farms and around 500 dairy farms in the Adama Town alone. Moreover, there is high mobility of human and animal populations in the area. Thus, these entire situations could creat favourable environment for the transmission of mycobacteria within the cattle population, and their zoonotic transmission between the cattle and the human populations. However, inspite of such favourable conditions, few has been conducted on the epidemiology of BTB and its zoonotic transmission in contact human in the area. Hence, this study was undertaken to investigate the epidemiology of BTB and its zoonotic transmission to human contacts in the East Shewa Zone. Furthermore, the identification of mycobacteria was undertaken at the species and strain levels. Consequently the scientific evidences obtained from this study would help to improve the existing of TB prevention and control in Ethiopia.

1.2 Objectives of the study:

General:

To investigate the epidemiology of TB in cattle and humans in East Shewa, Central Ethiopia using conventional and molecular tools

Specific:

- To estimate the prevalence of BTB and assess risk factors in beef feedlot and dairy cattle at Adama and surrounding towns of central Ethiopia
- To estimate the prevalence of BTB and assess possible risk factors related to individual and herd in cattle slaughtered at Adama and Bishoftu abattoirs in East Shewa, central Ethiopia
- To investigate the strais circulating between cattle and in contact human East Shewa
- To isolate and characterize the species and strains of *MT*C species isolated from cattle and humans in East Shewa

2. LITERATURE REVIEW

2.1 Mycobacteria

Mycobacterium (M) is acid fast, aerobic, non-motile, capsulated and non-spore forming bacteria. Its taxonomic classification is as presented in Table. The genus name, Mycobacterium, is originally proposed in 1896 by Lehmann and Neumann (ITIS, 2017). At present, Mycobacterium consists of 170 different species (Forbes, 2017). Robert Koch discovered the major causative agent of TB, *MTB* in 1882 which was called Koch's bacilli (Taylor *et al.*, 2003). Through time it is known that TB is caused by a group of genetically very similar bacteria called *MT*C.

Table 1: Mycobacterial Taxonomy

Kingdom:	Bacteria
Subkingdom:	Posibacteria
Phylum:	Actinobacteria
Subclass:	Actinobacteridae
Order:	Actinomycetales
Sub-order:	Corynebacterineae
Family	Mycobacteriaceae
Genus:	Mycobacterium

(ITIS, 2017)

2.2 - Mycobacterium tuberculosis-complex (*MT*C)

The mycobacteria grouped in the *MT*C are characterized by 99.9% similarity at the nucleotide level and identical 16S rRNA sequences but differ widely in terms of their host tropisms, phenotypes, and pathogenicity, suggesting that they all derived from a common ancestor (Böddinghaus *et al.*, 1990; Sreevatsan *et al.*, 1997; Brosch *et al.*, 2002). *MT*C species, namely, *MTB*, *M. africanum*, *M. microti*, *M.canetti*, and *M. bovis*, can be categorized according to a restricted number of laboratory phenotypes and genetic markers but, importantly, differ in physiological characteristics, virulence and host range (Mostowy *et al.*, 2005).

Though it has been conventionally established that *MTB* and *M. africanum* are isolated from humans, *M. microti* from voles, and *M. bovis* predominantly from cattle, reports of MTC organisms in a variety of other domesticated and wildlife hosts pose a challenge to this classification scheme (Mostowy *et al.*, 2005). *M. microti* causes TB mainly in small rodent-like voles, but until now, its importance for TB in humans has remained unclear (Niemann *et al.*, 2000). The *M. Canetti* can cause TB in humans, but so far only a few *M. canetti* strains have been isolated and its epidemiological contribution to TB in humans is uncertain (Van-Soolingen *et al.,* 1997).

The two subgroups of *M. africanum* have been described as subtype I and II. Numerical analyses of their biochemical characteristics revealed that *M. africanum* subtype I is more closely related to *M. bovis*, whereas subtype II more closely resembles *MTB* (Niemann *et al.*, 2002; Djelouadji *et al.*, 2008). Thus, *M. africanum* is an intermediate species between *M. tuberculosis* and *M. bovis* (Johnson *et al.*, 2008). The host range of *M. bovis* is considered to be the broadest of the complex, causing disease across a variety of animals. Other member of MTC is the *M. caprae* that isolated from goats (Aranaz *et al.*, 1999; Aranaz *et al.*, 2003). The *M. pinnipedii* is also species in the MTC, based on host preference, phenotypic and genotypic characteristics. *M. pinnipedii* has been isolated mainly from sea lions and seals (Cousins *et al.*, 2003). Alexander *et al.* (2010) reported *M. mungi* as new member of MTC causing disease outbreak in banded mongooses in Botswana (Figure 1).

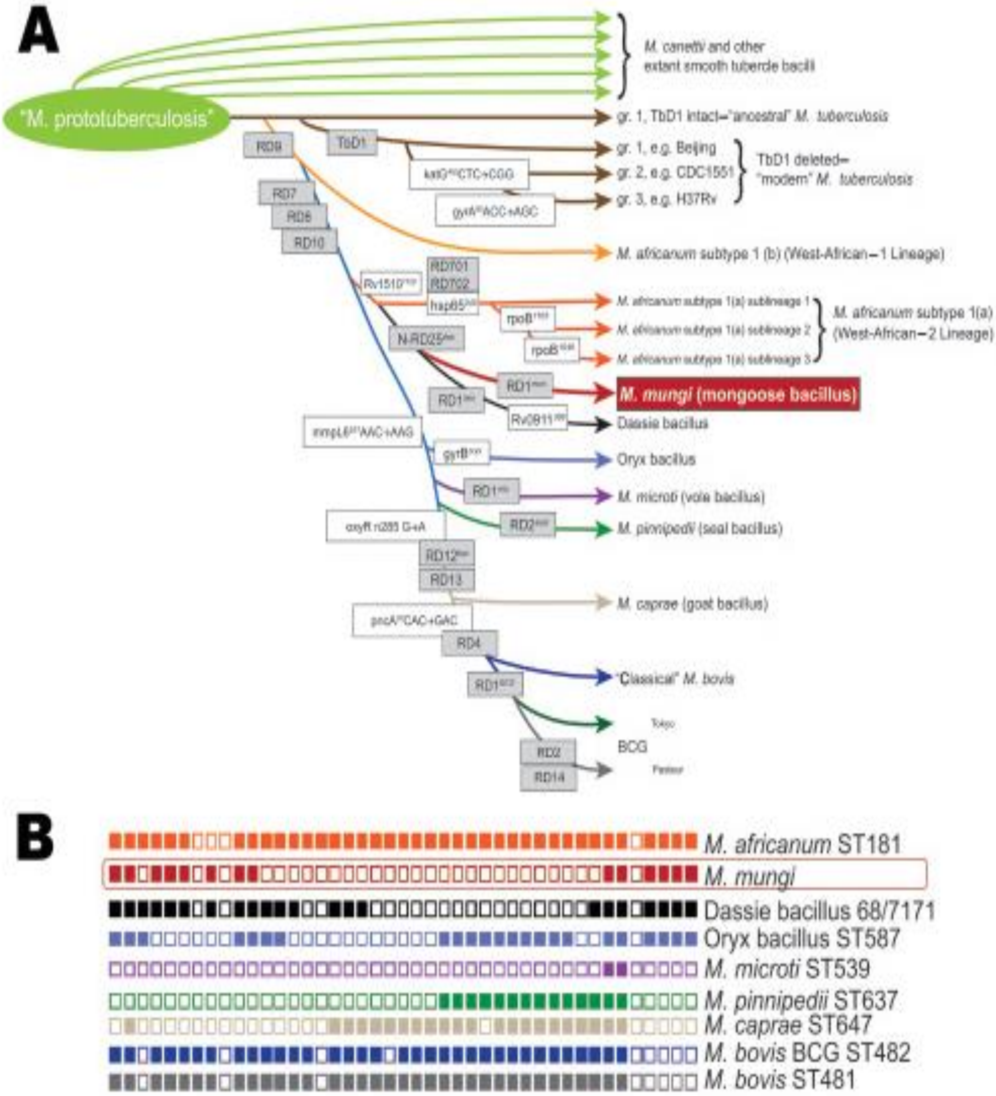


Figure1. Schematic of the phylogenetic relationships among MTC a newly discovered *M. mungi*.

A) Schematic of the phylogenetic relationships among MTC species, including newly discovered *M. mungi*, based on the presence or absence of regions of difference (gray boxes) as well as specific single-nucleotide polymorphisms (white boxes), modified from (Gordon *et al.*, 2009).

B) Spoligotype of *M. mungi* compared with representative spoligotypes from other *M. tuberculosis* complex species (Alexander *et al.*, 2010).

2.2.1. Mycobacterium bovis

Mycobacterium bovis can infect and cause BTB in all bovid animals including cattle and buffalo (O'Reilly and Daborn, 1995; Ayele *et al*., 2004; Radunz, 2006). This highly-adapted and 'successful' pathogen has a world-wide distribution and its host range is considered to be the broadest and complex, causing disease across a variety of animals species (Pollock and Neill, 2002; Mathews *et al*., 2006; Carslake *et al*., 2011). *M. caprae* can also affect other domestic and wild animals as well as humans (Amanfu, 2006). Other members of the MTC group such as *MTB*, *M. africanum* and *M. canetti* are predominantly human pathogens (Bezos *et al*., 2012).

A phylogeny of the MTC has recently shown that the animal-adapted strains are found in a single lineage marked by the deletion of chromosomal region (RD9) (Brosch *et al.*, 2002). The classical *M. bovis* showed the greatest number of RD deletions and seem to have undergone the greatest loss of Deoxyribonuecleotid acid relative to other members of the MTC. These lacked regions are RD4, RD5, RD6, RD7, RD8, RD9, RD10, RD12, and RD13 (Brosch *et al.*, 2002) and it is thought that *M. bovis*, is the most recent member of this lineage (Smith *et al.*, 2004) (Figure2).

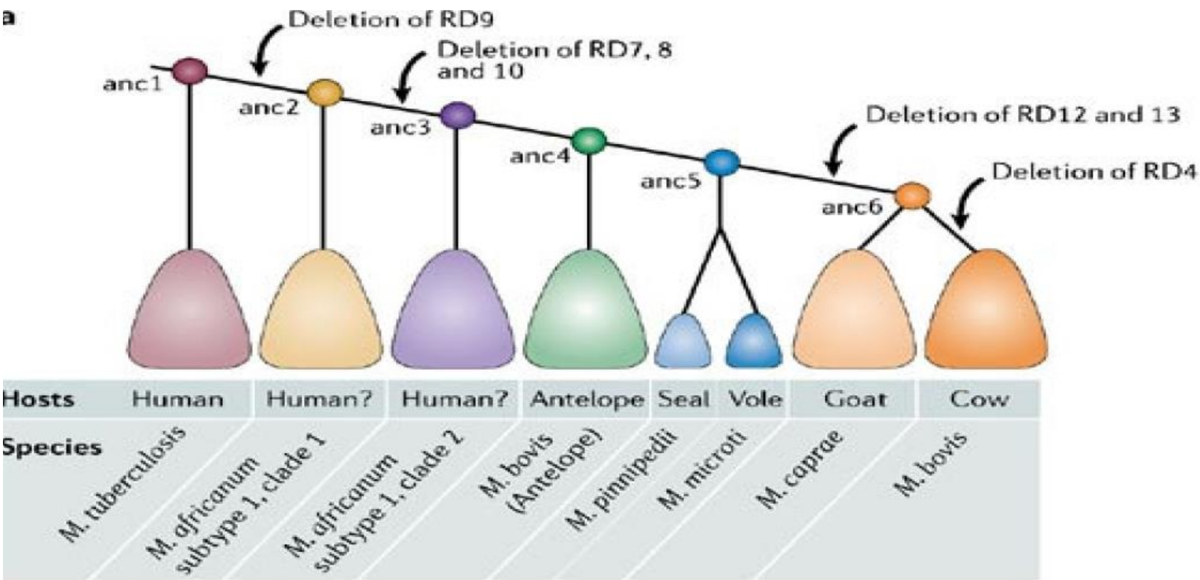


Figure 2: Evolutionary scheme of the members of the *MTC* (Brosch *et al*., 2002).

Prvious molecular studies based on deletion analysis of specific chromosomal regions and absence of specific spacers in standard spoligotype patterns revealed that certain *M. bovis* strains occur in high frequency in cattle population of specific regions. They are characterized as clonal complex of *M. bovis* and epidemiologically dominant and geographically localized in the occurring regions. Some of such clonal complexes of *M. bovis* that have been identified are African 1 (Af1) clonal complex (Muller *et al.*, 2009), African 2 (Af2) clonal complex (Berg *et al.*, 2011), European 1 (Eu1) clonal complex (Smith *et al.*, 2011) and European 2 (Eu2) clonal complex (Rodriguez-Campos *et al.*, 2012).

2.3. Epidemiology of human tuberculosis

2.3.1 Global epidemiology

TB is an ancient disease, which still create human health problem. In 2016, it was among the leading cause of human death worldwide, which stands ninth. Globally there were about 10.4 million incident cases of TB in 2016 of which 45% cases were in South East Asia regions, 25% cases were in Africa region, 17% cases were in Western Pacific region, 7% cases were in East Mediterranean region, 3% cases in European region and 3% cases were in Americas region (WHO, 2017). It is estimated that one-fourth of the world's population (~1.9 billion human being) are infected with the disease-causing agent (Houben and Dodd, 2016).

The five countries that stood out as having the largest number of incident cases in 2016 were (in descending order) India, Indonesia, China, the Philippines and Pakistan (figure 3), which together accounted for 56% of the global total. Of these, China, India and Indonesia alone accounted for 45% of global cases in 2016. Nigeria and South Africa each accounted for 4% of the global total (WHO, 2017).

Figure 3: Estimated TB incidence in 2016 for countries with at least 100 000 incident cases

The 30 high TB burden countries accounts for 87% of global TB incidence and global deaths associated with TB in 2016. Of the global deaths related to TB, Africa accounts for 32% of deaths due to TB without HIV co-infection and 86% of deaths due to TB with HIV co-infection (WHO, 2017). Among the 30 high TB burden countries 16 countries are in the continent of Africa. The incidence of TB cases in these 16 countries was ranging from 11,000 TB cases in Namibia to 438,000 TB cases in South Africa in 2016. Ethiopia is the fourth in TB burden countries in the Africa continent with 182,000 new TB cases in 2016. Ethiopia is fifth in multi-drug resistant TB (MDR-TB) burden from countries in the Africa continent and 17th from countries in the world with 5,800 new MDR-TB cases in 2016 (WHO, 2017).

2.3.2 Epidemiology of TB in Ethiopia

TB in Ethiopia has been recognized as public health concern before 1960s and TB control effort began in 1960s through establishing TB centers and sanatorium (FMOH, 2008). TB in Ethiopia is recognized as the third cause of hospital admission and the second cause of deaths (FMOH, 2008). Since the mid-1990s Direct Observed Treatment Short-course (DOTS) has been recommended by WHO. DOTS have been started in Ethiopia in 1992 as a pilot (FMOH, 2008) and since then Ethiopia has been implementing DOTS. TB incidence per 100,000 population in Ethiopia has been declined from 260 TB cases in 1997 to 177 TB cases in 2016 (WHO, 2017). The prevalence of MDR-TB in Ethiopia has been increasing from 1.6 to 2.3% in new TB cases and 11.8 to 17.6% in re-treatment TB cases from 2005 to 2014 and 17.6% of all TB patients were co-infected with HIV (EPHI and FMOH, 2015).

There were about 30,000 deaths due to TB with and without HIV in 2016 (WHO, 2017). In 2015, a total of 135,831 TB cases were notified of which bacteriologically confirmed TB cases were 35.1%, clinically diagnosed TB cases were 32.4%, EPTB cases were 29.8% and re-treatment TB cases were 2.7% (FMOH, 2015). TB case notification per 100,000 populations in 2015 varied from region to region. The highest TB case notification was reported in Dire Dawa about 400 TB cases per 100,000 populations and the lowest TB case notification was reported in Somalia less than 100 TB cases per 100,000 populations (FMOH, 2015).

2.3.2 Mycobacterium tuberculosis infection in animals

MTB considered primarily a human pathogen. Apart from humans, *MTB* infection has been reported in a wide range of domestic or wildlife animal species, most frequently living in close, prolonged contact with humans: e.g., in captive settings (Sternberg *et al*., 2002; Sulieman and Hamid, 2002; Alfonso *et al*., 2004). Among domestic animals, infection with *MTB* has been most frequently identified in cattle (Thoen *et al*., 1981). According to published data, the prevalence of *MTB* infection in cattle herds did not exceed 1% in the majority of studies (Pavlik *et al*., 2003). However, a few exceptions, like Algeria and Sudan (Sulieman and Hamid, 2002) and Ethiopia (Berg *et al.,* 2009; Ameni *et al*, 2011 and 2013) weredescribed as a most probable consequence of the high prevalence of human TB in these three African countries.

The risk of spillover of *MTB* from humans to animals is considered high where tuberculosis in humans continues to be of great public health concern. *MTB* does not appear to have an indigenous animal host or reservoir and the animals that become infected represent most probably accidental hosts (Thoen and Steele, 1995). Humans suffering from active TB are strongly believed to represent the main source of *MTB* in animals, including cattle. This was demonstrated in an 11-year study on *MTB* cases in the National Zoological Gardens of South Africa, which indicated that visitors to animals more frequently transmitted the disease than between animals (Michel *et al.*, 2003). Reinforcing the above idea, pathological changes in cattle do not appear to support disease transmission, since *MTB* infection usually does not progress beyond the development of small granulomas in several different lymph nodes (Cousin *et al.*, 2004).

The number of documented cases of *MTB* in cattle appears to have increased in recent years (Cadmus *et al.*, 2006; Berg *et al.*, 2009; Chen *et al.*, 2009) which may be due to improved diagnosis by molecular tools and/or an actual increase in transmission from humans to cattle in these countries. At the same time many developing countries have intensified their livestock production to meet the growing demand for food security, which has led to a higher risk of transmission for both *MTB* as well as *M. bovis* at the human–livestock interface (Michel *et al.*, 2010). Apart from molecular confirmation of numerous cases of human-to-human and some cases of animal-to-animal transmission of *MTB* (Alexander *et al.*, 2002; Zolnir-Dovc *et al.*, 2003) molecular tools has been used rarely for molecular confirmation of transmission of mycobacteria between humans and animals. The isolation of *MTB* from cattle and subsequent molecular typing adds more knowledge to previous scientific questions related to the role of humans as a source of infection for cattle. Alternatively, hypothesis like the possible existence of cattle adapted *MTB* strains and their subsequent role for cattle-to cattle and cattle-to-human transmission could similarly be investigated.

2.4 Epideiology of bovine tuberculosis

2.4.1. Maintenance, reservoir and spill-over host of Mycobacterium bovis

Domestic and non-domestic animals may be considered either as maintenance (or reservoir) hosts or as non-maintenance (or spillover) hosts for BTB. Maintenance hosts are those that can maintain infection in an area in the absence of cross transmission from other species of domestic or wild animals (Haydon *et al.*, 2002). Maintenance hosts are critical in disease epidemiology and control because without intervention, disease will persist indefinitely. Hence, the most efficient disease control efforts are aimed at maintenance hosts (Palmer *et al.*, 2012). In contrast, spillover hosts become infected with *M. bovis* but the infection only occurs sporadically or persists within these populations if a true maintenance host is present in the ecosystem (Haydon *et al*., 2002). Spillover hosts are usually 'dead-end' hosts, in which the incidence and pathology of the disease indicates that they play no significant role in transmission. Spillover hosts may also be 'amplifier' hosts if they act as incidental sources of BTB for livestock or other species and wildlife can be source of transmission back to domestic animals (spillback) (de Lisle *et al*., 2001). Both, maintenance and spillover hosts may act as a disease vector (Corner, 2006).

Domestic cattle are considered the true natural hosts of the bacterium and the principal reservoir of infection for other animals and man. Amongst domestic animals, captive (farmed) deer and, occasionally, goats can act as maintenance hosts of *M. bovis* (Cousins, 2001). Amongst free-living mammals, the brushtail possum and the ferret in New Zealand, the Cape buffalo in parts of South Africa, Kafue lechwe antelope and other bovide in Africa, the North American bison in parts of Canada and the white-tailed deer in Michigan , United State of American (USA), are also considered maintenance hosts of *M. bovis* (de Lisle *et al*., 2001). Species reported to be spillover hosts include sheep, goats, horses, pigs, dogs, cats, ferrets, camels, llamas, many species of wild ruminants including deer and elk; elephants, rhinoceroses, foxes, coyotes, mink, primates, opossums, otters, seals, sea lions, hares, raccoons, bears, warthogs, large cats and several species of rodents (de Lisle *et al*., 2002).

BTB is particularly rare in horses and sheep, which should be considered true dead-end hosts, the rest should be treated as potential amplifier hosts of *M. bovis*, which means that, if infected, these species may act as a source of infection for other animals and man. Infected humans can also act as amplifier hosts of BTB, representing a potential source of *M. bovis* for animal and human contacts (Smith *et al*., 2006a). Within maintenance community framework, BTB epidemiology may be represented as a dynamic multi-host system with three main 'compartments', namely wildlife, livestock and humans (Figure 4). BTB infections may be maintained (independently or not) within livestock populations and within wildlife populations, whereas human infections result from pathogen spillover from animals (Biet *et al*., 2005), and very rarely from human-to-human transmission (Michel *et al*., 2010).

Factors associated with BTB spillover from livestock to wildlife (Munyeme *et al*., 2009) should also influence BTB spillback from wildlife to livestock. The main risk factors are thus linked with: (i) the type of interface (fence, herding practices) and the distribution of resources (water and grazing), which directly influence contact patterns between livestock and wildlife (Berg *et al.*, 2009) the environmental conditions, which directly influence the persistence of BTB in the environment. In this wildlife–livestock–human interfaces husbandry practices (housing, mixing cattle with small ruminants), food preferences (consumption of raw milk) and overall health and hygienic conditions are identified as the main BTB risks of transmission between livestock and humans (De Garine-Wichatitsky *et al.*, 2013). The complex and dynamic interactions involving domestic animals, wildlife, and humans create environments favorable for the transmission of infectious diseases across different species.

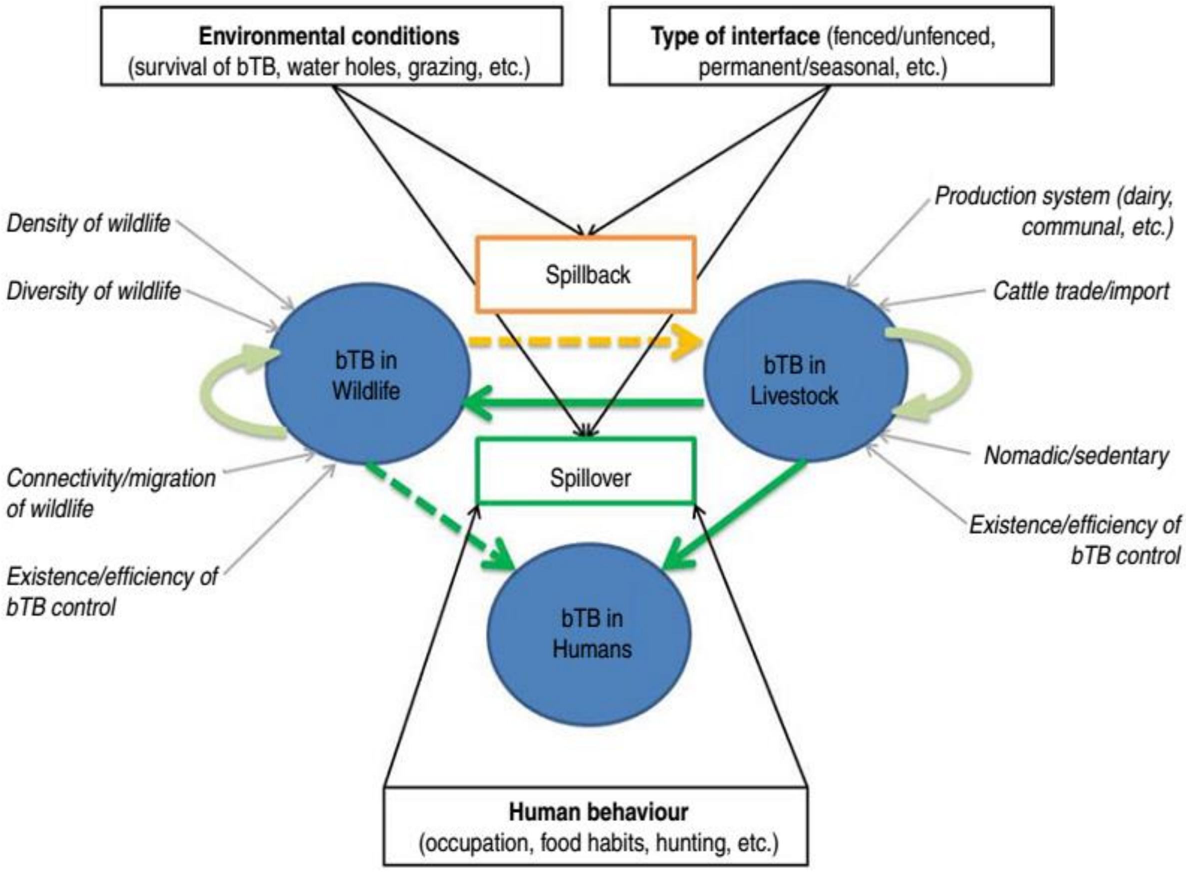


Figure 4: Interspecific transmission of BTB at wildlife–livestock–human interfaces (De Garine-Wichatitsky *et al.*, 2013).

2.4.2 Transmission of Mycobacterium bovis in animals

In theory, transmission can be either direct, through close contact, or indirect from exposure to viable bacteria in a contaminated environment. Age, environment and local farming practices are likely to influence the routes by which cattle become infected. Infection via the alimentary (ingestion) route would not be unexpected in young calves ingesting milk from tuberculous dams. Direct transmission via the respiratory route is also supported by natural cattle behavior, especially with high stocking density and substantial cattle movement (Skuce *et al.,* 2011). The respiratory and the alimentary routes are the main transmission pathways of BTB. Less described modes of disease transmission are vertical and genital transmission Percutaneous transmission was described in kudu and large predators resulting in granulomatous skin or muscle infections (Renwick *et al.*, 2007).

In cattle as well as in other animal hosts, the route of transmission of *M. bovis* can be deduced by the pattern of lesions observed in slaughtered animals. Animals with lesions restricted to the thoracic cavity are presumed to have been infected by the inhalation of aerosols, while those with lesions in mesenteric lymph nodes are thought to have acquired the infection by ingestion (Pollock and Neill, 2002). The nature and extent of tuberculous lesions vary with the route of exposure and the anatomical location of the lesions, which can subsequently, affects how *M. bovis* is excreted from the infected host (Gavier-Widen *et al.*, 2001).

In field cases of cattle, lesions are mainly found in the upper and lower respiratory tract and associated lymph nodes. Thus, it is considered that the inhalation of *M. bovis* is the most probable route of infection stated that the inhalation of one single *M. bovis* bacillus in an aerosol droplet would be enough to infect an animal., Dean, *et al.*, (2005) found experimentally that 1 colony forming unit (cfu) (6-10 bacilli) could induce tuberculosis immuno-pathology. This is supported by i) experimental studies that has shown that low numbers of bacilli are needed to experimentally infect animals via the respiratory tract, as compared to the large doses needed to infect animals via the digestive route (Menzies and Neill, 2000) and ii) the high frequency of tuberculosis lesions found in the respiratory tract and associated lymph nodes in cattle Johnson *et al.*, (2007).

Ingestion of contaminated products (e.g. carcasses/prey, pastures and water) is considered as a secondary way to spread the disease in cattle (Menzies and Neill, 2000), however it is an important pathway in wildlife (Renwick *et al.*, 2007). Survival of *M. bovis* in the environment is usually considered short, especially if environmental conditions are dry and sunny, as desiccation and ultra violat (UV) light kill them. However experimental studies conducted in

New Zealand, South Africa, Great Britain, Ireland and the USA have demonstrated that *M. bovis* can persist in environment substrate for varying amounts of time (Fetene *et al.*, 2011).

Although it is still one of the most important questions in BTB epidemiology, the exact nature and mechanism for transmission of infection, between and within herd, remains largely unanswered (Skuce *et al.*, 2011). Hence, determining the relative contribution of each of these transmission routes for within herd and between herd spread across different herd husbandry and management system will be helpful in diseases prevention and control program.

2.4.3. Husbandry system as transmission risk factors within and between herds

Husbandry practices in Ethiopia are divided into three categories, namely, extensive, intensive and smallholder production systems. The extensive system is traditional and the most popular husbandry practice as integrated extensive husbandry system (more in the highland areas) and as pastoral production systems (in the lowland areas) of the country, and is the main source of milk and meat but receives very little attention from veterinary services. The extensive farming system is practiced mostly in rural areas where animals share grazing land and watering points. The intensive systems are usually dairy and beef farms which are located in peri-urban areas and are intended for milk and beef production(Shitaye *et al.*, 2007). These distinct husbandry system have different impact on the prevalence and route of infection and hence on the distribution and severity of the diseases.

In extensive animal husbandry system where animal movement from one area to another and feeding of animals on open grazing pasture are common practices, possibility of contamination of grazing fields by the infective bacilli is the most likely condition and results in transmission of *M. bovis* infection through oral route (Kassa, 2014). In addition, in pastoral area in particular, In Ethiopia, several prevalence studies have been performed recently that show prevalence vary depending on the geographical areas, breeds and husbandry practices (Gumi *et al.*, 2012). Transmission of BTB seems to be higher (50%) (Ameni *et al.*, 2008) in intensive peri-urban settings as compared to (0.8%) reported in extensive rural and pastoral areas (Tschopp *et al.*, 2010). This could be attributed, on one hand, to the introduction of exotic breeds as opposed to local Zebu which are relatively less resistant to diseases and on the other hand to husbandry practices which are confined indoors, where close contact between animals and lack of ventilation increase chances of disease transmission (Kiros, 1998). Study by Ameni *et al.*, (2006) on cattle husbandry in Ethiopia as predominant factor affecting the pathology of BTB and gamma interferon responses to mycobacterial antigens indicated that cattle husbandry and cattle density are the dominant factors influencing disease severity and the distribution of the lesions.

In addition, this scenario can be more aggravated during a severe dry season, when drought takes place for a longer time in particular, so as animals will get enough time to be in contact with others, which favors the transmission of the infection (Shitaye *et al.*, 2007).

Unlike other determinant of diseases, the effect of management practice is not mutually exclusive from other risk factors. Therefore, a more detailed investigation in the components of farming systems could help to refine our understanding on diseases maintenance and transmission with and across herd of different management system.

2.4.4 Bovine tuberculosis worldwide

BTB negatively affects animal welfare and productivity worldwide with significant economic losses in some countries (Pollock and Neill, 2002). Infected cattle are important sources of infection for healthy cattle, but wildlife reservoirs of infection have also been reported in many regions (Wedlock *et al.*, 2002; Philips *et al.*, 2003; Kaneene and Pfeiffer, 2006; Thoen *et al.*, 2009), thereby complicating the epidemiological picture. BTB is an FAO-OIE "List B" disease because of its important socio-economic and public health impact (OIE, 2008). Bovine TB is also a serious zoonosis transmitted to humans through the consumption of contaminated raw or poorly cooked animal products (e.g.: fresh milk and meat), inhalation of aerosols from infected animals and contamination of breaks in the skin (CFSPH, 2009; Ayele *et al.*, 2004; Kaneene and Pfeiffer, 2006).

Strict control / eradication programmes have eliminated or nearly eliminated BTB from domesticated animals in many industrialized countries (Citron, 1988; Gilbert *et al.*, 2005; Abernethy *et al.*, 2006; Good, 2006; Goodchild and Clifton-Hadley, 2006; Pavlik, 2006a; CFSPH, 2009). However, endemic areas and infected herds still continue to be reported in some industrialized countries (Good 2006; Goodchild and Clifton-Hadley, 2006) because of the presence of BTB in wildlife species (e.g. wild white-tailed deer in the United States of America, badgers in the U.K. and Ireland, and brush-tailed opossums in New Zealand) which share the same environment as cattle (O'Reilly and Daborn, 1995; Bruning-Fann *et al.*, 2001; Good, 2006; Mathews *et al.*, 2006; Delahay *et al.*, 2007). Increasing rate or persistence of BTB has also been linked to husbandry trends of increasing herd sizes and changes in cattle genotypes (Goodchild and Clifton-Hadley, 2006).

2.4.5 Bovine tuberculosis in Sub-Saharan Africa

According to the World Animal Health Information databases, collating reports of member states regarding outbreaks of transboundary animal diseases to (1996–2011) the majority of African countries (38/54) reported BTB in livestock (Figure 5). However, of all nations in Africa, only seven apply disease control measures as part of a test-and-slaughter policy and consider BTB a notifiable disease (Fgure 6). The remaining nations have control the disease inadequately or not at all. Almost 15% of the cattle populations are found in countries where BTB is notifiable and a test-and-slaughter policy is required. Approximately 85% of the cattle and 82% of the human population of Africa are in areas where BTB is either partly controlled or not controlled at all (Cosivi *et al.*, 1998).

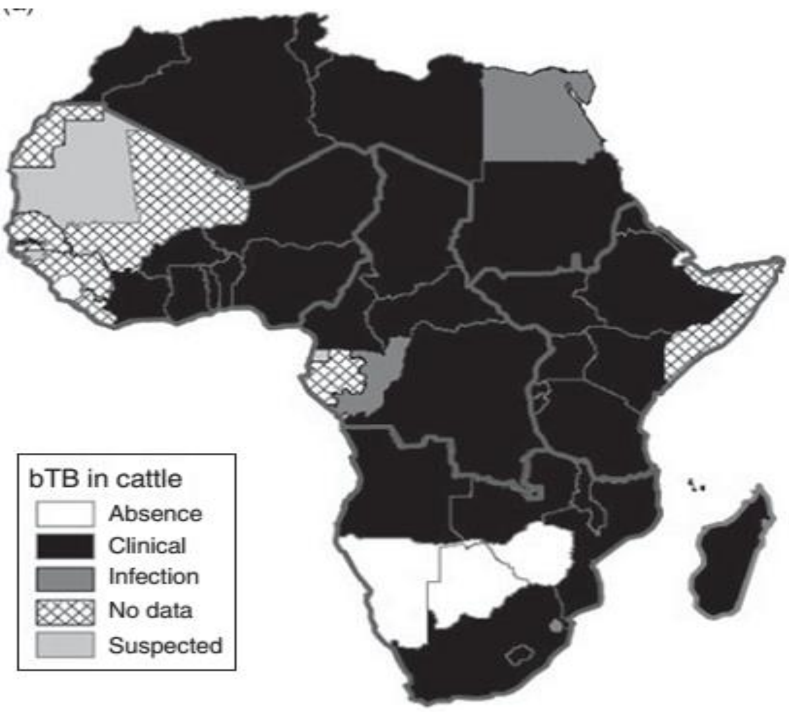


Figure 5: Distribution map of bovine tuberculosis in Africa during 1996–2011 (large grey lines indicate the African subregions as referred to in the text: West, Central, East and Southern Africa) (De Garine-Wichatitsky *et al*., 2013)

Figure 6. Control measures for bovine tuberculosis based on test-and-slaughter policy and disease notification, Africa (FAO/OIE/WHO Animal Health Yearbook 1993).

Though BTB represents a potential health hazard for both animal and human populations in most developing countries, *M. bovis* infection remains largely uninvestigated in Africa. Its epidemiology and public health significance remains largely unknown due to several factors including the high cost of testing programme, social unrest due to political instability, ethnic wars resulting in displacement of large numbers of people and animals, and a lack of veterinary expertise and communication networks (Michel *et al.*, 2010; Thoen *et al.*, 2010).

M. bovis was isolated in numerous African countries and there seem to be an endemic state of BTB in some of these nations (Rigouts *et al.*, 1996) of Burundi and (Sulieman and Hamid, 2002) of Sudan isolated *M. bovis* in 38%, 13% and 39.9% of abattoir lesions respectively. (Vekemans *et al.*, 1999) of Bobo Dioulasso, (Schelling *et al.*, 2000) of Chad, (Bonsu *et al.*, 2000) of Ghana, (Omer *et al.*, 2001) of Eritrea, (Oloya *et al.*, 2007) of Uganda found higher prevalence 13%, 17%, 13.8%, 14.5% and 6% positive reactors respectively to skin test. However, (Jiwa *et al.*, 1997) of Tanzania and (Cleaveland *et al.*, 2007) of Sudan, reported lower prevalence in cattle. In Ethiopia, of the 1,869 about 8.5% of tuberculin sensitive cows (12 of a sample of 141) (Elias *et al.*, 2008) and of 1171 animals, about 13.3% (4 of 30) (Regassa *et al.*, 2010) secreted acid-fast bacteria in their milk. Similarly, two (0.2%) of 805 milk samples secreted the bacilli in the Southern Highlands of Tanzania (Kazwala *et al.*, 1998). Both *M. bovis* and *MTB* have also been found in milk samples in Nigeria (Idrisu and Schnurrenberger, 1977) and Egypt (Nafeh *et al.*, 1992).

The WHO reported in1998 that 3.1% of TB in humans worldwide is attributable to *M. bovis* and that 0.4–10% of sputum isolates from patients in African countries could be *M. bovis* (Michel *et al.*, 2010). Zoonotic TB is now acquiring increasing recognition in developing countries, including Ethiopia, as animals and humans share the same environment (Kassa, 2014). This has also prompted researchers to evaluate its impact on human health. In Uganda, (Oloya *et al.*, 2008) found a prevalence rate of 7% *M. bovis* in humans suffering from cervical lymphadenitis in the Karamoja region, while study conducted in Tanzania in 2001 on human patients with cases of lymphadenitis reported that 16% of the cases were due to *M. bovis*(Kazwala *et al.*, 1998). In Democratic Republic of Congo, *M. bovis* was isolated from gastric secretions in two of five patients with pulmonary TB (Mposhy *et al.*, 1983). Isolation of *M. bovis* from sputum samples of patients with pulmonary TB has also been reported from Nigeria in which 3.9% of MTC isolates were *M. bovis*(Mawak *et al.*, 2006). In a more recent study in Ethiopia, (Gumi *et al*., 2012) and (Kassa, 2014) showed a link in the transmission of TB.

2.4.6 Bovine tuberculosis in livestock population of Ethiopia

BTB has been frequently reported in Ethiopia from small-scale studies. Prevalence varies depending on the geographical areas, the breeds and the husbandry practices. Yet large areas in the country remain un-investigated and as data is lacking across the different geographical areas, breed and host species, husbandry practices and wildlife reservoir, it is not ease to make association with these risk factors.

Different authors reported ranges of prevalence rate in Ethiopia based on abattoir based study. For instance Regassa *et al*., (2010) reported 1.1% prevalence at Hawassa; Woyessa *et al*., (2014) reported 5.9% at Nekemte Municipality abattoir; Shitaye *et al*., (2006) reported 3.46% in Addis Ababa; Teklul *et al*., (2004) reported 4.53% at Hossana; Ameni and Wudie, (2003)repoted 5.16% from Adama Manicipaliy abattoir; Ameni *et al*., (2010) repoted 5% at Kombolch meat processing plant, Southern Wollo; Regassa *et al*., (2010) reported 7.96% at Wolaita Sodo; Demelash *et al*., (2009) reported 4.2% at five different abattoirs including four Municipal (Addis Ababa, Adama, Hawassa and Yabello) and one export (Melge-Wondo) abattoirs and Mamo *et al*., (2011) reported 5% prevalence of gross tuberculous lesion in camels slaughtered at Dire Dawa abattoir in eastern Ethiopia. Hiko and Agga, (2011) reported 4.2% abattoir prevalence of BTB in Mojo export abattoir base on gross lesions.

Prevalence in dairy farms with cross-breeds varying between 3.5% and 50% (Shitaye *et al*., 2007; Demelash *et al*., 2009; Regassa *et al*., 2010). Skin test prevalence in traditionally kept zebu cattle varies between 0.9-4% based on international used cut of value (Tschopp *et al*., 2010). Kiros, (1998) found that in Eastern Shoa of central Ethiopia local breeds had much lower prevalence rate 5.6% than exotic breeds (Holstein, 86.4%). Ameni *et al*., (2003) found an individual animal prevalence of 7.9% using comparative intradermal tuberculin test (CIDT) in Wuchale district of Central Ethiopia. Large scale study involving 5424 cattle carried out in Central Ethiopia showed that the overall prevalence in cattle was 13.5%, with higher prevalence found in Holstein (22.2%) compared to local zebus (11.6%) (Ameni *et al*., 2007). But prevalence in extensive farming system is generally low as it was reported by Tschopp *et* *al.*, (2010) prevalence of BTB at human-livestock-wildlife interface in Hamer Woreda of South-West Ethiopia was showed individual BTB prevalence in cattle was 0.8% with the ≥4 mm cut-off and 3.4% with the ≥2 mm cut-off.

BTB studies in Ethiopia so far did not address all the different geographical area, breed and host species, husbandry practices and other area specific risk factors, which could be potential future research area. In addition, despite the isolation of *M. bovis* from human sample and *MTB* from animal tissue, there is no single study, which shows the direct transmission of *M. bovis* and *MTB* from animal to human and visversa. Hence, addressing the transmission dynamics of the diseases will be other future research area. Furthermore, no studies have so far been carried out to identify strain circulating in wildlife species at the livestock-wildlife interface, and thus the possible existence of a wildlife reservoir for the disease.

2.4.7 Human tuberculosis due to M. bovis

The *MTC* has been described to originate from a common ancestral progenitor that underwent deletions of various genomic regions of difference (RD) (e.g. loss of RD9, RD4, RD1) (Brosch *et al.*, 2002; Smith *et al.*, 2006a; Müller *et al.*, 2009a; Thoen *et al.*, 2009). These bacteria particularly *M. bovis* have since spread to all groups in the human and animal populations and constitute major threats to human health as well as animal health and production (Tan *et al.*, 2003; Ayele *et al.*, 2004; Thoen *et al.*, 2009). Although TB is a leading cause of human deaths due to a single infectious agent in the world today the extent of human TB due to *M. bovis* is not known but *M. bovis* seems to account for only a small percentage of the cases of TB reported in humans (O'Reilly and Daborn, 1995; Tan *et al.*, 2003; Thoen *et al.*, 2009).

TB is of great economic importance in the animal industries (wild and domestic) worldwide, especially in countries where little information is available on the incidence of *M. bovis* infection in humans (Cosivi *et al.*, 1998; Enarson, 2006; Pavlik, 2006a; Pavlik, 2006b; Thoen *et al.*, 2006; Zinsstag *et al.*, 2006). However, *M. bovis* has been estimated to account for less than 0.5%-7.2% of human TB in most industrialised countries and 10%-15% in most developing countries (Cosivi *et al.*, 1998; Ashford *et al.*, 2001). Also, approximately 85% of the cattle and 82% of the human populations of Africa live in areas where animal TB is either partly controlled or not controlled at all (Ayele *et al.*, 2004; Shitaye *et al.*, 2006). Reliable information is not generally available on the incidence of human TB due to *M. bovis* in developing countries since poor attention is given to the problem due to limited diagnostic facilities. Indeed, zoonotic TB is neglected in most African countries and techniques for differentiating between organisms are not widely accessible (Cosivi *et al.*, 1998; Zinsstag *et al.*, 2006; Thoen and LoBue, 2007).

In countries where BTB is endemic and not controlled or partially controlled, human TB due to *M. bovis* may occur resulting from ingesting contaminated milk and milk products, other fresh animal products (ex: contaminated raw beef) and by inhaling cough spray from infected cattle (Collins and Grange, 1987; Moda *et al.*, 1996; Cosivi *et al.*, 1998; Etter *et al.*, 2006; Thoen *et al.*, 2006; Shitaye *et al.*, 2007). Human infection by *M. bovis* is also clinically indistinguishable from that caused by *MTB* (Cosivi *et al.*, 1998; de la Rua-Domenech, 2006b; Thoen *et al.*, 2009) and can lead to pulmonary and extrapulmonary TB. Cervical lymphadenopathy, intestinal lesions, chronic skin TB (lupus vulgaris) and other extrapulmonary forms are common in human due to *M. bovis* infection (Cosivi *et al.*, 1998; Kazwala *et al.*, 2001a).

Progress in the global control of human TB has been challenged by several factors in developing countries and within industrialized countries where the disease occurs (WHO, 2008; Thoen *et al.*, 2009; WHO, 2009). These factors include population growth, close association of TB with poverty, co-infection of TB and HIV/AIDS epidemic, deficiencies in control measures and migratory movements (Corbett *et al.*, 2003; Corbett *et al.*, 2006; Thoen *et al.*, 2006; WHO, 2008; Thoen *et al.*, 2009; WHO, 2009). However, other situations also become significant when the human TB is due to *M. bovis* and BTB is endemic in the region. Zoonotic TB can occur as a result of occupational or accidental hazard among cattle farmers, handlers of fresh cattle products, veterinarians and migrants from countries where BTB is endemic (O'Reilly and Daborn, 1995; Ameni *et al.*, 2003; Ayele *et al.*, 2004; Etter *et al.*, 2006; Shitaye *et al.*, 2007; Regassa *et al.*, 2008). *M. bovis* infected cattle professionals may also be source of infection to cattle (Cosivi *et al.*, 1998; Ayele *et al.*, 2004) as well as other persons they come in contact with (Ocepek *et al.*, 2005; Evans *et al.*, 2007; Thoen *et al.*, 2009; Etchechoury *et al.*, 2010).

Certain studies have reported the isolation of *M. bovis* from humans suffering from pulmonary TB in parts of Africa such as Cameroon, Egypt, Nigeria, Democratic Republic of Congo and Tanzania (Cosivi *et al.*, 1998; Kazwala *et al.*, 2001a; Niobe-Eyangoh *et al.*, 2003; Zinsstag *et al.*, 2006). These findings suggest zoonotic BTB transmission to humans is supported by transient influences on the sensitivity to TST of cattle exposed to human TB cases and coincidental environmental mycobacteria (Cosivi *et al.*, 1998). The threats to public health of human TB due to *M. bovis* in Africa therefore require urgent collaborations of veterinary and medical professionals, biomedical and ecological (environmental) experts, public health services, social workers and policy makers.

2.4.8 Human tuberculosis due to M. bovis in Ethiopia

Ethiopia is the country with the highest TB cases and has a yearly incidence of 341 of all forms TB cases/100,000 population. The prevalence and mortality of TB of all forms is estimated to be 546 and 73 per 100,000 populations respectively. According to the latest estimates, among the 22 high TB burden countries that account for 81% of estimated cases, Ethiopia ranks 7th Kidane *et al.*, (2002). However, very few studies have been conducted on isolation of *M. bovis* from humans TB cases in Ethiopia so far. Kidane *et al.* (2002) indicated that *M. bovis* was found to be a cause for tuberculous lymphadenitis in 17.1% of 29 human TB cases in Ethiopia. The proportion of BTB to the total of TB cases in humans depends on the prevalence of the disease in cattle, socioeconomic conditions, consumer habits, practiced food hygiene and medical prophylaxis measures Kidane *et al.*, (2002).

In rural areas of Ethiopia, most people drink raw milk and do have extremely close attachment with cattle (such as sharing shelter) that intensifies the transmission and spread of BTB. Detection of causal agents of BTB from raw milk (Kinfe and Eshetu, 1987; Kiros, 1998) confirms the existing problem and the potential risk of the infection in humans. With respect to this, Kiros (1998) demonstrated that out of 7138 human patients with tuberculosis, 38.4% were found with extra-pulmonary tuberculosis and the proportion of patients with extra-pulmonary tuberculosis was significant in patients who have close contact with cattle and in those who frequently used to drink raw milk in particular Kiros (1998). Regassa (2005) demonstrated the association of *MTB* and *M. bovis* in causing tuberculosis between humans and cattle. The cattle owned by tuberculous patients had a higher prevalence (24.3%) than cattle owned by non-tuberculous owners with 8.6%. The author also noted that 73.8% and 16.7% of 42 human isolates were identified as *MTB* and *M. bovis* and from cattle isolates 18.1% and 45.5% of 11 were found to be *MTB* and *M. bovis* species, respectively. This showed that the role of *M. bovis* in causinghuman tuberculosis seemed to be significantly important. On the other hand, in Ethiopia, consuming raw meat is a welcome tradition, thus meat may also remain to be another area of concern or threat to be a source of BTB infection.

2.5 Mycobacterium bovis clonal complex in Ethiopia, as part of East Africa

The MTC is a very closely related group of bacteria that all cause a similar disease in humans and animal hosts. The most important member of this complex of bacteria is *M. tuberculosis*, which is host, adapted to humans. However, within the MTC is a lineage of strains, all marked by the absence of chromosomal region RD-9, which are primarily, although not exclusively, isolated from animals (Brosch *et al.*, 2002). The RD-9 deleted lineage comprises at least seven species with differing host preference or geographical localization and includes strains of *M. africanum*, *M. bovis* (antelope), *M. microti*, *M. pinnipedii*, *M. caprae* and *M. bovis*. Because of the close nucleotide similarity and pathology of these clades it has been suggested that they would best be described as ecotypes of *MTB* rather than species (Smith *et al.*, 2006b). Each of these ecotypes is marked by a nested series of deletions and single nucleotid polymorphisms (SNPs) leading from the two, possibly human-adapted, clades of *M. africanum* down to the cattle adapted *M. bovis* (Brosch *et al.*, 2002; Smith *et al.*, 2006b).

Molecular tools have enhanced our understanding on the epidemiology of TB by providing new insight on the transmission dynamics, source, and spread of *MTB* (Mathews *et al.*, 2006). A series of clonal complexes of *M. bovis* have been identified by deletion or SNP analysis and each of these clonal complexes has been associated with a specific spoligotype signature to aid identification (Muller *et al.*, 2009; Berg *et al.*, 2011; Smith *et al.*, 2011). A stable molecular marker that was present in, and unique to, the single cell that was the most recent common ancestor (MRCA) of the clonal complex can identify a clonal complex of M. bovis strains. All members of the clonal complex will carry the molecular marker by descent and the molecular marker defines membership of the clonal complex. The most useful molecular markers to use are unique chromosomal deletions although, if necessary, SNPs may also be used if a unique deletion cannot be identified (Rodriguez-Campos *et al.*, 2012).

The four west-central African countries where Af1 strains were found to be dominant are Mali, Nigeria, Chad and Cameroon and the four east African countries where Af2 strains are highly prevalent are Burundi, Uganda, Tanzania and Ethiopia. Isolates of the Af1 clonal complex are very rare or not present in countries with the Af2 complex and vice versa. Countries where no Af1 or Af2 strains have been identified and countries where no isolates were the rests. Many strains of *M. bovis* isolated from East Africa also had a distinct spoligotype signature the loss of spacers 3–7. Strains with this spoligotype signature were found at high frequency in Uganda, Burundi, Tanzania and Ethiopia. A specific deletion of chromosomal DNA, called RDAf2, was identified in a representative strains from East Africa and strains with the RDAf2 deletion were found in over 65% of *M. bovis* isolates from these four east African countries; while RDAf1 were found in West Africa Mali, Nigeria, Chad and Cameroon. In all cases, these RDf2 East African strains had the specific spoligotype signature (loss of spacers 3-7). This clonal complex was named African 2 (Af2) (Berg *et al.*, 2011) (Table 2).

Table 2: Definition and summary of characteristics of the Af2 clonal complex of *M. bovis*

Category	Description
Definition	Presence of deletion RDAf2 (14.1 kb between Mb0599 and Mb0610)
Spoligotype marker	Absence of spacers 3 to 7
Spoligotype signature	1100000101111110111111111111111111111100000 (SB0133)
Distribution	At high frequency in East Africa (Uganda, Burundi, Tanzania, and Ethiopia)
IS6110 copy no	4 or more copies (infrequently less than 4)

Source: J Bacteriol. 2011 Feb; 193 (3):670-8. Doi: 10.1128/JB.00750-10.

Although the number of strains sampled from Uganda, Burundi, and Tanzania is small, the population structure of Af2 in these countries showed remarkable differences; the most common Af2 spoligotype pattern in each of the four east African countries surveyed was different. This observation contrasts with the spoligotype distribution of the Af1 clonal complex, for which a single ancestral-type spoligotype pattern was dominant in three of four west-central African nations (Muller *et al.*, 2009). Both the spoligotype surveys and the genotype comparisons suggest that the population of Af2 strains in each of these four east African countries is unique. That is, for any isolate of Af2, it should be possible, with reasonable accuracy, to determine from its genotype its country of origin. This conclusion reinforces a continuing theme of national localization of *M. bovis* genotypes initially described for Af1 strains in west-central Africa (Muller *et al.*, 2009).

The geographical localization of the Af2 clonal complex to these four east African countries, and perhaps to some additional neighboring countries not yet surveyed, may have been governed by geographical features that affect cattle density, trading, and movement in this part of Africa. Clonal complexes may represent groups of strains with different selective advantages or behaviors, and comparing and contrasting the phenotypic differences between these distinct divisions within *M. bovis* may elucidate the molecular mechanisms of these differences and identify the selective forces operating on both BTB and its cattle host (Berg *et al.*, 2011).

There is high isolation frequency of spoligotype SB1176 from various BTB study in Ethiopia (Berg *et al.*, 2009) and demonstrates dominance of this spoligotype as a major cause of BTB in Ethiopian cattle particularly in the central region where the disease is highly prevalent (Demelash *et al.*, 2009). Apart from its uniqueness, repeated isolation of SB1176 at high frequency from Ethiopia may indicate the existence of a locally evolved clonal strain with selective genetic advances perhaps linked to virulence and transmissibility. Persistent occurrence of SB1176 could also indicate the extreme high degree of reinfection rate prevailing in the area. Spoligotype SB1176 is the major strain in RD Af2 clonal complex. It is therefore, reasonable to formulate future research work to determine if the Af2 clonal complexes are specifically adapted to *Bos indicus* (Zebu) cattle, which is common in East Africa as opposed to *Bos Taurus* (European) a cattle which is common in West Africa, where the Af1 clonal complex of *M. bovis* dominates.

2.6 Diagnosis of bovine tuberculosis

TB can be diagnosed clinically, but usually only in the later stages of the disease. The tuberculin skin test is universally recognized and is generally used for preliminary diagnosis in BTB control programs. However, in countries with low disease prevalence or disease free status, meat inspection is used for diagnosis and surveillance. Other tests, such as an antibody enzyme-linked immunoassay (ELISA) and the gamma interferon assay, have been used as supplementary tests in eradication and control (Ayele *et al.*,2004).

Bovine tuberculosis infection in cattle is usually diagnosed in the live animal on the basis of delayed hypersensitivity reactions (tuberculin skin testing). In cattle, infection is often sub-clinical; when present, clinical signs are not specifically distinctive to other disease caused conditions and might include weakness, anorexia, emaciation, dyspnoea, enlargement of lymph nodes, and cough, particularly with advanced TB (OIE, 2009). Post-mortem, infection is diagnosed by necropsy and histopathological and bacteriological techniques. Rapid nucleic acid methodologies such as the polymerase chain reaction (PCR) may also be used. These are demanding techniques and only validated procedures should be used. Classical mycobacterial culture remains the routine method for confirmation of infection (OIE, 2009).

2.6.*1 Clinical Signs*

It is usually a chronic debilitating disease in cattle, but it can occasionally be acute and rapidly progressive. Early infections are often asymptomatic. In countries with eradication on programs, most infected cattle are identified early and symptomatic infections are uncommon. In late stages, common symptoms included progressive emaciation allows grade fluctuating fever, weakness and in appetence. Animals with pulmonary involvement usually have a most cough that is worse in the morning, during cold weather of exercise and may have dyspnea or tachypnea. In terminal stage, animals may become extremely emaciated and develop act respiratory distress (Garner, *et al.,* 2004). In brush- tailed opossums, BTB is usually a fulminating pulmonary disease that typically lasts two to six months. In the final stage of the disease, animals become disoriented, cannot climb, and may be seen wandering about in day light. In contrast, most infected badgers have no visible lesion (NVL) and can survive for many years asymptomatic badgers, the disease is primary a respiratory distress (Garner, *et al.*,2004; Sharma *et al.*, 2005).

2.6.2 Post mortem examination

Detection of BTB in most African countries is based on the post mortem findings of TB lesions (Asseged *et al.*, 2004; Shitaye *et al.*, 2006). It is characterized by the formation of granulomas (tubercles) where bacteria have localized. These granulomas are usually yellowish and either caseous, or calcified, they are often encapsulated. In some species such as deer, the lesion tends to resemble abscesses rather than typical tubercles. Some tubercles are small enough to see by the naked eye unless the tissue is sectioned. In cattle, tubercles are found in the lymph nodes, particularly those of the head and thorax. It is also common in the lungs, spleen, liver and the surfaces of body cavities. In disseminated case, lesions are sometimes founds on the female genitalia, but are rare on the male genitalia. In countries with good control programs, infected cattle typically have few lesions at necropsy. Most of those lesions found in lymph nodes associated with the respiratory system. However, small lesions can often be discovered in the lungs of these animals if the tissues are sectioned (Garner, *et al.,* 2004; Ameni *et al.*, 2010).

2.6.*3. Acid-fast staining*

Demonstration of acid-fast bacilli (AFB) in a smear made from a clinical specimen provides a preliminary diagnosis of mycobacterial disease, while the isolation of mycobacteria on culture provides a definite diagnosis of TB or disease due to mycobacteria other than *MTC* (MOTT) (Siddiqi and Rusch-Gerdes, 2006). The acid-fast stain developed in 1882 by Paul Ehrlich and later improved by Ziehl and Neelsen is based on the ability of *Mycobacteria* to retain the primary dye even when decolorized by a powerful solvent such as acid-alcohol. Most other bacteria are easily decolorized by acid-alcohol. The sensitivity of acid fast smear for specimen from extra- pulmonary tuberculosis and disease caused by MOTT is lower than from sputa (Vasanthakumari, 2007).

2.6.*4. Histopathological examination*

Histopathological features of granulomas show caseous necrosis with or without calcification surrounded by macrophages, lymphocytes, plasma cells, neutrophils, epithelioid cells and Langhan's giant cells and enclosed partially or completely by a thin capsule (Katia *et al.*. 2008). However, chronic granulomas can also be found in various conditions and diseases such as mycotic and bacterial pyogranulomas (Liebana *et al.,* 2008). Although tuberculous granulomas in cattle often cover large areas of histological sections and typically contain only small number of acid-fast bacilli (Liebana *et al.,* 2008); the absence of acid-fast organisms does not exclude tuberculosis in lymphadenitis of unknown aetiology (OIE, 2009). A sample is considered as positive only if it consists of granulomatous inflammation associated with central necrosis and with no evidence of other non-mycobacterial aetiologies (Fitzgerald *et al.,* 2000).

2.6.*5 Mycobacterium culture*

In the isolation of mycobacterium by culture, the ideal medium should be able to support rapid and luxuriant growth, and allow the determination of its characteristic features. For example, colony morphology, growth rate and pigment production. The luxuriant growth of *MTB on* glycerol containing media, giving the characteristic 'rough, tough and buff' colonies is known as eugenic while the growth of *M. avium* on media containing glycerol is also described as eugenic whereas *M. bovis* has sparse, thin growth on glycerol containing media that is called dysgenic, however, grows well on pyruvate-containing media without glycerol (Patterson and Grooms, 2000).

Most of mycobacterial culture media fall into egg-potato-base media and agar-base media. The most popular egg-based media are the Lowenstein Jensen (LJ) buffered egg potato medium and the American Trudeau Society egg yolk-potato flour medium. Among the agar-based media, Middle brook 7H10, Middle brook 7H11, and Dubose oleic-albumin agar are recommended. The advantages of egg-based media are the long shelf life (1 year when refrigerated) and the low cost of preparation. Egg media require heat for solidification, which, along with the presence of albumin, inactivates certain ant-tuberculous drugs (OIE, 2009).
Similarly, in veterinary bacteriology, the egg based LJ and stone brinks media are most commonly used. LJ medium can be obtained commercially. An agar-based medium such as middle brook 7H10 and 7H11 or blood based agar medium may also be used (OIE, 2009). The media are prepared as solid slants in screw capped bottles. Malachite green dye (0.025g/100ml) is commonly used as selective agent. *MTB, M. avium* and many of the atypical mycobacteria require glycerol for growth. However, glycerol is inhibitory to *M. bovis* while sodium pyruvate (0.4%) enhances its growth. Thus, the media with glycerol and without glycerol (but with sodium pyruvate) should be inoculated. The media can be made more selective by the addition

of cycloheximide (400µg/ml), lincomycin (2µg/ml) and nalidixic acid (35µg/ml). Each new batch of culture medium should be inoculated with the stock strains of mycobacteria to ensure that the medium supports satisfactory growth. The inoculated media may have to be incubated at 37°C for up to 8 weeks and preferably for 10 to 12 weeks with or without carbon dioxide for the Mycobacteria in the TB group. *MTB* and *M. avium* prefer the caps on the culture media to be loose while *M. bovis* grows best in airtight containers (Quinn and Markey, 2003; WHO, 2013a).

2.6.*6 Tuberculin skin test*

The tuberculin skin test based on a delayed type hypersensitivity to mycobacterial tuberculous protein, is the standard ante mortem test both in human and cattle. It is convenient, cost effective method for assessing cell mediated responses to a variety of antigens and it is "gold standard" for diagnostic screening for detection of new or asymptomatic MTC infection (Katial *et al*., 2001). The reaction in cattle is usually detectable 30-50 days after infection. The tuberculin is prepared from cultures of *MTB* or *M. bovis* grown on synthetic media. The tuberculin test is usually performed between the mid necks, but the test can also be performed in the caudal fold of the tail (Lee and Holzman, 2002; Radostits *et al*., 2007).The skin of the neck is more sensitive to tuberculin than the skin of the caudal fold. To compensate for this difference, higher doses of tuberculin may be used in the caudal fold of the tail. Bovine tuberculin is more potent and specific, potency of tuberculin must be estimated by biological methods, based on comparison with standard tuberculin, and potency is expressed in the international unit (IU).

In several countries, BTB is considered to be of acceptable potency if its estimated potency guarantees per bovine dose at least 2000 IU in cattle. In cattle with diminished allergic sensitivity, a higher dose of bovine tuberculin is needed and the volume of each injection dose must not exceed 0.2ml. Cell mediated hypersensitivity, acquired through infection can be demonstrated systematically by fever or ophthalmic-ally by conjunctivitis, or dermally by local swelling, when tuberculin test or its purified protein derivative (PPD) is given by the subcutaneous, conjunctival or intradermal route, respectively (Radostits, 2007).

2.6.*7 Single Intradermal Test (SIDT)*

It is applied by the intradermal injection of 0.1ml of BTB PPD into a skin fold at the base of the tail or into the cervical fold and the subsequent detection of swelling as a result of delayed hypersensitivity. The reaction is read between 48 and 96 hours after injection with a preference for 48-72 hours for maximum sensitivity and at 96 hours for maximum specificity. The positive reaction constitutes a diffuse swelling at the site of injection. The main disadvantage of the SID test is its lack of specificity and the number of reactor lesion occur. Mammalian tuberculin is not sufficiently specific to differentiate between reactions due to infection with *M. bovis* and infection with *M. avium, M. tuberculosis a*nd *M. paratuberculosis* including vaccination or *Nocardia farcinicus (*Radostits, 2007). The other disadvantages of SID test include failure to detect cases of minimal sensitivity, in old cows and in cows which have recently calved as well as in early infection, in some cattle in an unresponsive state, referred to as anergy which is developed due to antigen excess or immunosuppression, which in turn caused by non-specific factors such as malnutrition and stress (Hirsh and Zee, 2000; Andrews, 2003; Quinn and Markey, 2003).

2.6.*8 Singleintradermal cervical comparative tuberculin test (SICCTT)*

Two sites on the mid neck, 10-12 cm apart, are shaved and the thickness is measured in millimeters with caliper before the injection of tuberculin. In the *SICCTT*, 0.1ml of PPD from *Mycobacterium avium* (PPD-A) and 0.1ml of PPD from *M. bovis* (PPD-B) are injected intradermal into separate clipped sites on the side of the neck. Care must be taken in placing the injection as varied from place to place in the skin. After 72 hours, the thickness of the skin at the sites is measured again (Quinn and Markey, 2003; Ameni *et al.*, 2010). When the change in skin thickness is greater at the PPD-A injection site, the result is considered negative for BTB. When the change in skin thickness increased at both sites, the difference between the two changes is considered. Thus, if the increased in the skin thickness at the injection site for the bovine (B) is greater than the increase in the skin thickness at the injection site at the avian (A) and (B-A), is less than 1mm, between 1 and 4 mm, or a 4 mm and above, the result is classified as negative, doubtful, or positive for BTB, respectively and the animal with the evidence of infection is termed as reactor. The comparative test is used to differentiate between animals infected with *M. bovis* and those responding to bovine tuberculin as a result of exposure to other Mycobacterium. This sensitization can be attributed to the antigenic cross reactivity among mycobacterial species and related genera (OIE, 2012).

2.6.9 Gamma Interferon Assays

It is a laboratory-based test detecting specific cell mediated immune responses by circulating lymphocytes. In this test, the release of the lymphocyte gamma interferon (IFN-) is measured in a whole-blood culture system. The assay is based on the release of IFN- from sensitized lymphocytes during a 16-24 hours incubation period with specific antigen. The test makes use of comparison of IFN production following stimulation with PPD-A and PPD-B. The detection of bovine IFN- is carried out with a sandwich Enzyme-linked immune sorbent assay (ELISA) that uses two monoclonal antibodies to bovine IFN-γ (ggamma-interferon).

It is recommended that the blood samples be transported to the laboratory and the assay set up as soon as practical, but not later than the day after blood collection. Because of the IFN- test capability of detecting early infections, the use of both tests in parallel allows the detection of a greater number of infected animals before they become a source of infection for other animals as well as a source of contamination of the environment. The use of defined mycobacterium antigens such as ESAT-6 and CFP-10 shows promise for improved specificity. The use of these antigens may also offer the ability to differentiate Bacillus Calmette Guerin (BCG)-vaccinated from unvaccinated animals.

In animals that are difficult or dangerous to handle, such as excitable cattle or other bovidae, the advantage of the IFN- test over the skin test is that the animals need be captured only once. The IFN- test is used for serial testing (to enhance specificity) and parallel testing (to enhance sensitivity). The advantages of the IFN- assay are its increased sensitivity, the possibility of more rapid repeat testing and no need for a second visit to the farm and more objective test procedures. The limitations of IFN comprise a reduced specificity, high logistical demands (culture start is required within 24 hours after blood sampling), an increased likelihood of non specific response in young animals (owing to natural killer (NK) cell activity) and its high cost (Corbett *et al.,* 2003; OIE, 2009).

2.6.10 *Enzyme Linked Immunosorbent Assays (ELISA)*

The ELISA appears to be the most suitable of the antibody-detection tests and can be a complement, rather than an alternative, to test based on cellular immunity (OIE, 2009). It is a valuable complementary tool in order to identify possible anergic cows that may be acting as reservoirs of the agent (Molhuizen, *et al.,* 1997). An advantage of the ELISA is its simplicity, but sensitivity is limited mostly because of the late and irregular development of humoral immune response in cattle during the course of the disease. Specificity is also poor in cattle when complex antigens such as tuberculin or culture filtrates are used. *M. bovis* has been shown to be useful in increasing specificity in the ELISA. The ELISA may also be useful for detecting *M. bovis* infections in wildlife. For example, a lateral flow-based rapid test has been shown to be useful for detecting tuberculous animals, particularly some domestic animals, wildlife and zoo animals, where no cellular immunity tests like the IFN-γ test are available and where skin testing has been proven unreliable. However; its sensitivity in cattle is relatively low (Lilenbaum and Fonseca, 2005).

2.6.*11. Lymphocyte Proliferation Assay*

This type of in-vitro assay compares the reactivity of peripheral blood lymphocytes to tuberculin PPD-B and PPD-A. They can be performed on whole blood or purified lymphocytes from peripheral blood samples. This test endeavors to increase specificity of the assay by removing the response of lymphocytes to "non-specific" or cross-reactive antigens associated with non-pathogenic species of mycobacteria to which the animal may have been exposed. Results are usually analyzed as the value obtained in response to PPD-B minus the value obtained in response to PPD-A. The assay has scientific value, but is not used for routine diagnosis because the test is time-consuming and the logistics and laboratory execution are complicated, meaning it requires long incubation times and the use of radioactive nucleotides. As with the IFN- test, the lymphocyte proliferation assay should be performed shortly after blood is collected. The test is relatively expensive and has not been subjected to laboratory comparisons (Palomino *et al.,* 2007; Radostits *et al.,* 2007; Regassa, 2010).

2.6.*12 Spoligotyping*

Spoligotyping also called spaceroligo nucleotides is a rapid PCR based method for simultaneous detection and typing of MTC bacteria. The typing method is based on the strain dependent DNA polymorphism present at the direct repeat locus (DR) within the genome (Mostowy and Behr, 2005). The DR locus consists of identical 36bp DRs interspersed with 35 to 41 bp non-repetitive spacer sequences (Groenen *et al.,* 1993). The presence or absence in the DR region of spacers of known sequence can be detected by hybridization of amplified spacer DNA to a set of immobilized oligonucleotides, representing each of the unique spacers Spoligotyping has been used to type a large number of strains (Kremer *et al.,* 2002).(Figure7).

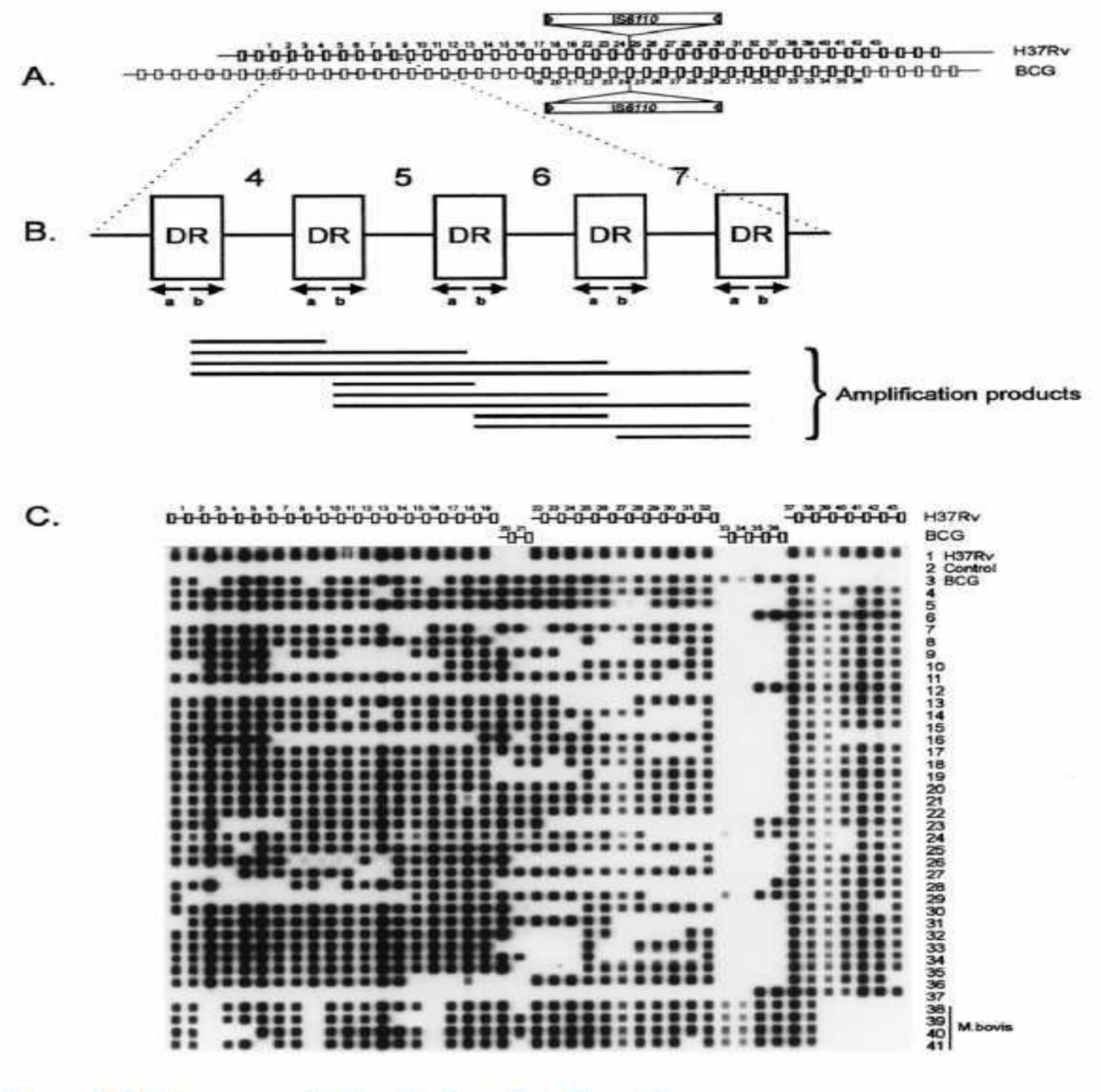


Figure 7: Diagrammatic description of spoligotyping

(A) Representation of the DR locus of the mycobacterial genome structure. DRs are depicted as rectangles, which are interspersed with unique spacers of 35 to 41 bp. The 37 spacers correspond to 3 *MTB* H37Rv and six spacers specifically correspond to *M. bovis* BCG. The site of IS6110 is illustrated. (B) DR regions PCR amplification. Primers bind to the DR regions. Number of fragments with different size produced. The depicted fragments are expected to be produced from five DRs. (C) Spoligotype patterns of the amplified products of strains of 35 *MTB* and five *M. bovis* genome on autorad. The sequence of spoligotypes on autorad corresponds to their order in the genome (Kamerbeek *et al.,* 1997).

The method enables classification of isolates into distinct strains, and thus allows characterization of genetic diversity of *MTB* species in clinical samples. It has gained widespread use for studying TB outbreaks and identifying sources and chains of infection (Luciani *et al.,* 2008). Another advantage is its ability to measure prevalence of endemic strains (Filliol *et al.,* 2003). The technique is useful not only for studies of TB epidemiology, but also for deciding about possible cross-contamination in mycobacteriology laboratories (Mazurek *et al.,* 1991).

2.6.13 Restriction Fragment Length Polymorphism (RFLP)

The gold standard molecular technique that is used for the molecular typing of *MTB is* due to its high discriminative power and reproducibility. It can also be used for outbreaks identification and can facilitate contact tracing of TB (Sharma and Gupta, 2011). However, this technique requires large amount of DNA and is therefore restricted to the mycobacterial cultures which take around 20-40 days to obtain sufficient DNA needed and for the combined process of probe labeling, DNA fragmentation, electrophoresis, blotting, hybridization, washing and auto-radiograph. Moreover, this technique is also technically demanding, slow, cumbersome, expensive and requires sophisticated analysis software for result analysis (Patterson and Grooms, 2000; Iwnetu *et al.,* 2009; Sharma and Gupta, 2011).

2. 6.14 Mycobacterial interspersed repetitive unit-variable number of tandem repeat (MIRU-VNTR) typing

Another PCR based method for the identification of various types of MTB strains is MIRU-VNTR typing. This genotyping method is based on the variability in copy number of the mycobacterial interspersed repetitive units (MIRU) (Magdalena *et al.,* 1998; Mazars *et al.,* 2001a; Supply *et al.,* 2001). MIRU-VNTR typing has been increasingly used to type MTC strains. Originally, the method was employed using 12 MIRU loci. More recently, a 24 loci based MIRU-VNTR typing with higher level of discriminatory power has been proposed (Supply *et al.,* 2006). The discriminatory power of the 24 loci MIRU-VNTR may exceed that of the gold standard, which is IS6110 RFLP method when used in combination with spoligotyping (Christianson *et al.,* 2010). Thus, currently molecular typing based on MIRU-VNTR has been proposed, in combination with spoligotyping, as a preferred technique for large-scale, high-throughput genotyping of *MTB* strains (Supply *et al.,* 2006).

2.7 Control of bovine tuberculosis

Control and eradication programs for BTB, human TB and zoonotic TB of humans due to *M. bovis* are based on early accurate detection and removal of infected animals, chemotherapy of infected humans and vaccination of target populations to attenuate or prevent the manifestation of the disease (Citron 1988; Abernethy *et al*., 2006; Good, 2006; Goodchild and Clifton-Hadley 2006 and Pavlik, 2006a). The test-and-slaughter policy is the basis for international BTB control and eradication programs using the TST to detect affected herds (and re-test) periodically and removing reacting cattle (Gilbert *et al*., 2005; Abernethy *et al*., 2006 and Good, 2006) that may shed the infective organism. In many industrialised countries there is "effective" compulsory reporting of *M.bovis* infection of all animals, quarantine of infected herds, tracing and re-testing of animals in contact with BTB skin positive reactors, movement restrictions of cattle herds *not* yet tested for TB as well as controlled animal movement out of known TB infected herds and endemic areas (Citron, 1988; Gilbert *et al*., 2005; Abernethy *et al*., 2006; Good, 2006; Goodchild and Clifton-Hadley, 2006; Pavlik, 2006a; and OIE, 2008; 2009).

However, the test-and segregation program, a modified form of the test-and-slaughter policy, may be more useful for developing countries, where the test-and-slaughter policy cannot be practicable for the whole cattle population (WHO, 1994). Thus, interim measures to segregate infected herds and phased slaughter of reactors are done. In most countries with strict TB eradication programmes, the test-and segregation strategy made up the early stages followed by the test-and slaughter methods in the final stage (CFSPH, 2009) and infected slaughter /meat cases during inspection are traced back to the originating farms. Informed farm management decisions such as proper sanitation and disinfection are also important to reduce the spread of *Mycobacterium,* within and between herds as well as the risks of exposure and transmission of BTB infection to humans (Defra, 2008).

The occurrence of *M. bovis* in wildlife reservoir hosts complicates eradication efforts. Culling to reduce population density can decrease animal TB transmission but the situation must be assessed carefully to avoid unanticipated effects such as the economic benefit and increase scattering members of the infected species (Donnelly *et al.*, 2007; Smith *et al.,* 2007; CFSPH, 2009). The development of TB vaccines for wildlife reservoirs (Hughes *et al.*, 1996; Ayele *et al.*, 2004) and use in situations where the test-and-slaughter policy is totally impracticable (WHO, 1994) is also being considered as an alternative. Also, human TB due to *M. bovis* is rare in countries where raw and poorly cooked meat are not consumed and pasteurization of milk and milk products are components of BTB eradication programs (WHO, 1994; Ayele *et al.*, 2004). *MTB* infection and zoonotic TB of humans can be treated successfully with antimicrobial drugs but there is widespread drug resistance and untreated infections are usually fatal (Chalmers *et al.*, 1996).

3. MATERIALS AND METHODS

3.1 Study area

The study was conducted from November 2014 to August 2017 in Adama and Bishoftu towns East Shewa zone of Oromia Regional state, central Ethiopia (Figure 8). The two study sites, Adama and Bishoftu towns are located at 95km and 47km southeast of Addis Ababa, respectively. Adama Town is located at 8°.54′N 39°.27′E at an elevation of 1712 meters, above sea level 99 km southeast of Addis Ababa.

Bishoftu Town is at latitude and longitude of 8°.45′N 38°.59′E with an elevation of 1,920 meters above sea level. Bishoftu resort town is best known for its five crater lakes and the population is 104 215.

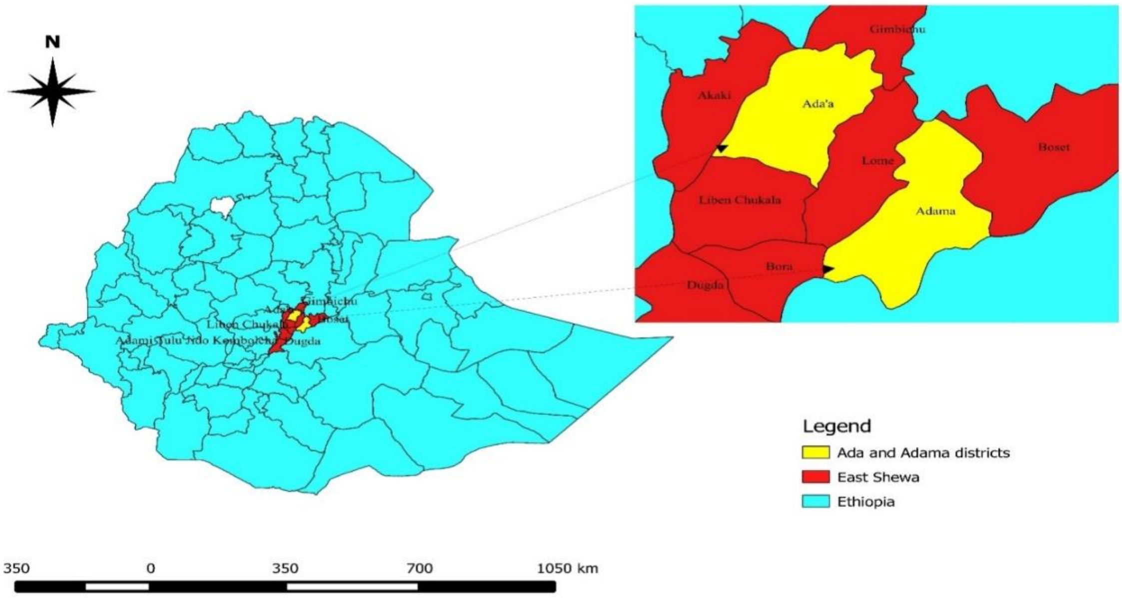


Figure 8: Location map of study area

3.2 Study subjects

The study subjects comprised of dairy cattle, beef feedlot cattle, abattoir slaughtered cattle and human TB patients at Adama, and surrounding towns and Bishoftu town East Shewa Zone of, Central Ethiopia. In Adama, there are about 500 dairy farms. Adama Town is divided into 14 kebeles (a *kebele* is the smallest administrative unit in the district in Ethiopia, similar to a neighborhood or ward). In the current study, 10-20 dairy cattle farms were selected from each kebele considering the the number of dairy farms existing in each kebele; willingness of the owners and availability of the road for transport 7during the study period. Accordingly, 206 farms were selected. Each selected farm has got 3-50 dairy cattle kept for milk production purpose mainly to family livelihood. All cattle in the farm except sick and less than six months age have been tasted. In Adama Town, there are about 100 beef feedlot farms. Most of the beef feedlot animals were Borena breeds, which were kept for around three months for live animal export and local abattoirs for slaughter. Abattoir slaughtered cattle came from the surrounding Adama and other different sites such as Walaga, Shewarobit, Kemisie, Haiq, Afar, Dessie, Harar, Arsi, Bale, and Borena.

3.3. Study design, sampling method and sample size determination

3.3.1 Study design, sampling method and sample size determination for dairy cattle

A cross sectional study was conducted in dairy farms selected from target population at Adama town between April and July 2016. The sample size was determined according to Thrusfield, (2005) considering 13.50% expected prevalence based on a previous report from Central Ethiopia (Ameni *et al.*, 2007), 5% absolute precision and 95% confidence interval. Accordingly, a minimum of 1038 cattle was required for the study. These animals were obtained from 206 dairy farms found in the town. The sampling frame (list of dairy farms in the town) was constructed in collaboration with the Veterinary Department in the town. In farms with small herd size (≤10 animals), all animals were included in the study except sick and less than six months age; while at least 10% were sampled from large farms (>10 animals).

3.3.2 Study design, sampling method and sample size determination for beef feedlote cattle

A cross sectional study was conducted in five feedlot farms found in Adama and its surround area from August to November 2015. The sample size was determined according to Thrusfield (2005). Since there was no previous study on feedlot cattle in Ethiopia, an expected 50% prevalence of BTB in feedlot cattle with 95% confidence interval and 5% required precision. According to this, the calculated sample size was increased to 501 cattle considering the dropout and missing on the second reading. For each farm number of animal tested were proportionally distributed. The five-feedlot farms were selected based on the presence of cattle in the farms during the study period, the willingness of owners, the distance of sites and the population of animals. Five hundred one (501) Borena cattle were included and in each feedlot, individual cattle were selected randomly based on individual animals identification number record and cattle population mentioned above in each farms. The feedlot farms mainly consist of only male cattle with age ranging from 1.5-6 years old.

3.3.3 Study design, sampling method and sample size determination for abattoir study

A cross-sectional study was carried out from November 2014 to August 2016 using abattoir-based survey. Actually 1,896 slaughtered cattle were sampled and inspected based on the formula given by Thrusfield (2005). An expected prevalence of 6.79% reported by Terefe (2014), 95% CI, and precision level of 1% were used to calculate sample size. Systematic random sampling technique was employed to calculate the number of cattle required for inspection of TB-like lesions.

3.3.4 Study design, sampling method and sample size determination for humans study

The study design was cross sectional. Human subjects having contact with cattle and diagnosed as TB cases between November 2014 and August 2016 at Adama referral hospital and health center and Bishoftu referral hospital were included in the current study. High TB patient flow, existence of better diagnostic facilities and skilled human resource were the major reasons for selecting the specified health facilities. TB patients who visited these health facilities during the study period were enrolled in the study, excluding those below 5 years of age and those who had started treatment prior to launching the study.

3.4. Study methodology

3.4.1 Single intradermal cervical comparative tuberculin test (SICCTT)

SICCTT was carried out by injecting both PPD-B and PPD-A (Prionics Lelystad B.V., Platinastraat 33, 8211 AR Lelystad, The Netherlands). Two sites on the skin of the mid-neck of the cattle, 12 cm apart, were shaved, and skin thickness was measured with a caliper. One site was injected with an aliquot of 0.1 ml of 3000 IU/ml bovine PPD into the dermis, and the other was similarly injected with 0.1ml of 2,500 IU/ml avian PPD. After 72 h, the skin thickness at the injection sites were measured and recorded. Results were interpreted according to the recommendations of the Office International des Epizooties (OIE, 2009) and Ameni *et al.,* (2008) at ≥ 4 mm cut-off and at ≥ 2 mm cut-off respectively. Thus, at cut-off ≥ 4 mm, if the increase in skin thickness at the injection site for bovine PPD (PPD-B) was greater than the increase in skin thickness at the injection site for avian PPD (PPD-A) and PPD-B minus PPD-A was less than 2 mm, between 2 and 4 mm, or 4 mm and above, the animal was classified as negative, doubtful, or positive for BTB, respectively. At cut-off ≥ 2 mm, if the difference between B and A was greater or equal to 2mm, the animal was considered as positive, while if the difference is less than 2 mm, the animal was considered as negative. When the change in skin thickness was greater at PPD A injection site, the animal was considered positive for mycobacterium species other than *MTC*. A herd was considered as positive if it had at least one tuberculin reactor animal.

3.4.2 Post-mortem examination

Animal identification and animal related variables such as age, breed, sex and body condition were recorded during ante-mortem inspection of selected animals in the abattoir. Postmortem examination was carried out as described previously by Vordermeier *et al.*, (2002). Each of the seven lobes of the lungs were thoroughly inspected and palpated for suspicious gross TB-like lesions. Similarly, mandibular, retropharyngeal, cranial and caudal mediastinal, left and right bronchial, hepatic, and mesenteric lymph nodes were sliced into 2mm size sections and then be inspected for the presence of visible lesions according to the protocol described earlier Vordermeier *et al.,* (2002).

3.4.3. Body condition scoring and age determination

Body condition scoring was made using a method developed for Zebu cattle (Nicholson and Butterworth, 1986). Accordingly, based on observation of anatomical parts such as vertebral column, ribs, and spines. The study animals were classified as lean (1), medium (2 and 3) or good (4 and greater). Age of the study animals was determined by using the dental eruption and wear as described by De Lanta and Habel (1986) and for the present study, animals were categorized as young age ≤ 2 and adult age > 2.

3.4.4 Sample collection and processing

3.4.4.1 Collection of suspected tuberculous lesions in slaughtered cattle

The tissues showing macroscopic lesions suggestive of BTB were collected from slaughtered cattle carcasses during the postmortem inspection at Adama Municipal and Bishoftu ELFORA Export abattoira. From 1896 inspected carcasses, 80 suspected BTB lesions were collected in sterile plastic bags and transported to Akililu Lemma Institute of Pathobiology (ALIPB) in cold chain for culturing. At the TB lab of the Institute, the samples were kept at –20°C until processed for culturing.

3.4.4.2 Collection of milk samples from PPD TB positive dairy cattle

Milk samples were collected from PPD strongly TB positive dairy cattle. About 20ml of milk was drawn from 4 quarters of 12 strongly tuberculin-positive cows under aseptic conditions. Samples were collected and transported in sterile containers in a cold chain ALIPB laboratory in Addis Ababa for analysis.

3.4.4.3. Collection of human sputa for mycobacterium culture

Regular visits were made to Adama and Bishoftu referral hospitals and Adama health center in East Shewa zone of, Central Ethiopia. Sputum samples from 392 willing participants with microscopic demonstrations of acid-fast bacilli (AFB) following Ziehl-Neelsen (ZN) staining were collected for mycobacterial culture and characterisation. Sputum specimens were collected in sterile plastic sample bottles for mycobacteriological culture or stored at 2–8°C for a maximum of 24 hours before incubation on LJ media.

3.4.5 Mycobacterium culture of suspected tuberculous lesions, sputem and milk samples

The suspected TB lesions and infected sputa were incubated using pyruvate and glycerol enriched LJ slants following standard procedures. All processing of the bovine and human specimens was done in biosafety level 2 cabinet. ZN staining and microscopic demonstrations of acid-fast bacilli were used to conform successful inoculation and growth (WHO, 1998).
Briefly, suspected tuberculous cattle specimens were cut into tiny pieces and then homogenized separately in 0.85% saline in sterile blenders to obtain fine pieces. Frozen samples were allowed to thaw to room temperature before processing. The cattle tissue homogenates and suspected human sputa were decontaminated with equal volumes of sterile 4% NaOH; mixed well by shaking for a few seconds and allowed to stand for 10 minutes at room temperature before neutralization with 1 mol/L HCl using phenol red as the indicator. Neutralization was achieved when a yellowish colour change of the suspension was attained; which was centrifuged at 3,000 rpm for 15 min. The supernatant was discarded leaving about 2ml or re-suspended in 1–2ml of distilled water (if required) and spread generously (~ 0.3 ml) on the LJ slants as follows: 2 LJ medium enriched with glycerol and 2 LJ medium enriched with Pyruvate. Incubation at 37°C for up to 12 weeks with weekly observation for growth of colonies was done. On observation of visible growth, a few colonies were gently mixed into one drop of sterile saline and smeared on a clean, grease-free microscopic slide, heat-fixed using the Bunsen burner flame without burning and stained by the ZN method to confirm the presence of acid-fast bacilli. The smeared slide was flooded with ZN carbol fuchsin, steamed without boiling gently with the Bunsen burner flame from the underside for 5 min. It was then rinsed gently with plenty of water until all free stain was washed away. The slide was flooded with 3% acid-alcohol decolorizing solution for 2 – 3 minutes until the red color disappeared, then rinsed again with water and the excess water drained. The slide was then flooded with Methylene blue counter stained for 1 minute, rinsed thoroughly with water, excess water drained from the slide and the smear allowed to air dry without blotting. The smear was systematically examined under a microscope (100 x oil immersion objective) for the presence of acid-fast bacilli. The presence of at least three bacilli in 100 immersion fields was recorded

as positive. Smears in which no acid-fast bacilli were seen in 100 fields were considered negative.

Culturing of milk specimens was processed according to the procedures given by Kazwala *et al.* (1998). Briefly, the milk was added to screw-capped sterile test tubes and centrifuged at 3,000 rpm for 15 minutes at room temperature. The cream was removed with sterile spatula, the supernatant was decanted, and the sediment was decontaminated with 4% NaOH. It was centrifuged again in the same condition and neutralized with concentrated HCl. Bromocresol was used to monitor neutralization, which was achieved when the suspension changed from deep purple to yellow. The sediment was inoculated onto 2 LJ media (one with pyruvate and the other with glycerol). The culture was incubated at 37˚C for a week in a slant position and for 12 weeks in an upright position.Growth of mycobacteria was monitored every week for up to 12 weeks.

3.4.6 Harvesting of growth colonies from LJ media

Cultures with growth/colonies on LJ medium were checked for being AFB using ZN staining. Isolates which are AFB positive were harvested and kept into two separate nunc tubes (one contains 1 ml freezing media and the other contains 0.3 ml dH2O). Isolates in freezing media were kept at -20^{o}C. Isolates in dH2O were subjected to Heat killing. Isolates in dH2O were treated with heat in a water bath at 80^{o}C for 50 minutes to release the mycobacterium genomic DNA material. Released mycobacterium genomic DNA material were kept at -20^{o}C until used for molecular characterization (Lowenstein, E. 1933).

3.4.7 Molecular characterisation of mycobacterial isolates

3.4.7.1 Spoligotyping of mycobacterial isolates

Spoligotyping was performed for 107 MTC isolates at ALIPB-AAU as described by (Kemerbeek *et al.* 1997). *MTB* strain H37Rv and *M. bovis* reactions were positive controls. DH2O served as a negative control of the experiment. In short, 5 µl of a DNA extract from a MTC isolated was added to a final volume of 25µl consisting of 3.5 µl dH2O, 12.5 µl Hot TaqPCR Master Mix, 2 µl primer DRa and 2 µl primer DRb. The thermal cycler was programmed for 3 min enzyme activation at 96^{o}C followed by 30 cycles of 1 min denaturation at 96^{o}C, 1 min annealing at 55^{o}C, 30 sec elongation at 72^{o}C, and 10 min final elongation at 72^{o}C. Twenty-five µl PCR products were added to 150-µl 2xSSPE/0.1%SDS, denatured at 96^{o}C for 10 min and immediately put on ice. Denatured PCR products were transferred to a mini-blotter and hybridized with the immobilized spacer-oligos on a membrane for 60 min at 60^{o}C. The membrane was removed from the mini-blotter and washed twice with 250 ml 2xSSPE/0.5% SDS for 10 min at 60^{o}C. After keeping the membrane in a rolling bottle, 2.5-µl streptavidin-peroxidase conjugate in 10 ml of 2xSSPE/0.5% SDS was added and incubated for 60 min at 42^{o}C. The membrane was washed twice with 250 ml of 2xSSPE/0.5% SDS for 10 min at 42^{o}C and then twice with 250 ml of 2xSSPE for 5 min at room temperature. The membrane for detection of hybridized spacers was incubated in 20 ml of ECL detection liquid for 1 min. A light sensitive film was exposed to the membrane. Black squares and blank spaces on a film indicated the presence and the absence of variable spacer sequences in the MTC isolate genome, respectively, and were interpreted in binary format using a spoligotyping database (http://www.pasteurguadeloupe. fr:8081/SITVIT_ONLINE/) and lineage classified using TB insight database. Isolates with spoligotype patterns similar to those in the SITVIT database were assigned a SIT number. Isolates not assignable to SIT numbers were referred as "Orphans" spoligotypes.

3.5. Ethical clearance

We obtained ethical clearance from the Institutional Review Board (IRB) of Oromia Health Bureau (Reference No. IRB/BEFO/HBTFH/1-8/3647). Written informed consent was obtained from each of the study participants.

3.6. Data analysis

Data analysis was performed using STATA (Stata Corp, 2011). Individual animal prevalence was defined as number of positive reactors per 100 animals tested. The herd prevalence was calculated as number of herds with at least one reactor animal per herd tested. Logistic regression analysis was used to assess the association between prevalence and animal risk factors using STATA statistical software version 12. Odds ratio was calculated to assess strength of association of factors to the prevalence of BTB.

4. RESULTS

The result section of this dissertationwas organized on manuscript basis including the discussion into four chapters as follows:

4.1. Prevalence and risk factors of BTB in dairy cattle by using SICCTT in Adama city, Central Ethiopia

4.2. Prevalence and risk factors of BTB in beef feedlot of Borena cattle by using Single intradermal cervical comparative tuberculin test (SICCTT) in Adama town, Central Ethiopia

4.3. Abattoir based study of BTB in Adama municipal abattoir and Bishoftu ELFORA export abattoir, in east Shewa, central Ethiopia

4.4. Characterization of MTC species and /or strains isolated from humans and cattle in East Shewa

4.1. Prevalence and risk factors of bovine tuberculosis in dairy cattle in Adama town, Central Ethiopia

Tefera Woldemariam[1, 2], Gezahegne Mamo[1], Rahmeto Abebe1* and Gobena Ameni[3]
[1]Faculty of Veterinary Medicine, Hawassa University, P. O. Box 05, Hawssa, Ethiopia
[2]College of Veterinary Medicine and Agriculture, Addis Ababa University, P. O. Box 34, Bishoftu, Ethiopia
[3]Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia

Status*: published on the Journal of Veterinary Medicine and Animal Health, 2018, 13 (17), 2141-2529*

4.1.1. Abstract

Bovine tuberculosis (BTB) is a serious infectious disease of cattle with significant economic impact and public health risk. It is particularly important in Ethiopia where effective control programs are lacking. This cross-sectional study was carried out between April and July 2016 on 1038 cattle selected from 206 dairy farms in Adama city located in Central Ethiopia to estimate the prevalence of BTB and assess the associated risk factors using single comparative intradermal tuberculin skin test. Accordingly, the individual animal level prevalence at ≥ 2mm cut off point found to be 2.1% (CI: 1.35 – 3.22). Of the 206 dairy farms included in the study, 15 (7.3%; CI: 4.29 – 11.98) had one or more cattle positive for the tuberculin test. Furthermore, 5.6% of cattle were found reactive for atypical mycobacterium. Among the risk factors considered, age and lactation status of the animals were significantly associated with the prevalence of BTB. It was noticed that the apparent prevalence of BTB positive reactivity was greater in young and non-lactating cattle than their counterparts. In conclusion, the present study revealed a very low prevalence of BTB in the dairy farms investigated and culling of positive reactors is recommended as a feasible control intervention.

***Key words:** Bovine tuberculosis, comparative intradermal tuberculin skin test, dairy cattle, Adam, Ethiopia.*

4.1.2. Introduction

Bovine tuberculosis (BTB) is a disease of zoonotic and economic importance caused predominantly by *M. bovis*. The disease is transmitted between animals primarily by inhalation of aerosols although transmission through ingestion is also common in cattle grazing on pasture contaminated with *M. bovis*. The disease in cattle is characterized by the formation of granulomas in tissues and organs, more significantly in the lungs, lymph nodes, intestine, kidneys and others. The economic loss of BTB in dairy cattle include reduction in productivity, movement restrictions, screening costs, culling of affected animals, and trade restrictions (OIE, 2016).

Ethiopia has the largest cattle population in Africa which is estimated at 53.99 million heads (CSA, 2013). The vast majority of the national herd is of indigenous zebu cattle maintained in rural areas under extensive husbandry systems. However, in response to the increasing demand for milk products and the Ethiopian government's efforts to improve productivity in the livestock sector, recent years have seen increased intensive husbandry settings holding exotic and cross breeds. This drive for increased productivity is however threatened by animal diseases that thrive under intensive settings, such as BTB (Firdessa *et al.*, 2012). Over the years, several studies have been conducted in Ethiopia to show the importance of BTB. According to most recent published studies, the prevalence of BTB ranges from 2 to 47% (Ameni *et al.*, 2003; Ameni *et al.*, 2007; Tschopp *et al.*, 2010; Gumi *et al.*, 2012; Firdessa *et al.*, 2012; Nega *et al.*, 2012; Ashenafi *et al.*, 2013; Mamo *et al.*, 2013). The studies have shown that BTB is an endemic disease in Ethiopian dairy farms due to lack of effective control programs and needs due attention.

Although previous studies have indicated that the disease is endemic in the country, there is paucity of information about the status of BTB at Adama town where dairy farming with different levels of intensification is flourishing rapidly in response to a higher demand for milk and milk products, which is the result of increased human population. Therefore, this study was planned to estimate the current prevalence of BTB in the dairy farms of the town and identify the potential risk factors.

4.1.3 Results

4.1.3.1. Individual animal prevalence

A total of 1038 cattle were screened for BTB and all were found during follow up for the second reading after 72 h. Based on *SICCTT* result, the apparent individual animal prevalence of tuberculin reactors was 2.1% (95% CI:1.35 – 3.22) using 2 mm cut-off point. Of the 206 herds tested, 15 (7.3%, 95% CI: 4.29 - 11.98) had one or more animals reactive to bovine PPD. The prevalence of BTB in individual herds ranged from 0 –55.6%. On the other hand, the change in skin thickness was found greater at avian PPD injection site than bovine PPD infection site in 5.6% (57/1023) cattle showing that these animals are positive for *Mycobacterium* spp. other than *MTC* (Table 6).

Table 3: Apparent prevalence of bovine and avian tuberculin reactors in Adama dairy farms, central Ethiopia

Tuberculin test type	Number of cattle tested	Number of positive	Prevalence (%)	95% Confidence Interval	$\chi2$	P
Bovine PPD	1038	22	2.1	1.35 – 3.22	17.2	<0.001
Avian PPD	1038	57	5.6	4.32 – 7.22		

In univariable analysis of the potential risk factors, age of the animals was the only variable that significantly contributed to positive reactivity to BTB ($P<0.05$) (Table 7). However, in multivariable analysis, age and lactation were found to be significantly associated with tuberculin positive reaction (Table 8).

Table 4: Univariable analysis of risk factors for bovine tuberculin reactors in Adama dairyfarms using χ2–test

Variables	No of cattle examined	No of positive	Prevalence (%)	χ^2	P- value
Herd size				2.5	0.116
Small (≤10)	741	19	2.6		
Large (>10)	297	3	1.0		
Age				8.0	0.018
<2	156	7	4.5		
2-5	486	12	2.5		
≥ 6	396	3	0.8		
BCS				0.14	0.934
Lean	5	0	0		
Medium	105	2	1.9		
Good	928	20	2.2		
Breed					
Local	18	0	0		
Cross	1020	22	2.2		
Pregnancy					
Non-pregnant	993	22	2.2		
Pregnant	45	0	0		
Parity				0.94	0.816
1-2	448	11	2.5		
3-5	273	4	1.5		
≥6	5	0	0		
Lactation				5.3	0.070
Dry	68	4	5.9		
Lactating	659	11	1.7		

Table 5: Multivariable logistic regression analysis of risk factors for bovine tuberculin reactors in Adama dairy farms

Variables	**Odds ratio (OR)**	**Std. Err.**	**Z**	**P**	**95% CI for OR**
Herd size	0.31	0.23	-1.56	0.118	0.07 – 1.34
Age	0.11	0.07	-3.43	0.001	0.03 – 0.38
BCS	1.35	1.03	0.39	0.695	0.30 – 6.04
Parity	0.68	0.23	-1.14	0.254	0.35 – 1.32
Lactation	0.32	0.18	-2.01	0.044	0.10 – 0.97
Cons	20.57	74.17	0.84	0.402	0.02 - 24100

4.1.4. Discussion

BTB is known to be endemic in dairy farms of Ethiopia. The present study has shown an apparent individual animal prevalence of BTB as 2.1% in the dairy farms of Adama city. This finding is comparable to a previous report of 2% in Ethiopia (Gumi *et al.*, 2012) and 2.4% in Tanzania (Katale *et al.*, 2013). However, it is considerably lower than the figures reported by much of the previous studies in Ethiopia, 7.9% (Ameni *et al.*, 2003), 13.5% (Ameni *et al.*, 2007), 3% (Tschopp *et al.*, 2010), 30% (Firdessa *et al* ., 2012), 7.1% (Nega *et al.*, 2012), 13.64% (Ashenafi *et al.*, 2013), 18% (Mamo *et al.*, 2013), and 5.5% (Dejene *et al.*, 2016). Out of 206 herds tested, only 15 (7.3%) had one or more tuberculin positive animals and the number of tuberculin reactors varied widely (0 to 55.6%) between the herds. The present herd-level prevalence too, was considerably lower than the range of estimates (12.5 to 56%) documented in the aforementioned previous studies Gumi *et al.*, 2011).

The variation in the prevalence of TB reactors between the present and previous studies is thought to be attributed to differences in the herd size of the study animals. As the majority of the herds in the present study were small comprising less than 10 animals, management conditions favoring the spread of BTB, such as overcrowding and poor ventilation, were less likely to have influenced the prevalence of infection in the current study. The status of other husbandry practices with potential effect on the BTB infection, such as cattle movement, purchase of animals from outside, keeping of cattle with other livestock species and contact with wildlife, has not been investigated in the present study. Thus, future studies to be conducted in the same study area need to consider these factors.

In the multivariable analysis of the risk factors considered, lactation status of the animals was another factor found to be significantly associated with tuberculin reactivity although this was not evident in the univariable analysis. The apparent prevalence was higher among dry cows than lactating cows or calves and heifers. It is difficult to give a plausible biological explanation for this finding and perhaps it might be due to the confounding effect of some other variable unnoticed. In contrast to the present finding, previous studies in Ethiopia, which considered lactation status in their analysis, did not find significant variation in the prevalence of positive reactors between lactating and non-lactating cattle (Mamo *et al*., 2013; Zeru *et al*., 2014; Dejene *et al*., 2016).

Contrary to previous studies which demonstrated a significantly higher prevalence of BTB in larger herds than smaller ones (Ameni *et al*., 2003; Cleaveland *et al*., 2007; Elias *et al*., 2008; Romha *et al*., 2014; Zeru *et al*., 2014), this study showed no significant difference between large and small farms (P >0.05). This could be explained by the fact that the majority (89.8%) of herds included in the study were small with less than 10 cattle, making comparison of different herd size difficult. However, the present finding is consistent with that of (Tschopp *et al*. 2010, Ashenafi e*t al*. 2013 and Admasu *et al*. 2014). Previous studies in Ethiopia have shown that the native zebu cattle are more resistant to BTB than exotic breeds or their crosses (Vordermeier *et al*., 2012; Admasu *et al*., 2014; Sibhat *et al*., 2017). Consistent to this, the entire tuberculin positive reactors in the current study were Holstein-Friesian X zebu crosses while none of the local breeds was found reactive.

However, as the number of local cattle included in the study was so small compared to the crosses, the current evidence is not sufficient to declare breed differences in susceptibility to BTB. None of the pregnant cows tested in the current study were positive for tuberculin reaction and the entire test positive animals were non-pregnant. However, previous studies have shown lack of significant difference between pregnant and non-pregnant cows (Zeru *et al.,* 2014). Likewise, no significant association was found between parity numbers and positive reaction to tuberculin test and this is consistent with previous studies (Zeru *et al.,* 2014).

Although some studies in Ethiopia (Elias *et al.*, 2008; Admasu *et al.*, 2014; Zeru *et al.*, 2014; Nuru *et al.*, 2015; Dejene *et al.*, 2016) have reported that tuberculin reactivity is associated with poor BCS, this was not evidenced in the present study. Unlike previous studies, 90.9% of the tuberculin reactive cattle in the present study were in good body condition status while none of those with poor BCS were found positive. Consistent to the present study, Ashenafi *et al.* (2013) have reported significantly higher tuberculin reactivity in cattle with good BCS. Nevertheless, we should bear in mind that body condition scoring is often subjective and variation in judgment among researchers is obvious. In the present study, about 5.6% of the study animals were reactive for avian *Mycobacterium* PPD. This is much higher than a previous report of 0.7% in Ethiopia (Gumi *et al.*, 2012). Reaction bias to *M. avium* PPD could be due to infection with *M. avium* subsp. *avium* and *M. avium* subsp *paratuberculosis*. The latter causes a chronic debilitating disease known as paratuberculosis in cattle, which are most susceptible to infection when they are young. Apart from its economic impact on cattle production, *M. avium* subsp *paratuberculosis* has a zoonotic significance (Radostits *et al.*, 2007). Thus, it should be given due emphasis in future studies.

In conclusion, the present study has shown a very low prevalence of BTB in the dairy farms studied compared to previous reports in the country. As the proportion of cattle affected with BTB in each herd was small, culling of the positive reactors could be a feasible control intervention. Thus, owners of the positive herds need to be advised in this regard. Furthermore, the observation of atypical TB with a proportion higher than BTB warrants the need for future studies to focus on *Mycobacterium* spp. other than *MTC*, particularly *M. avium* subspp *paratuberculosis* due to its zoonotic and economic importance.

4.2. Prevalence of bovine tuberculosis in beef feedlot cattle of Borena by using comparative intradermal skin test, Adama, Ethiopia

Tefera Woldemariam[1], Gezahegne Mamo[2], Temesgen Mohammed[3] and Gobena Ameni[3]

[1]Hawassa University, School of Veterinary Medicine, P.O. Box 05, Hawassa, Ethiopia
[2]Department of Microbiology, Immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia
[3]Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa Ethiopia

Status: ***Published on the Ethiopian Veterinary Journal 2016, 20 (2):17-29***

4.2.1. Abstract

A cross-sectional study was carried out from August to November, 2015 among feedlot cattle in and around Adama export feedlot farms by using SICCTT to estimate the prevalence of bovine tuberculosis and to assess the associated risk factors. The individual animal prevalence with ≥4 mm cut-off point and with ≥2 mm cut-off point was 4.39% (95% CI: 2.59- 6.19) and 9.58% (95% CI: 6.99-12.16), respectively. In univariate logistic regression analysis, older animals (OR=7.11, 95% CI: 1.7-29.8), cattle from Yabello market (OR=5.66, 95% CI: 2.4-13.2) and cattle in feedlot 5 (OR=2.65, 95% CI: 1.03-6.8) were more likely to be tuberculin reactors than younger animals, cattle originated from Dubuluk market and those from feedlot I, respectively. In multivariable logistic regression, however, only feedlot farm difference showed a statistical significance difference among the groups with the OR=3.4 (95% CI: 1.2-9.5), while other factors were not statistically significant. In conclusion, the study revealed the occurrence of bovine tuberculosis in Adama export feedlot farms composed mainly of Borena cattle breeds which were established for export of fattened live beef cattle and hence, the findings of this study warrants the need to design a farm based control strategies at feedlot level and testing of animals during the purchase of the cattle at the market place of the animals.

Keywords: *Bovine tuberculosis, Comparative tuberculin testing, Feedlot farm, Prevalence, Risk factor*

4.2.2. Introduction

Bovine tuberculosis (BTB) is a chronic infectious disease of cattle characterized by the formation of granulomatous nodules which is caused by *M. bovis*. BTB has been controlled in developed countries through test-and-slaughter method; however, the disease poses a significant problem to the economy of the livestock sub-sector and remains a potential public health threat in developing countries where controlling programs are lacking (Cosivi *et al.,* 1998). Infection of livestock with BTB has an estimated global cost of €2 billion annually due mostly to the lack of BTB control in developing countries, where infection is endemic, resulting in reduced productivity of livestock(Garnier *et al.,* 2003).

Ethiopia has the largest livestock population in Africa, With 52.1 million cattle, 24.2 million sheep, 22.6 million goats, 987, 000 camels, 44.9 million poultry and nearly 5 million beehives, that contributes to the livelihoods of 60–70% of the population (Halderman, 2004). In Ethiopia, BTB is endemic in cattle with the prevalence varies from 0.8 to 46.8% depending on the geographical location, breed and the husbandry practices based on abattoir and tuberculin skin tests (Ameni *et al.,* 2003; Berg *et al.,* 2009; Bifa *et al.,* 2010; Regassa *et al.,* 2010; Tschopp *et al.,* 2010), with extensive rural and pastoral setting showing low prevalence as compared to intensive dairy farms (Ameni *et al.,* 2007; Mamo *et al.,* 2013).

The existing information on BTB so far in Ethiopia was mainly in dairy animals and in extensive traditional and pastoralist production system. However, with increasing beef demand both in country and abroad, there is a growing number of feedlot in central Ethiopia where cattle brought together from different sources and geographic location to the feedlots located mainly in Adama and its surrounding towns of east Shewa central Ethiopia. So far, in east Shewa Central, Ethiopia, there is no study carried out to determine the prevalence of bovine tuberculosis and its associated risk factors in beef animals kept under feedlots conditions. The present study, therefore, was designed to investigate the prevalence of bovine tuberculosis in beef cattle kept in feedlots in and around Adama town of central Ethiopia and to assess the potential determinant risk factor using comparative intradermal skin test method.

4.2.3. Results

4.2.3.1. Individual animal prevalence

On the basis of SICCTT, the prevalence of BTB with ≥4 mm cut-off point and with ≥2 mm cut-off point were 4.39% (95% CI: 2.59- 6.19) and 9.58 %,(95% CI: 6.99- 12.16) respectively. At ≥4 mm cut-off point, there was statistically significant differences in proportions of bovine positive reactor animals between the two age categories (X^2= 6.79, p= 0.045) in which a higher bovine positive reactors were observed in adult cattle than young one. While there was no statistical difference in the proportion of bovine tuberculin positivity between groups in relation to feedlot farm, origin of the animal and body condition score (P>0.05) (Table 3).

Table 6: Association of different risk factors skin test positivity of feedlot at ≥4 mm cut-off point for BTB in East Shewa, Central Ethiopia

Variables	No of cattle examined	No of positive (%)	χ^2	P- value
Farm				
I	90	6(6.67)	6.79	0.15
II	111	7(6.31)		
III	100	0(0.00)		
IV	100	5(5.00)		
V	100	4(4.00)		
Origin				
Dubluq	289	9(3.11)	3.95	0.139
Moyale	155	8(5.16)		
Yabelo	57	5(8.77)		
Age				
≤2	109	1(0.92)	4.00	0.045*
>2	392	21(5.36)		
BCS				
Medium	16	0(0.00)	0.76	0.384
Good	485	22 (4.53)		
Total	501	22(4.39)		

Key: BCS=body condition scoring; *=statistically significant

At ≥2 mm cut-off point, statistically high significant differences were observed in proportion of bovine positive reactors between the five feedlot farms ($\chi2$ =27.18, p= < 0.001); source of origin ($\chi2$ =22.22, p = <0.001); age categories ($\chi2$=9.65, p=0.002). But in relation to body condition score, there was no statistically significant difference between the groups ($\chi2$=0.2, P= 0.687) (Table 4).

Table 7: Association of different risk factors skin test positivity of feedlot at ≥2 mm cut-off point for BTB in east Shewa, Central Ethiopia

Variable	**No of cattle examined**	**No of positive (%)**	χ^2	**P-value**
Farm				
I	90	15(16.7)	27.18	< 0.001*
II	111	20(18.0)		
III	100	0 (0)		
IV	100	6(6)		
V	100	7(7)		
Origin				
Dubluq	289	13 (4.5)	22.22	0.001*
Moyale	155	23(14.8)		
Yabelo	57	12(21.1)		
Age				
≤ 2	109	2(1.8)	9.65	0.002*
>2	392	46(11.7)		
BCS				
Medium	16	2(12.5)	0.2	0.687
Good	485	46(9.5)		
Total	501	48 (9.6)		

*statistically significant

In univariate logistic regression analysis, older animals (OR=7.11, 95% CI: 1.7- 29.8), cattle from Yabello market (OR=5.66, 95% CI: 2.4-13.2) and cattle in feedlot 5 (OR=2.65, 95% CI: 1.03-6.8) were more likely to be tuberculin reactors than younger animals, cattle originated from Dubliqu market and those from feedlot I, respectively. In multivariable logistic regression, however, only feedlot farm difference showed a statistical significance difference among the groups with the OR=3.4 (95% CI: 1.2-9.5), while other factors were not statistically significant (Table 5). Although it was not significant, older animals showed 2.1 times the odds of being tuberculin reactor than younger animals (OR=2.10; 95% CI: 0.46-9.59).

Table 8: Multivariable logistic regression analysis of tuberculin reactors with various host and environment related factors at ≥2mm cut-off point.

Variable	**No of cattle examined**	**No of positive (%)**	**Crude OR 95% CI**	**Adjusted OR95% CI**
Farm				
I	100	7 (7)	1	1
II	111	20(18)	2.92(1.18-7.24)*	6.99 (1.63-30.04)*
III	100	0(0)	-	-
IV	100	6(6)	0.85(0.27-.61)	0. 79 (0.25-2.45)
V	90	15(16.7)	2.65(1.03-6.85)*	3.4 (1.22-9.50)*
Origin				
Dubuliq	289	13(4.5)	1	1
Moyale	155	23(14.8)	3.70(1.82-7.53)	0.35(0.11-1.10)
Yabelo	57	12(21.1)	5.66(2.43-3.19)*	
Age				
≤2	109	2(1.8)	1	1
>2	392	46 (11.7)	7.11(1.70-9.79)*	2.10 (0.46-9.59)
BCS				
Medium	16	2 (12.5)	1	1
Good	485	46(9.5)	0.73(0.16-3.33)	0.89(0.19 - 4.22)
Total	501	48 (9.6)		

Key: COR=Crude Odds Ratio; AOR=Adjusted Odds Ratio; CI: Confidence interval

4.2.3.2. Herd prevalence of bovine tuberculosis

The herd prevalence was 80% (95% CI: 76.5-83.5) at ≥4 mm and ≥2 mm cut-off points. Association of the risk factors with bovine tuberculin reactivity showed farm, origin of the cattle, age and body condition score showed a statistically significant difference among the categories at ≥4mm and ≥2mm of cut off points ($P<0.05$). According to *SICCTT* result of avian tuberculin skin reaction, the overall animal prevalence of *Mycobacterium avium* complex infection was 0.6% (95% CI: 0.08- 1.27) at ≥4 mm cut-off point.

4.2.4. Discussion

Bovine tuberculosis is known to be endemic in cattle of Ethiopia particularly in dairy farms with a prevalence ranging from 0.8 to 46.8% in different husbandry system and breed of cattle (Ameni *et al.*, 2003) based on *SICCTT*. However, no information is available on the prevalence of BTB in beef cattle feedlots of Ethiopia particularly on Borena breed managed under private fattening export farms located in and around Adama town.

In the present study, based on *SICCTT*, the prevalence of BTB was 4.39% at ≥4 mm cut-off point and 9.58% at ≥2mm cut-off point. The prevalence in this study was lower than the prevalence reported in previous studies done on dairy cattle in Ethiopia (Ameni and Wudie, 2003). The main reason for this might be related to dairy farm mainly compared of female crossbreed, which may be affected by their reproduction, and milk production status that may lead to stress as compare to male animals that constitute the beef feedlots. Hence, dairy animals might be more susceptible than beef animals (Radostits *et al.*, 2007), the difference in husbandry system, origin of animals, breed of animals and age of animals. In dairy farms where high prevalence of BTB was recorded mainly reared under in-housed intensive husbandry system in which the majority of the cattle were cross breed as compared to the open-air handling system and consisting of local zebu (Borena) breeds in the feedlots. According to previous studies intensive husbandry system and cross breed cattle were important risk factors for high prevalence of BTB in the dairy farms (Ameni *et al.*, 2006; Ameni *et al.*, 2007).
Similarly, dairy cattle were kept for longer age as compared to the beef cattle which were relatively younger in their age (2 to 5 years), hence, beef cattle had short lifetime for exposure for infection as compared to the dairy cattle which were exposed for longer years to aerosol or oral infection by *Mycobacterium* species. In the present study, the prevalence of BTB showed an increase with age which is in agreement with previous reports by other researchers in cattle (Regassa *et al.*, 2010 and Biffa *et al.*, 2011).

At ≥2 mm cut-off point, statistically significant differences in proportion of bovine positive reactors were observed among feedlot farms, in which Farm II and Farm V showed higher proportion of positive bovine tuberculin reactor than Farm I. This difference might be related to the origin of cattle from which the animal were purchased and age distribution of the farms that may affect the farm level bovine tuberculin positivity. According to the observation in this study the settings in terms of husbandry, duration of stay in the feed lot and site of the feedlot farms were almost similar, and hence, the difference assumed to be related to the origin of the animals rather than the difference at feedlot level. Previous studies in the Yabello area based on *SICCTT* showed 5.5% prevalence in extensive pastoral production system and similarly a closer prevalence (8.7%) at ≥ 4mm cut-off points was reported in the present study. Similar to other studies in Ethiopia, there was no association between body condition score and tuberculin skin test positivity (Tschopp *et al.*, 2010). In present study, a high herd level prevalence (80%) was observed which might indicate the widespread occurrence of the infection in the feedlot herds as compared to moderately low herd prevalence observed in extensive pastoral production system (Mamo *et al.*, 2013).

In conclusion, the study revealed a low prevalence of BTB in beef cattle and indicated the presence of epidemiological risk factors for infection among cattle of the feedlots such as the origin of the cattle and age of the animal as important factors. Considering the fact that these feedlots established for export of fattened live beef cattle, the findings of this study warrants the need of designing a control strategies at feedlot level and testing during quarantine period of the animals.

4.3. Abattoir based study of bovine tuberculosis in Adama municipal abattoir and Bishoftu ELFORA export abattoir, central Ethiopia

Tefera Woldemariam[1], Temesgen Mohammed[2], Gezahegne Mamo[3] and Gobena Ameni [2]
[1]Hawassa University, School of Veterinary Medicine, P.O. Box 05, Hawassa, Ethiopia
[2]Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia
[3]College of Veterinary Medicine and Agriculture, Addis Ababa University, P. O. Box 34, Bishoftu, Ethiopia

Status: Draft manuscript

4.3.1. Abstract

A cross-sectional study was conducted from November 2014 to August 2016 at Adama Municipal Abattoir and Bishoftu ELFORA Export Abattoir, central Ethiopia, to estimate the prevalence and distribution of lesions of bovine tuberculosis (BTB) based on gross TB-like lesion. Detailed *postmortem examination was employed to undertake this study on 1,896 cattle (1,266 from Adama and 630 from Bishoftu) and 80-suspected TB-like lesions were collected. Data on their body condition scores, origin of animals and ages were recorded before slaughtering. The prevalence of BTB was 4.2% (80/1896) based on gross TB-like lesions. Larger proportion (52.5%) of TB lesion was recorded in the respiratory pathway followed by lymph nodes of the head (26.25%), mesenteric lymph nodes (7.5%), prescapular lymph nodes (7.5%) and hepatic lymph nodes (6.25%). The prevalence of the disease was significantly (P < 0.05) varying with origin of animal, location of abattoir and body condition score. The prevalence of BTB recorded by this study was similar with those reported in other abattoirs from different regions of Ethiopia*

Key words: *Adama; Bishoftu; Bovine Tuberculosis; Abattoir; Prevalence*

4.3.2. Introduction

BTB is a chronic bacterial disease characterized by progressive development of tubercles in any tissue/organ of the body (Hlokwe *et al.*, 2013; Pal *et al.*, 2014; Terefe, 2014). It has been reported from 176 countries as one of the important bovine diseases causing great economic loss (Awah-Ndukum *et al.*, 2013). TB in general can be difficult to diagnose based only on the clinical signs. Regular surveillance by skin test, bacteriology and molecular methods is not feasible due to lack of resources. Thus, abattoir inspection will continue to play a key role for national surveillance.

Ethiopia has the largest livestock population in Africa, with an estimated 52 million cattle (CSA, 2011) that contributes to the livelihoods of 60–70% of the population. Ethiopia's increasing human population, coupled with expanding urbanization and higher average income is putting an increasing pressure on the meat supply. To meet this demand, millions of food animals slaughtered every year throughout the country. In 2007, for example, a total of 18.8 million cattle, sheep, goats and camels slaughtered at municipal abattoirs, primarily for domestic consumption (FAO, 2009). For this reason, close monitoring of meat hygiene, including proper implementation of meat inspection procedures during slaughter, should be a vital part of the national public health protection program.

BTB characterized by the formation of nodules called tubercles whose location depends largely on the route of infection. In calves, BTB usually transmitted by ingestion and lesions involve the mesenteric lymph nodes with possible spread to other organs. In older cattle, infection usually transmitted by the respiratory tract with lesions in the lung and dependent lymph nodes (Carter and Wise, 2004). There has been increasing reports of human cases due to *M. bovis* especially in patients with HIV (Russell, 2003). Thus, a greater degree of transmission of infection with bacteria to human and domestic farm animals could occur (Taracha *et al.*, 2003). In industrialized countries, animal TB is controlled and eliminated with test and slaughter, which has in turn drastically reduced the incidence of the disease caused by *M. bovis* in both cattle and human. In developing countries however, animals' TB is widely distributed, as control measures are not applied or applied suboptimally. In Ethiopia, animals are kept in the same house with their owners; cow dung is used for painting of the wall and floor of houses as well as as sources of energy for cooking. All these practices do exacerbate the chance of spread of TB to human (Asseged, 1999). The nation wide distribution of the disease and the economic loss associated with it has not been fully determined due to lack of good diagnostic facilities (Asseged, 2004).

The primary reason for post mortem examination of carcasses at slaughterhouse is for the protection of public health. The knowledge of TB in cattle slaughtered provides useful information and is a proxy indicator for the epidemiology of the disease in the cattle population from which the slaughtered cattle are originated. Furthermore, it could serve as a good indicator of risk to humans through consumption of infected meat. Apart from providing data for regulatory programs. This provides better programmatic awareness with subsequent development of targeted guidance on how to reduce the risk of TB spread within the specific geographic area, as well as opportunities to trace back the source of infection to the herds. Hence, having the knowledge of distribution, prevalence and risk factors of the disease are fundamental to look for effective control strategy. Therefore, the objectives of this study were to estimate the prevalence of BTB at Adama Municipal Abattoir and Bishoftu ELFORA Export Abattoir, Central Ethiopia and to assess the distribution of tuberculous lesions in slaughtered animals.

4.3.3 Results

4.3.3.1 Postmortem findings

The prevalence of BTB was 4.22% (80/1896) on the basis of gross TB-like lesion. There was statistically significant difference between origin ($p<0.001$) of animal, location ($p<0.001$) of abattoir and body condition score ($p<0.001$) of the animals (Table 9).

Table 9: Association of different risk factors in cattle slaughtered at abattoir for BTB -

Variable	No of cattle inspected	No of carcasses positive (%)	χ^2	P-value
Origin of animal				
Arsi, Bale and Borena (southeast)	1018	16((1.6)	45.9	0.000
Afar, Harar, Dessie, Kemisie, Shewarobit and Haiq (east-north)	593	51(8.6)		
Walaga (west)	285	13(4.6)		
Abattoir				
Adama abattoir	1266	32(2.5)	26.9	0.000
Bishoftu export abattoir	630	48(7.6)		
BCS				
Lean	460	33(7.2)	19.4	0.000
Medium	440	23(5.2)		
Good	996	24(2.4)		
Total	1896	80		

4.3.3.2. Distribution of tuberculoses lesions

The distribution of TB-like lesions in different tissues of cattle was presented in Tables10 and 11. About 52.5% of the lesions were observed in the lung and associated lymph nodes (Table 11). The lung region contributes a higher percentage of tubercle lesions than the head and the gastrointestinal area (Table 10).

Table 10: Percent of distribution of tuberculosis lesion in organs and lymph nodes

	Postmortem	
Organs	**Number**	**Percent**
Lung tissue	14	0.74
Bronchial LN	17	0.89
Mediastinal LN	11	0.58
Retropharyngeal LN	17	0.89
Mandibular LN	4	0.21
Mesenteric LN	6	0. 31
Prescapular LN	6	0. 31
Liver tissue	5	0.26
Total	80	

Table 11: Pooled TB lesions distribution

Anatomic site	**Lesions**	**Percent from all***	**Organ proportion****
Lymph nodes around head	21	1.1	26.25
Lung and lymph nodes around it	42	2.21	52.5
Mesenteric lymph nodes	6	0. 32	7.5
Prescapular LN	6	0. 32	7.5
Liver and hepatic lymph nodes	5	0.26	6.25
Total	80		

*Percent from all=lesion divided by/number of animal examined (1896) multiplied by 100

**Organ proportion=lesion in the region divided by overall lesions (80) multiplied by 100

4.3.4 Discussion

This study generated information of the prevalence and distribution of lesions of BTB in cattle slaughtered at Adama Municipal Abattoir and Bishoftu ELFORA Export abattoir in East Shewa, Central Ethiopia. Based on postmortem examination in the present abattoir study, the prevalence of gross lesions of BTB was found to be 4.22%, which is comparable with the results reported by Demelash *et al.* (2009) in Yabello municipal abattoir, Teklu *et al.,* (2004) in Hosanna and Berg *et al.* (2009) in Addis Ababa, Gonder, Woldiya, Gimbi, Butajira, and Jinka.However, the prevalence of TB lesions found in this study is lower than those reported(Biffa *et al.*, (2010a) in Addis Ababa, Adama, Hawassa, Yabello, Melge-Wondo, Ameni *et al.*, (2010) in Kombolcha, Tigre *et al.*, (2010) in Nekemte, Aylate *et al.*, (2013) in Woldyia, Mekibeb *et al.*, (2013) in Addis Ababa, Romha *et al.*, (2013) in Humera, Zeru *et al.*, (2013) in Mekelle, and Ameni *et al.*, (2003) in Nazareth, While, it was higher than the finding reported Regassa *et al.*, (2010) in Hawassa, Shitaye *et al.*, (2006) in Addis Ababa, Nuru *et al.*, (2017) in Bahir Dar Abattoir, Gumi *et al.*, (2012b) in Negelle and Asseged *et al.*, (2004) in Addis Ababa. This difference in prevalence of tuberculoid lesions could be due to the difference in origin or types of production system and breed of animals that are slaughtered in the abattoirs.

In the present study, larger proportion (52.5%) of TB lesion was recorded in the respiratory pathway of the lung and associated lymph nodes followed by lymph nodes around head (26.25%), mesenteric lymph nodes (7.5%), prescapular lymph nodes (7.5%) and liver and hepatic lymph nodes (6.25%). This finding is significantly different from previous studies done in Ethiopia (Tamiru *et al.*, 2013) where 70 and 70.7% TB lesions were reported in lungs and associated lymph nodes, respectively. However, the distribution of TB lesion in the current study comparable with the results reported by Alemu *et al.*, (2015) where 50% of lesions involved in the lymph nodes of thoracic cavity. The observation of the largest proportion of TB lesions in the respiratory pathway was consistent with the reports of previous findings (Asseged *et al.*, 2004; Shitaye *et al.*, 2006; Regassa *et al.*, 2009; Mihreteab and Indris, 2011). This finding indicated that inhalation might be the principal route of TB infection in cattle. Therefore, during post-mortem examination focus should be given on lungs and associated lymph nodes.

The prevalence of the disease was statistically significant difference ($P<0.05$) in the prevalence of the disease between body condition scores (BCS), the prevalence being higher in lean (7.2%) than medium (5.2%) and good (2.4%) body conditioned animals. The present result is consistent with previous reports, which indicated that animals with good BCS have relatively strong immunological response to the infectious agent than animals with medium BCS, and the result could indicate the wasting nature of the disease (Radostits *et al.*, 2007).

In conclusion, the result of this study suggested the low prevalent of BTB in cattle slaughtered in Adama municipal abattoir and Bishoftu ELFORA export abattoir, in east Shewa, central Ethiopia. Larger proportion of BTB lesions recorded in the respiratory pathway of the lung and associated lymph nodes of this study suggested that inhalation might be the principal route of TB infection and during post-mortem examination; more attention should be given on the lungs and associated lymph nodes.

4.4. Characterization of MTC species and/or strains isolated from humans and cattle in East Shewa Zone, Central Ethiopia

Tefera Woldemariam[1, 2], Temesgen Mohammed[2], Mahlet Chanyalew[2], Aboma Zewude[2], Gezahegne Mamo[3] and Gobena Ameni [2]

[1]Hawassa University, School of Veterinary Medicine, P.O. Box 05, Hawassa, Ethiopia

[2]Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia

[3]College of Veterinary Medicine and Agriculture, Addis Ababa University, P. O. Box 34, Bishoftu, Ethiopia

Status: Manuscript is ready for submission for publication

4.4.1 Abstract

Tuberculosis remains a major public health problem in Ethiopia. While human to human transmission of mycobacterium tuberculosis (MTB) is of major importance in driving TB epidemic, the impact of Mycobacterium bovis (M. bovis) transmission from infected cattle is largely unknown. Therefore, the objective of this study was to investigate the zoonotic transmission of TB between cattle and human populations in East Shewa Zone, central Ethiopia between November 2014 to August 2016 on 392 in contact humans at Bishoftu and Adama Health institutions. In addition, 80 TB-like lesions from cattle slaughtered at Adama and Bishoftu as well as 12 milk samples from strong single intradermal cervical comparative tuberculin test (SICCTT) positive dairy cattle were cultured. Further the isolates were typed using spoligotyping. Of 392 human subjects from which sputum samples were cultured, mycobacterial growth was detected in 86/392 (21.9%) subjects. Spoligotyping was performed on these 86 isolates and 84 were confirmed as M. tuberculosis while two were M. bovis.

However, no growth of mycobacteria in 12 milk samples collected from these strongly tuberculin-positive reactor cows.

In conclusion isolation of M. bovis from humans and isolation of MTB from cattle tissues could suggest the transmission of these two species of mycobacteria between cattle and humans in East Shewa Zone central Ethiopia.

Keywords: *Zoonotic, M. bovis, M. tuberculosis, spoligotyping, East Shewa zone, Central Ethiopia*

4.4.2 Introduction

Bovine tuberculosis (BTB) is a disease characterized by the progressive development of specific granulomatous lesions or tubercles in the lung tissues, lymph nodes or other organs. Mycobacterium bovia (*M bovis),* the causative agent of BTB, is a member of the mycobacterium tuberculosis complex (MTC) (Smith *et al*., 2006). *M. bovis* is mainly responsible for BTB in cattle and in a wide range of both domestic and wild animals. It is also a known cause of zoonotic TB in humans, which can appear indistinguishable with regard to pathogenesis, lesions, and clinical findings to that caused by mycobacterium tuberculosis (*MTB)* (Cosivi *et al.,* 1988; Moda *et al.,* 1996). *M. bovis* shows a high degree of virulence for both humans and animals (Moda *et al.,* 1996). *MTB* is the most common cause of TB in humans but an unknown proportion of cases are due to *M. bovis*. More than 94% of the world population lives in countries in which the control of BTB in cattle is limited or absent (Cousins, 2001). In countries where BTB is still common and pasteurization of milk is not practiced, an estimated 10–15% of human TB is caused by *M. bovis* (Ashford *et al.,* 2001).

In many developing countries, BTB remains endemic causing significant economic losses (Zinsstag *et al.,* 2006). In animals, BTB has been reported from 33 of 43 African countries (Ayele *et al.,* 2004). WHO reported in 1998 that 3.1% of TB in humans worldwide is attributable to *M. bovis* and that 0.4–10% of sputum isolates from patients in African countries could be *M. bovis* (Michel *et al.,* 2010). Zoonotic TB is now acquiring increasing recognition in developing countries, including Ethiopia, as animals and humans share the same environment (Malama *et al*., 2013). Human cases of BTB have been described in some Sahelian countries like Ghana, Niger, Uganda andTanzania (Idigbe *et al.,* 1986; Addo *et al.,* 2007; Oloya *et al.,* 2008) and in immigrants from Chad (Godreuil *et al.,* 2010).
Gumi *et al*. (2012) found a link in the transmission of TB between livestock and pastoralists of South East Ethiopia. Human TB of animal origin (zoonotic TB) is an important public health concern in developing countries (Etter *et al.,* 2006). Rural inhabitants and some urban dwellers in Africa still consume unpasteurized and soured milk potentially infected with *M. bovis* (Ayele *et al.,* 2004). Human infection due to *M. bovis* is thought to be contracted mainly through drinking raw milk however; cases of pulmonary TB have also been reported particularly in patients from rural areas that live in close contact with cattle (Kazwala *et al.,* 1998).

In Ethiopia, several prevalence studies have been performed and showed that BTB is endemic in cattle; however, prevalence vary depending on the geographical areas, breeds and husbandry practices. Abattoir and dairy farm studies from central Ethiopia have reported prevalence between 3.5%-13.5% and locally in peri-urban Addis Ababa up to 50% (Ameni *et al.,* 2007; Shitaye *et al.,* 2007; Berg *et al.,* 2009; Demelash *et al.,* 2009; Regassa *et al.,*2010). In contrast, lower prevalence of 0.9% was reported in traditionally-kept zebu cattle (Tschopp *et al.,* 2010a). The observed variability of BTB disease frequency in Ethiopia might well be influenced by different livestock production systems (rural/pastoral/peri-urban) and different geographic and climatic contexts. Transmission of BTB seems to be higher in intensive peri-urban settings when compared to extensive rural and pastoral areas. Thus, the disease is particularly serious in intensive dairy farms, which serve the public as sources of dairy products, including milk. The situation could be exacerbated by the absence of any control method employed in the country. Thus, although the extent of transmission of *M bovis* to human beings has not yet been established in Ethiopia, the high prevalence of BTB on dairy farms and the practice of raw milk consumption by the public suggest that the impact of *M. bovis* on human TB could be significant.

Urban livestock production constitutes an important sub-sector of the agricultural production system in Ethiopia. In East Shewa zone of central Ethiopia specifically in Adama and Bishoftu towns, with the response to increased demand for milk and milk products linked to growth of human population, relatively large numbers of dairy farms and feedlot farms are found with intensive management system. In addition there are around 100 buchers, and several export abattoirs are located in the area which are exporting meat of small ruminants to Middle East countries (Saudi Arabia, Dubi and Yemen) and African countries (Djibouti, Congo Brazzaville, Cote-d'ivoire and Egypt), and meat of cattle for local consumption. Unfortunately, BTB has shown close links with intensive management system (Kleeberg, 1984 and Barwinek *et al.,* 1996).

The knowledge of the epidemiology of the disease is a footstep for designing any control strategy to limit the disease spread and to avoid risk for human infection. However, since in East Shewa Zone of central Ethiopia, specifically in Adama and Bishoftu towns the demand for dairy products is increasing, animal-to-human transmission of TB might still be significant although it has not been documented. Therefore, the objective of this study was to investigate the zoonotic transmission of TB between cattle and human populations in east Shewa zone central Ethiopia.

4.4.3 Results

4.4.3.1 *Isolation of mycobacterium from human*

Out of the 392 sputum samples cultured, growth of mycobacteria was observed in 21.9% (86/392) on primary culture. Mycobacterial growth was not observed in the majority os samples.

4.4.3.2 Isolation of mycobacterium from tissue and milk samples

Growth of MTC species were observed in 26.25% (21/80) of tissue TB-like lesion samples on primary culture. However, no growth of mycobacteria in 12 milk samples collected from these strongly tuberculin-positive reactor cows.

4.4.3. *3 Spoligotyping of human isolates*

Spoligotyping of 86 human isolates 35 different spolygotype patterns. The spoligotype patterns were grouped into 14 clustered patterns containing of 65 (75.6%) isolates and 21 (24.4%) single each consisting of one isolate.The overall diversity of the patterns was 40.7%. Out of the 35 spolygtype patterns, 27 were registered in the international data base and thus were considered as shared types while the remaining 8 were not found in the SITVIT Database and were orphans (Table 12). The dominantly identified patterns (~strains) were SIT 149, SIT 53, and SIT 118, each consisting of 18, 11 and 6 isolates, respectively (Table 12). Classification of the spoligotype patterns using TB-insight RUN TB-Lineage showed that the Euro-American (EA) lineage was the dominant Lineage consisting of 87.2% of the isolates. On the other hand, 3.5% of the isolates belonged to the Central-Asian (CAS) Lineage. Similarly, 3.5% of the isolates belonged to the *M. africanum* Lineages while only1.2% of the isolate belonged to Indo-oceanic Lineage. Interestingly, two *M. bovis* isolates (2.3%) were isolated from the sputa of two individuals.The two *M. bovis* were SB01443, which has already been registered in the *M.bovis*.org Database.

4.4.3 4. Spoligotyping of animal isolates

Spoligotyping of 21 isolates from cattle yielded 18 different spolygotype patterns. Of the 21 isolates, 12 (57.2%) isolates were confirmed to be *M. bovis* while the remaining 9 (42.8%) were *MTB*. The isolates were grouped into 3 clustered strains containing of 6 (28.63%) isolates and 15 (71.4%) single each consisting of a single isolate (Table 13). Out of the 18 spolygtype pattern, 6 were registered in the SITVIT2 international Database and thus were shared types while the remaining 12 were not found in the Database and hence were orphans (Table 13). The 12 strains of *M. bovis* isolated from cattle were represented by 9 different spoligotype patterns. Of the 9 clusters of *M bovis*, 7 clusters were new and had not been reported to the *M.bovis* spoligotype Database previously, while the remaining 2 clusters were SB1265 and SB1176. Of the nine strains of *MTB*, 5 strains were not registered in the international spoligotype SITVIT2 database and thus designated as orphan strains, while the remaining four strains of *MTB* were SIT 50, SIT 118, SIT 117 and SIT 1318 in the SITVIT2 database and most of them belonged to the Euro-American Linege (Table 13).

Table 12: Description of 27 shared-types (SITs; n=77 isolates) and 8 orphan strains (N=9) corresponding spoligotypingdefined lineages/sublineages starting from a total of 86 *MTB* strains isolated in central Ethiopia.

SIT	**Isolates with similar pattern**	**CBN* Lineage**	**SITVIT2 sublineage**	**Octal number**	**Binary**
149	18	Euro-American	T3 ETH	777000377760771	■■■■■■■■■□□□□□□□□□□■■■■■■■■■■■■■■□□□□■■■■■■■
777	3	Euro-American	H4	777777777420771	■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□■□□□□■■■■■■■
336	2	Euro-American	X1	777776777760731	■■■■■■■■■■■■■■■■■□■■■■■■■■■■■■■■□□□□■■■□■■■
53	11	Euro-American	T1	777777777760771	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■
50	2	Euro-American	H3	777777777720771	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■
134	3	Euro-American	H3	777777777720631	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■□□■■■
1745	1	Euro-American	T3	773737777760771	■■■■■■□■■■■■□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■
245	1	Euro-American	T1	777777777760671	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■□■■■■
37	4	Euro-American	T3	777737777760771	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■
221	1	Euro-American	X1	777766777760771	■■■■■■■■■■■■■■□■■□■■■■■■■■■■■■■■□□□□■■■■■■■
1221	1	Euro-American	T1	757767777760771	■■■■□■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□□■■■■■■■
1547	4	Euro-American	T3	777727777760771	■■■■■■■■■■■■□■□■■■■■■■■■■■■■■■■■□□□□■■■■■■■
51	1	Euro-American	T	777777777760700	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■□□□□
75	1	Euro-American	H3	777767777720771	■■■■■■■■■■■■■■□■■■■■■■■■■■■■■■□■□□□□■■■■■■■
7	1	Euro-American	T1	377777777760771	□■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■
118	6	Euro-American	T2	777767777760771	■■■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□□■■■■■■■
1318	3	Euro-American	T1	577767777760771	■□■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□□■■■■■■■
117	3	Euro-American	T1	777767777760731	■■■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□□■■■□■■■
1687	1	Euro-American	T1	777767775760771	■■■■■■■■■■■■■■□■■■■■■■■■■■□■■■■■■□□□□■■■■■■■
1802	1	Euro-American	H3	777767777720631	■■■■■■■■■■■■■■□■■■■■■■■■■■■■■■□■□□□□■■□□■■■
611	1	Euro-American	T1	777767777560771	■■■■■■■■■■■■■■□■■■■■■■■■■■■■□■■■□□□□■■■■■■■

Continued table 12

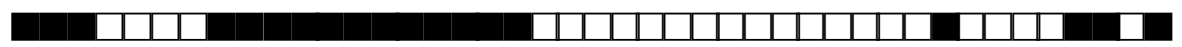
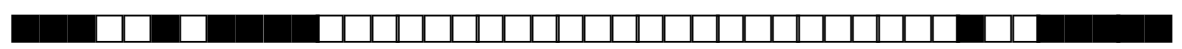
834	1	Euro-American	U	777767777740771	
116	1	Euro-American	H3	777767775720771	
Orphan	1	Euro-American	H3	477767777720571	
Orphan	1	Euro-American	X2	777737777760000	
Orphan	1	Euro-American	T	755777777760671	
Orphan	1	Euro-American	X2	777727777760000	
309	1	EAI	CAS1	703767740003171	
25	1	EAI	CAS1	703777740003171	
141	2	CAS	MTB CAS	703767740003771	
Orphan	1	CAS	CAS	703777400002061	
Orphan	2	*M-africanum*	Manu2	575347777763661	
Orphan	1	*M-africanum*	X2	554377777760660	
Orphan	1	IO	CAS	713600000001171	
SB1443	2	*M. bovis*	BOV	616777777777600	

Table 13: Description of 3 clustered strains containing of 6 (28.63%) isolates and 15 (71.4%) single each consisting of a single isolate from a total of 21 spoligotype isolates from cattle in central Ethiopia.

SIT/SB	Isolates with similar pattern	CBN* Lineage	SITVIT2 Lineage/	Octal number	Binary
SB1176	1	*M. bovis*	BOV_1	602773761000200	■■□□□□□■□■■■■■■□■■■■■■■□□□■□□□□□□□□□□■□□□□□
SB1256	2	*M. bovis*	BOV_1	616763777777600	■■□□□■■■□■■■■■□□■■■■■■■■■■■■■■■■■■■■■□□□□□
50	1	Euro-American	H3	777777777720771	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■
118	1	Euro-American	T2	777767777760771	■■■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□□■■■■■■■
117	1	Euro-American	T1	777767777760731	■■■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□□■■■□■■■
1318	1	Euro-American	T1	577767777760771	■□■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□□■■■■■■■
Orphan	1	*M. bovis*	BOV	416743777756600	■□□□□■■■□■■■■□□□■■■■■■■■■■■■■■□■■■□■■□□□□□□
Orphan	1	*M. bovis*	BOV_1	612763761000200	■■□□□■□■□■■■■■□□■■■■■■■□□□■□□□□□□□□□□■□□□□□
Orphan	1	*M. bovis*	BOV_1	612773761000200	■■□□□■□■□■■■■■■□■■■■■■■□□□■□□□□□□□□□□■□□□□□
Orphan	1	*M. bovis*	BOV_1	400201434200000	■□□□□□□□□□■□□□□□□■■□□□■■■□□□■□□□□□□□□□□□□□□
Orphan	1	*M. bovis*	BOV_1	402773761000200	■□□□□□□■□■■■■■■□■■■■■■■□□□■□□□□□□□□□□■□□□□□
Orphan	2	*M. bovis*	BOV_1	416763577756600	■□□□□■■■□■■■■■□□■■■□■■■■■■■■■■□■■■□■■□□□□□□
Orphan	2	*M. bovis*	BOV_1	612773761000200	■■□□□■□■□■■■■■■□■■■■■■■□□□■□□□□□□□□□□■□□□□□
Orphan	1	Euro-American	H3	577767777700771	■□■■■■■■■■■■■■□■■■■■■■■■■■■■■■□□□□□□■■■■■■■
Orphan	1	Indo-Oceanic	MANU1	777767777777771	■■■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■■■■■■■■■■■
Orphan	1	Euro-American	T	775767577760771	■■■■■■■□■■■■■■□■■■■□■■■■■■■■■■■■□□□□■■■■■■■
Orphan	1	Euro-American	T	777767777760661	■■■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□■■□■■□■
Orphan	1	Euro-American	X2	417763575600200	■□□□□■■■■■■■■■□□■■■□■■■■□■■□□□□□□□□□□■□□□□□

4.4.5 Discussion

In the present study, *MT*C species were isolated from 86 TB patients from Bishoftu and Adama Referral hospital and Adama Health Center who visited health institutions. In parallel, 21 *MT*C species were isolated from TB lesions of cattle slaughtered at Adama Municipal Abattoir and Bishoftu ELFORA Export Abattoir. The isolates were identified at strain and lineage levels on the basis of spoligotyping and compared with the strains in the SITVIT2 Database.

4.4.5.1 Animal isolates

In this study, culture positivity in primary culture media was found in 26.25% (21/80) TB suspected lesions. Culture positivity in present study is comparable with the results reported (Biffa *et al.* 2010a) with culture positivity from Addis Ababa, Adama, Hawassa, Yabello, Melge-Wondo and (Mekibeb *et al.* 2013) with culture positivity from Addis Ababa. While it was higher than that reported by Teklu *et al.* (2004) with culture positivity from Hossana, Shitaye *et al.* (2006), with culture positivity from Addis Ababa, Berg *et al.* (2009) with culture positivity from Addis Ababa, Gonder, Woldiya, Gimbi, Butajira, and Jinka and Nuru *et al.* (2017) with culture positivity from Bahir Dar Abattoir. However, it was lower than that reported by Ameni *et al.* (2010) with culture positivity from Kombolcha, Gumi *et al.* (2012b) with culture positivity from Negelle, Romha *et al.* (2013) with culture positivity from Humera and Zeru *et al.* (2013) with culture positivity from Mekelle.

Out of 22 bovine tuberculin positive reactors dairy cattle, no growth of mycobacteria was observed on Lowenstein-Jensen medium from 12 milk samples collected from these strongly tuberculin-positive reactor cows. The present result coincides with the result obtained by Ameni and Wudie (2003) in which out of 24 milk samples cultured from tuberculin reactive cows, no growth of mycobacterial was recorded.

In this study, 21 isolates showed evidence for *MTC* species, of which 12 were *M bovis* and nine were *MTB*. Further characterization of the strains of *M bovis* using spoligotyping revealed the presence of 12 isolates grouped into nine clusters of spoligotype patterns. Of the nine clusters of *M bovis*, seven clusters were new and had not been reported to the *M. bovis* spoligotype database previously, while the remaining two clusters of *M bovis* were of spoligotype SB1265 (two isolates) and SB1176 to the database *M.bovis*.org. The spoligotype SB1265 have been reported by Biffa *et al.* (2010a) in Yabello abattoir. The isolation of SB1176 (*M. bovis*) demonstrates dominance of this spoligotype as a major cause of BTB in Ethiopian cattle (Ameni *et al*., 2007b; Berg *et al*., 2009; Biffa *et al*., 2010a; Firdessa *et al*., 2012; Mekibeb *et al*., 2013). The spoligotype SB1176 carry a specific spoligotype feature (spacers 4–7 missing) typical for members of a clonal complex identified as *M. bovis* African 2 (Af2) so far only found in East Africa (Berg *et al*., 2011).

The nine *MTB* strain isolated from TB lesions in cattle belongs to eight Euro-American lineage (SIT 50, 118, 117 and 1318), and one Indo-Oceanic lineage. *M tuberculosis* infection has been reported in a wide range of domestic and wildlife animal species, most frequently in those living in close, prolonged contact with human beings (Thoen *et al*., 1981, Montali *et al*., 2001, Oh *et al*., 2002, Pavlik *et al*., 2003, Alfonso *et al*., 2004). Previously *MTB* isolation from TB suspected lesions were reported in cattle in Ethiopia (Berg *et al*., 2009; Ameni *et al*., 2010; Tsegaye *et al*.., 2010; Aylate *et al*., 2013). *MTB* has been sporadically isolated from cattle in other countries (Prasad *et al*., 2005; Ocepek *et al*., 2005) or zoo animals (Lyashchenko *et al*., 2006). Similarly, in Algeria and Sudan, the prevalence of *MTB* in cattle was 6.2 and 7.4 per cent, respectively (Boulahbal *et al*., 1978, Sulieman and Hamid, 2002). Animal attendants with active TB in the respiratory tract, urinary tract or gastrointestinal tract also represent an active source of *MTB* for animals, spreading the bacillus via the sputum, urine or faeces (Thoen *et al*., 1981). The isolation of *MTB* from tissues of bovine could likely justify the possible transmission of *MTB* from human to cattle.

4.4.5.2 Human isolates

Out of 392 smears positive samples cultured, only 86 (21.9%) were culture positive. This result is lower than with the previous findings reported in Nigeria Odubanjo and Dada-Adegbola (2011) and in Ethiopia by Disassa *et al.* (2015).

Spoligotyping of 86 mycobacterial human isolates revealed 35 distinct spolygotype patterns, which corresponded to 40.7% of genotype diversity. The diversity of spoligotypes strains that we observed in this study was consistent with the reported by Belay *et al.*, (2014) and Disassa *et al.*, (2015), but higher than the percentages reported earlier by other studies in Ethiopia (Diriba *et al.*, 2013; Yimer *et al.*, 2013; Debebe *et al.*, 2014). This low diversity of spoligotypes strains of *MTB* could suggest that a small number of lineages of *MTB* are causing the disease in the study area, despite the fact that there were significant migrations of different infected ethnic group of peoples to Adama city and its surroundings from other regions of the country. In addition, the long period of MTC clonal evolution may contribute to the diversity of strains (Godreuil *et al.*, 2010).

Sixty five of the mycobacterial isolates were grouped into 14 clusters with an overall clustering percentage of 75.6%. The clustering rate observed in this study was comparable with a study performed in the Afar Pastoral Region (Belay *et al.*, 2014) and a national survey (Getahun *et al.*, 2015). The rate is higher than in the context of studies in Bahir-dar (Nuru *et al.*, 2015), Benishangul (Disassa *et al.*, 2015) and Gambella (Asebe *et al.*, 2016). The observed differences in clustering rates might be related to differences of sanitation, housing and population density. And also high level of strain clustering could suggest recent and ongoing TB transmission in the study area (Small *et al.*, 1994; Barnes *et al.*, 1997; Easterbrook *et al.*, 2004).

The dominantly identified strains were SIT 149, SIT 53 and SIT 118 in order of decreasing frequency. In agreement with the present study SIT 149 (T3-ETH) is isolated frequently in Ethiopia and among Ethiopian immigrants in Denmark (Brudey *et al.*, 2006). Also similarly to this study, SIT149 was dominantly isolated by earlier studies conducted in Afar Pastoral Region of Ethiopia (Belay *et al.*, 2014). The second *MTB* strains found in the present study was SIT53, which was also reported earlier in the SITVIT database as the most common types in Ethiopia by other researchers (Bruchfeld, *et al.*, 2002).

The *MTB* isolated in the present study was belonged to the Euro-American, Indo-oceanic, East-African-Indian, *M. bovis* and *M. africanum*. The dominant Lineage was Euro-American (EA) Lineage consisting 87.2% of the isolates. This finding is in line with the findings of where they reported high prevalence of lineage 4, the most common lineage in Ethiopia (Mihret *et al.*, 2012; Diriba *et al.*, 2013; Firdessa *et al.*, 2013; Garedew *et al.*, 2013; Debebe *et al.*, 2014 and Disassa *et al.*, 2015). EA is the most dominant lineage in the world (Reed *et al.*, 2009) and in Ethiopia ranging from 32.5% in Gambella border to South Sudan (Asebe *et al.*, 2016), to 86.8% in central Ethiopia (Bedewi *et al.*, 2017). It can be hypothesized that the EA lineage might have been introduced to Ethiopia by Europeans during the Italian invasion of Ethiopia.

Screening of the SITVIT2 database also identified 3.5% (3/86) of the isolates as members of *M. africanum*. This finding is in line with the findings of where they reported by (Nuru *et al.*, 2015). *M. africanum* has been reported to be an important cause of human TB in the west African countries including Burkina Faso (Ledru *et al.*, 1996), Guinea-Bissau (Kallenius *et al.*, 1999), Senegal (Viana-Niero *et al.*, 2001), Cameroon (Niobe-Eyangoh *et al.*, 2003), CoteD'Ivoire (Niemann *et al.*, 2004), the Gambia (Jong *et al.*, 2006), Nigeria (Cadmus *et al.*, 2006) and Sierra Leone (Homolka *et al.*, 2008), Further studies are needed to explore evolutionary aspects that may have contributed to the spread of *M. africanum* in the study population.

Among 86 isolates, 2 (2.3%) isolates was identified as *M. bovis*. The two human *M. bovis* isolates had clearly the same spoligopatterns, suggesting that they originated from the same sources. The spoligotype is identical to SB1443 in thewww.mbovis.org database. The *M. bovis* isolates from human pulmonary TB patients matched with the spoligotype of the animal isolates (SB1443), which has been isolated from cattle in West Africa countries of Nigeria (Müller *et al.*, 2009), thus indicating cattle-to-human transmission. The overall contribution of *M. bovis* for TB in east Shewa zone central Ethiopia was low (2.3%) similar to other Ethiopian reports (Belay *et al.*, 2014; Berg *et al.*, 2015; Nuru *et al.*, 2015; Zewdie *et al.*, 2016). This finding was supported by the findings of other studies (Firdessa *et al.*, 2013) which shows the involvement of *M. bovis* for TB and presumably linked to contact with infected cattle and consumption of raw milk and meat (Ayele *et al.*, 2004). Higher prevalence of *M. bovis* in human TB patients has also been reported in Nigeria (Idigbe *et al.*, 1986; Mawak *et al.*, 2006)

Ghana (Addo *et al*., 2007) and Uganda (Oloya *et al*., 2008). This variation may be due to differences in transmission pathways or in sampling and diagnostic techniques.

5. GENERAL DISCUSSIONS

This study was conducted in East Shewa Zone on TB in cattle and in contact humans using purified protein derivatives skin test, postmortem examinations of TB-like lesion at abattoir, sputum samples from health institutions, bacteriological culture in TB lab and molecular typing in the study area.

Bovine tuberculosis is known to be endemic in cattle of Ethiopia particularly in dairy farms with a prevalence ranging from 0.8 to 46.8% in different husbandry system and breed of cattle (Ameni *et al*., 2003) based on *SICCTT*. However, no information is available on the prevalence of bovine tuberculosis in beef cattle feedlots of Ethiopia particularly on Borena breed managed under private fattening export farms located in and around Adama town.

The present study based on *SICCTT* has shown an apparent individual animal prevalence of BTB as 2.1% in the dairy farms of Adama city and in the beef feedlot was 4.39% at ≥4 mm cut-off point and 9.58% at ≥2mm cut-off point. In the dairy farm, the finding is comparable to a previous report of 2% in Ethiopia (Gumi *et al*., 2012) and 2.4% in Tanzania (Katale *et al.*, 2013). However, it is considerably lower than the figures reported by much of the previous studies in Ethiopia, 7.9% (Ameni *et al*., 2003), 13.5% (Ameni *et al*., 2007), 30% (Firdessa *et al* ., 2012), 7.1% (Nega *et al*., 2012), 13.64% (Ashenafi *et al*., 2013), 18% (Mamo *et al*., 2013), and 5.5% (Dejene *et al*., 2016). Out of 206 herds tested, only 15 (7.3%) had one or more tuberculin positive animals and the number of tuberculin reactors varied widely (0 to 55.6%) between the herds. The present herd-level prevalence too, was considerably lower than the range of estimates (12.5 to 56%) documented in the aforementioned previous studies.

Concerning the beef feedlot, the study revealed a low prevalence of bovine tuberculosis in beef cattle and indicated the presence of epidemiological risk factors for infection among cattle of the feedlots such as the origin of the cattle and age of the animal as important factors. Indicating the fact that these feedlots established for export of fattened live beef cattle, the findings of this study warrants the need of designing a control strategies at feedlot level and testing during quarantine period of the animals.

The study generated information of the prevalence and distribution of lesions of BTB in cattle slaughtered at Adama Municipal Abattoir and Bishoftu ELFORA Export abattoir in East Shewa, central Ethiopia. Based on postmortem examination at the abattoir, the prevalence of gross lesions of BTB was found to be 4.22%, which is comparable with the results reported by (Demelash et al. 2009) in Yabello municipal abattoir, (Teklu et al., 2004) in Hosanna and (Berg et al. 2009) in Addis Ababa.

In the present study, *MT*C species were isolated from 86 TB patients from Bishoftu and Adama Referral hospitals and Adama Health Center who visited health institutions. In parallel, 21 *MT*C species were isolated from TB lesions of cattle slaughtered at Adama Municipal Abattoir and Bishoftu ELFORA Export Abattoir. The isolates were identified at strain and lineage levels on the basis of spoligotyping and compared with the strains in the SITVIT2 Database.

The isolation of *M. bovis* from humans, as well as the isolation of *MTB* from cattle, indicates transmission between cattle and humans. Thus, the studies conducted in Ethiopia and elsewhere in the world (Ocepek *et al* 2005; Cadmus *et al* 2010) have confirmed the transmission of mycobacteria between animals and humans. Therefore, TB is of public health importance in Ethiopia and warrants locally adapted diagnosis and treatment protocols.

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study demonstrated low prevalence of BTB both in dairy farms located in Adama Town, and cattle that are slaughtered at Adama Municipality and Boshoftu ELFORA export abattoirs. Furthermore, the isolation of *M. bovis* from humans, as well as the isolation of *MTB* from cattle, indicates the possible transmission of mycobacteria between cattle and humans. Therefore, TB is of public health importance in Ethiopia and warrants locally adapted diagnosis and treatment protocols.

6.2 Recommendation

- Initiation of practically applicable methods for the control of BTB in cattle herds at Adama is recommeded. These can include registration of each dairy cattle, control of the movement of dairy cattle and regular testing of herds.
- Public education on the transmission of mycobacteria between cattle and humas is recommended.

7. REFERENCES

Abernethy D.A.; Denny, G.O.; Menzies, F.D.; McGuckian, P.; Honhold, N.; Roberts, A.R.; (2006): The Northern Ireland programme for the control and eradication of *M. bovis*. Veterinary Microbiology **112**:231-237.

Addo K.; Owusu-Darko K.; Yeboah-Manu D.; Caulley P.; Minamikawa M.; Bonsu F.; (2007): Mycobacterial Species Causing PTB at the Korle Bu Teaching Hospital, Accra, Ghana. Ghana Medical Journal **41** (2):52-57.

Admasu P.; Berihun W.; Niguse A.; (2014): Prevalence of BTB in dairy cattle of Yekidistrict, Southern Ethiopia. African Journal of Basic and Applied Sciences, **6**(5):135-140.

Alexander KA.; Laver PN.; Michel AL.; Williams M.; van Helden PD. and Warren RM.; (2010); *MTC*pathogen, *M. mungi.* Emerging Infectious Diseases 1296–9 10.3201/eid 1608.100314.

Alexander K.A.; Pleydell E.; Williams M.C.; Lane E.P.; Nyange J.F. and Michel A.L.; (2002): MTB: an emerging disease of free-ranging wildlife. Emerging Infectious Diseases **8***:* 598-601

Alemu J.; Mamo G.; Ameni G, and Pal M.; (2015): Molecular Epidemiology of Bovine Tuberculosis in Cattle and its Public Health Implications in Gambella Town and its Surroundings, Gambella Regional State, Ethiopia. *Global Journal of Medical Research:* Volume 15 Issue 5 Version 1.0 Year 2015 Type: Double Blind Peer Reviewed International Research Journal Publisher: Global Journals Inc. (USA) Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Alfonso R.; Romero R.E.; Diaz, A.; Calderon M.N.; Urdaneta G.; Arce J.; Patarroyo M.E.; and Patarroyo M.A.; (2004): Isolation and identification of mycobacteria in New World primates maintained in captivity. Veterinary microbiology **98:** 285-295.

Amanfu W.; (2006): The situation of TB and TB control in animals of economic interest. TB (Edinburgh, Scotland) **86:330**-335.7

Ameni G.; Tadesse K.; Hailu E.; Deresse Y.; Medhin G.; Aseffa A.; Hewinson G.; Vordermeier M. and Berg S.; (2013): Transmission of ***MTB*** between Farmers and Cattle in Central Ethiopia. PLoS ONE **8**(10): e76891.

Ameni G.; Vordermeier M.; Firdessa R.; Aseffa A.; Hewinson G.; Gordon S. V and

Berg S.; (2011): *Mycobacterium tuberculosis* infection in grazing cattle in Central Ethiopia. Vet J. **188** (3-4): 359–361. Doi: [10.1016/j.tvjl.2010.05.005]

Ameni G.; Desta F. and Firdessa R.; (2010): Molecular typing of M. bovis isolated from TB lesions of cattle in northeastern Ethiopia. The Veterinary Record **167***:* 138-141.

Ameni G.; Amenu K.,; and Tibbo M.; (2003): BTB prevalence and risk factor assessment in cattle and cattle owners in Wuchale-Jida district, Central Ethiopia. Journal of Applied Research in Veterinary Medicine; **1**(1): 17-26

Ameni G.; Aseffa A.; Engers H.; Young D.; Hewinson G.; and Vordermeier M. (2006): Cattle husbandry in Ethiopia is a predominant factor affecting the pathology of BTB and gamma interferon responses to mycobacterial antigens. Clinical and Vaccine Immunology: CVI **13***:* 1030-1036.

Ameni G.; Aseffa A.; Engers H.; Young D.; Gordon S.; Hewinson G.; and Vordermeier M.; (2007): High prevalence and increased severity of pathology of BTB in Holsteins compared to zebu breeds under field cattle husbandry in central Ethiopia. Clinical and Vaccine Immunology*:* CVI **14***:* 1356-1361.

Ameni G.; and Erkihun A.; (2007): BTB on small-scale dairy farms in Adama Town, central Ethiopia, and farmer awareness of the disease. Revue Scientifique et Technique (IOE) **26***:* 711-719.

Ameni G.; and Wudie A.; (2003): Preliminary study on BTB in Nazareth municipality abattoir of central Ethiopia. Bulletin of Animal Health and Production in Africa **51***:* 125-132.

Ameni G.; Hewinson G.; Aseffa A.; Young D.; and Vordermeier M.; (2008): Appraisal of interpretation criteria for the comparative intradermal tuberculin test for diagnosis of tuberculosis in cattle in central Ethiopia. Clinical and Vaccine Immunology: CVI**15*:*** 1272-1276.

Andrews A.; (2003): Bovine medicine, 2nd Ed, UK: Blackwell Science, Color of Atlas for the Diagnosis of Bacterial pathogens in animals, Germany: Paul Parey Scientific Publishers, pp108-118

Aranaz A.; Cousins, D.; Mateos, A.; Dominguez, L.; (2003): Elevation of *MTB* subsp. *Caprae (*Aranaz *et al.* 1999) to species rank as *Mycobacterium caprae* comb.

nov., sp. Nov. International Journal of Systematic and Evolutionary Microbiology 53: 1785-1789.

Aranaz A.; Liebana E.; Gomez-Mampaso E.; Galan J.C.; Cousins D.; Ortega A.; Blazquez J.; Baquero F.; Mateos A.; Suarez G.; and Dominguez L.; (1999): *MTB* subsp. *Caprae* subsp. Nov: A taxonomic study of a new member of the *MTC* isolated from goats in Spain. International *Journal of Systematic Bacteriology* **49**: 1263-1273.

Asebe G.; Worku A.; Zewude A.; Tafess K and Ameni G.; (2016): Molecular characterization of *MTC* in Gambella region, Southwest Ethiopia. Afr. J. Microbiol. Res**.** **10**: 8.

Ashenafi D.; Mamo G.; Ameni G.; and Simenew K.; (2013): Epidemiology and molecular characterization of causative agents of BTB in ruminants. Journal of Bacteriology and Parasitology, **4**:161.

Ashford D..; Whitney E..; Raghunathan P.; and Cosivi O.; (2001): Epidemiology of selected mycobacteria that infect humans and other animals. Revue Scientifique et Technique (IOE) **20***:* 325-337.

Asseged B.; Woldesenbet Z.; Yimer E.; Lemma E.; (2004): Evaluation of Abattoir Inspection for the Diagnosis of *M.bovis* Infection in Cattle at Addis Ababa Abattoir. Tropical Animal Health and Production **36**: 537-546.

Asseged B.; Lubke-Becker A.; Lemma E.; Taddele K. and Britton S.; (2000): BTB: A cross-sectional and epidemiological study in and around Addis Ababa. Bull Animal Health Production Africa: **48** (2): 71-80.

Asseged B.; (1999): BTB a cross-sectional and epidemiological study in and around Addis Ababa; MSc thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.

Awah-Ndukum J.; Kudi AC.; Bradley G.; Ane-Anyangwe IP.; Titanji VK.; Fon-Tebug S and Tchoumboue J.; (2013): Prevalence of BTB in cattle in the highlands of Cameroon based on the detection of lesions in slaughtered cattle and tuberculin skintests of live cattle. Veterinary Medicine: **57:** 59-76.

Awah-Ndukum J.; Kudi AC.; Bah GS, Bradley G and Tebug SF.; (2012): BTB in cattle in the highlands of Cameroon seroprevalence estimates and rates of tuberculin skin test reactors at modified cut-offs. *Vet Med Int* 2012, article ID 798502, 10.1155/2012/798502.

Ayele W.Y.; Neill S.D.; Zinsstag J.; Weiss M.G.; and Pavlik I.; (2004): Bovine tuberculosis: an old disease but a new threat to Africa. The international journal of TB and lung disease: the official journal of the International Union against TB and Lung Disease **8**: 924-937.

Aylate A.; Shah S.N.; Aleme H.; and Gizaw T.T.; (2013): BTB: prevalence and diagnostic efficacy of routine meat inspection procedure in Woldiya municipality abattoir north Wollo zone, Ethiopia. Trop. Anim. Health Prod. **45**: 855–864.

Barouni A. S.; Augusto C. J.; Lopes M. T. P.; Zanini M. S.; and Salas C. E.; (2004): "A pncA polymorphism to differentiate between Mycobacterium bovis and Mycobacterium tuberculosis," Molecular and Cellular Probes, vol. **18**, no. 3, pp. 167–170.

Barnes P.F.; Yang Z. and Preston-Martin S.; (1997): Patterns of TB transmission in central Los Angeles, J.A.M.A. **278**: 1159–1163.

Barwinek; (1996): Assessment of the socioeconomic importance of BTB in Turkey and possible strategies for control or eradication. Turkish- German Animal Health Information Project. General directorate of protection and control, Ankara, Eschborn. Deutsche Gesellschaft fur Technische Zusammenabeit.3-43.

Bedewi Z.; Worku A.; Mekonnen Y.; Yimer G.; Medhin G.; Mamo G.; Pieper R.; andAmeni G.; (2017): Molecular typing of *MTC* isolated from pulmonary TB patients in central Ethiopia. BMC Infect.Dis. **17**: 184.

Belay M.; Ameni G.; Bjune G.; Couvin D.; Rastogi N. and Abebe F.; (2014): StrainDiversity of *MTB i*solates from Pulmonary TuberculosisPatients in Afar Pastoral Region of Ethiopia. BioMed Research International 2014/238532 http://dx.doi.org/10.1155/2014/238532

Berg S.; Firdessa R.; Habtamu M.; Gadisa E.; Mengistu A.; Yamuah L.; Ameni G.; Vordermeier M.; Robertson B.D. and Smith N.H.*;* (2009): The burden of mycobacterial disease in Ethiopian cattle: implications for public health. PloS one **4:** e5068.

Berg S.; Garcia-Pelayo M.C.; Muller B.; Hailu E.; Asiimwe B.; Kremer K.; Dale J.; Boniotti M.B.; Rodriguez S.; and Hilty M..; (2011): African 2, a clonal complex of *M. bovis* epidemiologically important in East Africa. Journal of Bacteriology **193***:* 670-678.

Bezos J.; Alvarez J.; Romero B.; Aranaz A.; and Juan L.; (2012): TB in goats: assessment of current in vivo cell-mediated and antibody-based diagnostic assays. Veterinary Journal **191***:* 161-165.

Bhatia R. and Ichpujanti R.; (1994): Mycobacterium. In: Essentials of medical Microbiology, first edition, New –Delhi, pp: 285-292.

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

Biffa D.; Skjerve E.; Oloya J.; Bogale A.; Abebe F.; Dahle U.; Bohlin J.; and Djønne B.; (2010a): Molecular characterization of M. bovis isolates from Ethiopian cattle. BMC Vet. Res. **6**: 28.

Biffa D.; Inangolet F.; Bogale A.; Oloya J.; Djonne B.; and Skjerve E.; (2011): Risk factors associated with prevalence of TB-like lesions and associated mycobacteria in cattle slaughtered at public and export abattoirs in Ethiopia. Trop. Anim. Health Prod., **43**: 529-538

Blood D.C.; and Radostits O.M.; (1989): Veterinary Medicine. 7th edn. Bailliére Tindall, 7th Edition. London, UK.

Böddinghaus B.; Rogall T.; Flohr T.; Blöcker H.; and Böttger E.C.; (1990): Detection and identification of mycobacteria by amplification of rRNA. J Clin Microbiol: **28**: 1751– 1759.

Bonsu O.A.; Laing E.; and Akanmori B.D.; (2000): Prevalence of TB in cattle in the Dangme-West district of Ghana, public health implications. Acta Tropica **76***:* 9-14.

Boulahbal F.; Benelmouffok A. and Brahimi K.; (1978): Role of *MTB* in BTB. Archives De l'Institut Pasteur d'Algérie Institut Pasteur d'Algérie **53:** 155-164

Brosch R.; Gordon S.V.; Marmiesse M.; Brodin P.; Buchrieser C.; Eiglmeier K.; Garnier T.; Gutierrez C.; Hewinson G. and Kremer K.*;* (2002): A new evolutionary scenario for the MTC. Proceedings of the National Academy of Sciences of the United States of America**99***:* 3684-3689.

Bruchfeld J.; Aderaye G.; Palme I.; Bjorvatn B.; Ghebremichael S. and Hoffner S.; (2002): Molecular epidemiology and drug resistance of *MTB* isolates from Ethiopian PTB patients with and without human immunodeficiency virus infection. J Clin Microbiol; **40:**1636-1640.

Brudey K.; Driscoll J.; Rigouts L.; Prodinger W. A.; Gori A. and Al-Hajoj S.; (2006): MTC genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. BMC Microbiology; **6:**23-40.

Bruning-Fann C.S.; Schmitt S.M.; Fitzgerald S.D.; Fierke J.S.; Friedrich P.D.; Kaneene J.B.; Clarke K.A.; Butler K.L.; Payeur J.B.; Whipple D.L.; Cooley T.M.; Miller J.M.; and Muzo D.P.; (2001): BTB in free-ranging carnivores from Michigan. Journal of Wildlife Diseases **37**: 58-64.

Cadmus B.; Simeon I.; Mohammed K.; Abdullahi A.; Akinbowale O. and Dick van Soolingen; (2010): Mycobacterium bovis, but also M. africanum present in aw Milk of pastoral cattle in north-central Nigeria. *Trop Anim Health Prod* DOI 0.1007/s11250-010-9533-2

Cadmus S.; Palmer S.; Okker M.; Dale J.; Gover K.; Smith N.; Jahans K.; Hewinson R.G.; and Gordon S.V.; (2006): Molecular analysis of human and bovine tubercle bacilli from a local setting in Nigeria. Journal of Clinical Microbiology **44***:* 29-34.

Carslake D.; Grant, W.; Green L.E.; Cave J.; Greaves J.; Keeling M.; McEldowney J.; Weldegebriel H. and Medley G.F.; (2011): Endemic cattle diseases: comparative epidemiology and governance. Philosophical transactions of the Royal Society of London. Series B, Biological Sciences **366***:* 1975-1986.

Carter G and Wise D.; (2004): Essentials of Veterinary Bacteriology and Mycology. Sixth edition. Iowa State Press, a Blackwell Publishing Company, pp: 207-213.

Cassidy JP.; (2006): The pathogenesis and pathology of BTBwith insights from studies of in humans and laboratoryanimal models. *Vet Microbiol*, **112**: 151-161

CFSPH (2009): BTB. Institute for International Cooperation in Animal Biologics an OIE Collaborating Centre, Iowa State University, College of Veterinary Medicine, USA.

Chalmers J.W.T.; Jamieson, A.F.; and Rafferty P.; (1996): An outbreak of BTB in two herds in South West Scotland - veterinary and human public health response. Journal of Public Health **18**: 54-58.

Chen Y.; Chao Y.; Deng Q.; Liu T.; Xiang J.; Chen J.; Zhou J.; Zhan Z.; Kuang Y. and Cai H.; (2009): Potential challenges to the Stop TB Plan for humans in China; cattle maintain M. bovis and MTB. TB **89***:* 95-100.

Christianson S.; Wolfe J.; Orr P.; Karlowsky J.; Levett P.; Horsman G.; Thibert L.; Tang P. and Sharma M.; (2010): Evaluation of 24 locus MIRU-VNTR genotyping of *MTB* isolates in Canada. *Tuber:* **90**: 31-38.

Citron K.M.; (1988): Control and prevention of TB in Britain. British Medical Bulletin **44**: 704-716.

Cleaveland S.; Shaw D.J.; Mfinanga S.G.; Shirima G.; Kazwala R.R.; Eblate E. and Sharp M.; (2007):M. bovis in rural Tanzania: risk factors for infection in human and cattle populations. TB **87***:* 30-43.

Coetzer JAW.and Tustin RC.; (2004.): *Infectious diseases of livestock*, 2nd ed edn. Oxford: Oxford University Press

Cole S. T.; (2002): Comparative mycobacterial genomics as a tool for drug target and Antigen discovery. *Eur Respir J (in press).*

Collins C.H.; and Grange J.M.; (1987): Zoonotic implication of *M. bovis* infection. Irish Veterinary Journal**;** **41**: 363 – 366.

Corbett; (2006): Corbett, E.L.; Marston, B.; Churchyard, G.J.; De Cock, K.M., (2006): TB in sub-Saharan Africa: opportunities, challenges, and change in the era of antiretroviral treatment. The Lancet367: 926 – 937.

Corbett E.L.; Watt C.J.; Walker N.; Maher D.; Williams B.G.; Raviglione M.C.; and Dye C.; (2003): The Growing Burden of TB: Global Trends and Interactions with the HIV Epidemic. Archives of Internal Medicine **163**: 1009-1021.

Corner L.A.; (2006): The role of wild animal populations in the epidemiology of tuberculosis in domestic animals: how to assess the risk. Veterinary microbiology **112***:* 303-312.

Corner L.A.; (1994): Post mortem diagnosis of M. bovis infection in cattle. Veterinary Microbiology **40:** 53-63.

Cosivi O.; Grange J.M.; Daborn C.J.; Raviglione M.C.; Fujikura T.; Cousins D.; Robinson R.A.; Huchzermeyer H.F.; de Kantor I. and Meslin F.X.; (1998): Zoonotic TB due to *M. bovis* in developing countries. Emerging infectious diseases; **4***:* 59-70.

Cousin D.; Huchezermeyer H.; Griffin J.; Brückner G.; and Van Rensburg Kriek N.; (2004): TB in infectious diseases of livestock. Infectious diseases of livestock, 1973-1987.

Cousins D.V.; (2003): Tuberculosis in seals caused by a novel member of the Mycobacterium tuberculosis complex: Mycobacterium pinnipedii sp. Nov. Int J SystEvol Microbiol53, 1305-1314.

Cousins D.V.; (2001): M. bovis infection and control in domestic livestock. Revue scientifique et technique (IOE); **20***:* 71-85.

Cousins D.V. and Roberts J.L.; (2001): Australia's campaign to eradicate BTB: the battle for freedom and beyond. TB 81*:* 5-15.

CSA (2007): Population and Housing Census: Population Size by Age and Sex. CSA Summary and Statistical Report of the 2007. Central Statistics Agency (CSA), Addis Ababa, Ethiopia.

CSA (2011): Livestock sample survey Ministry of Finance and Economic Development.Addis Ababa, Ethiopia.

CSA (2013): Report on livestock and livestock characteristics (Private peasant holdings). Agricultural sample survey 2011/12, Addis Ababa, Ethiopia, Statistical Bulletin, P 468.

De Garine-Wichatitsky M.; Caron A.; Kock R.; Tschopp R.; Munyeme M.; Hofmeyr M. and Michel A.; (2013): A review of BTB at the wildlife-livestock-human interface in sub-Saharan Africa. Epidemiology and Infection; **141***:* 1342-1356.

De la Rua-Domenech R.; (2006b): Human *Mycobacterium bovis* infection in the United Kingdom: Incidence, risks, control measures and review of the zoonotic aspects of Bovine tuberculosis. *Tuberculosis* 86, 77-109.

De Lisle G.W.; Bengis R.G.; Schmitt S.M. and O'Brien D.J.; (2002): TB in free-ranging wildlife: detection, diagnosis and management. Revue Scientifique etTechnique (IOE) **21***:* 317-334.

De Lisle G.W.; Mackintosh C. and Bengis R.; (2001): *M. bovis* in free-living and captive wildlife, including farmed deer. Revue scientifique et technique IOE **20***:* 86-111.

Dean G.S.; Rhodes S.G.; Coad M.; Whelan A.O.; Cockle P.J.; Clifford D.J.; Hewinson R.G. and Vordermeier H.M.; (2005): Minimum infective dose of *M. bovis* in cattle. Infection and immunity; **73***:* 6467-6471.

Debebe T.; Admassu A. and Mamo G.; (2014): Molecular characterization of MTB isolated from pulmonary tuberculosis patients in Felege Hiwot Referral Hospital, northwest Ethiopia .J. Microbial. **47**; 333-338.

Defra (2008): Zoonoses Report United Kingdom 2007. Department for Environment, Food and Rural Affairs. London, UK.

Delahay R.J.; Smith G.C.; Barlow A.M.; Walker N.; Harris A.; Clifton-Hadley R.S.; and Cheeseman C.L.; (2007): BTB infection in wild mammals in the South-West region of England: A survey of prevalence and a semiquantitative assessment of the relative risks to cattle. The Veterinary Journal **173**, 287-301.

De-Lahunta A. and Habel R.E.; (1986): Teeth: Applied Veterinary Anatomy; W. B. Sounders Company. USA, pp. 4-16.

Demelash B.; Inangolet F.; Oloya J.; Asseged B.; Badaso M.; Yilkal A.; and Skjerve E.; (2009): Prevalence of BTB in Ethiopian slaughter cattle based on post-mortem examination. Trop Anim Health Prod **41*:*** 755-765.

Diriba B.; Berkessa T.; Mamo G.; (2013): Spoligotyping of multidrug-resistant *MTB*isolates in Ethiopia, Int. J. Tuberc. Lung Dis. **17:**246–250.

Disassa H.; Tafess K.; Worku A.; Ameni G.; (2015): A Preliminary Study onMolecular Characterization of *MTB*in BenishangulGumuz Region, Western Ethiopia. BMRJ **10**: 1-10.

Djelouadji Z.; Raoult D.; Daffé M.; & Drancourt M. A.; (2008): Single-Step Sequencing Method for the Identification of *MT*C Species. PLoS Negl Trop Dis. **2**: e253

Donnelly C.A.; Wei G.; Johnston W.T.; Cox D.R.; Woodroffe R.; Bourne F.J.; Cheeseman C.L.; Clifton-Hadley R.S.; Gettinby G.; Gilks P.; Jenkins H.E.; Le Fevre A.M.; McInerney J.P.; Morrison W.I.; (2007): Impacts of widespread badger culling on cattle TB: concluding analyses from a large-scale field trial. International Journal of Infectious Diseases **11**: 300-308.

Durr PA.; Hewinson RG. and Clifton-Hadley RS.; (2000): Molecular epidemiology of BTB- I. *M. bovis* genotyping. Revue Scientifique et Technique de IOE 19: 675-688

Easterbrook P.J.; Gibson A.; Murad S.; (2004): High rates ofclustering of strains causing TB in Harare, Zimbabwe: a molecular epidemiological study, J. Clin. Microbiol. **42**: 4536–44.

EFSA (2007): TB, Mycobacterila diseases, United Kingdom. Report on trends and sources of zoonoses and zoonotic agents in humans, foodstuffs, animals and feedingstuffs. Department for Environment, Food and Rural Affairs (Defra)

Britain and Department of Agriculture and Rural Development, (DARD) Northern Ireland, United Kingdom.

Elias K.; Hussein D.; Asseged B.; Wondwossen T. and Gebeyehu M.; (2008): Status of BTB in Addis Ababa dairy farms. Revue Scientifique etTechnique (IOE) **27:** 915-923.

Enarson D.A.; (2006): M. bovis infection in animals and humans: Introduction, In: Thoen, C.O.; Steele, J.H.; Gilsdorf, M.J. (eds.), *M.bovis* infection in animals and humans. Blackwell Publishing Ltd, Iowa, USA. 1 -5.

EPHI and FMOH.; (2015): Report on National TB/HIV Sentinel Surveillance (April 2010 - June 2015). EPHI, Addis Ababa, Ethiopia.

Etchechoury I.; Valencia G.E.; Morcillo N.; Sequeira M.D.; Imperiale B.; López M.; Caimi K.; Zumárraga M.J.; Cataldi A. and Romano M.I.; (2010): Molecular Typing of *M. bovis* Isolates in Argentina: First Description of a Person-to-Person Transmission Case. Zoonoses and Public Health**; 57**: 375–381.

Etter E.; Donado P.; Jori, F.; Caron A.; Goutard F.; Roger F.; (2006): Risk Analysis and BTB, a re-emerging Zoonosis. Annals of the NewYork Academy of Sciences **1081**: 61-73.

Evans J.T.; Smith, E.G.; Banerjee, A.; Smith, R.M.M.; Dale, J.; Innes, J.A.; Hunt, D.; Tweddell, A.; Wood, A.; Anderson, C.; Hewinson, R.G.; Smith, N.H.; Hawkey, P.M.; Sonnenberg, P., (2007): Cluster of human TB caused by *M. bovis*: evidence for person-to-person transmission in the UK. The Lancet**; 369**: 1270-1276.

FAO (2009): Livestock production primary. Food and Agriculture Organization of the United Nations, Rome, Italy.

Fetene T.; Kebede N. and Alem G.; (2011): TB infection in animal and human populations in three districts of Western Gojam, Ethiopia. Zoonoses and Public Health **58***:* 47-53.

Filliol I.; Driscoll J. R.; van Soolingen D.; Kreiswirth B. N.; Kremer K.; Valetudie G.; Dang D. A.; Barlow R.; Banerjee D. and Bifani P. J.; (2003): Snapshot of moving and expanding clones of *MTB* and their global distribution assessed by spoligotyping in an international study. J. Clin.Microbiol**. 41**: 1963-1970.

Firdessa R.; Berg S.; Hailu E.; Schelling E.; Gumi B.; Erenso G.; Gadisa E.; Kiros T.; Habtamu M.; Hussein J.; Zinsstag J.; Robertson B. D.; Ameni G.; Lohan A. J.; Loftus B.; Comas I.; Gagneux S.; Tschopp R.; Yamuah L.; Hewinson G.; Gordon S. V.; Young D. B. and Aseffa

A.; (2013): Mycobacterial lineages causing pulmonary and extrapulmonary tuberculosis, Ethiopia. *Emerg. Infect. Dis.* **19:** 460-463.

Firdessa R.,; Tschopp R.; Wubete A.; Sombo M.; Hailu E.; Erenso G.; (2012): High Prevalence of BTB in Dairy Cattle in Central Ethiopia: Implications for the Dairy Industry and Public Health. PLoS One, **7** (12): e52851.

Fitzgerald S. D.; Kaneene J. B.; Butler K. L.; Clarke K. R.; Herke J. S.; Schmitt S. M.; Bruning-Fann C. S.; Mitchell R. R.; Berry D. E. and Payeur J. B.; (2000): Comparison of post-mortem techniques for the detection of *Mycobacterium bovis* in white-tailed deer (Odocoileus virginianus*). Journal of Veterinary Diagnostic Investigation,* **12**:322-327.

FMOH (2008): TB, Leprosy and TB/HIV Prevention and Control Programme Manual. Federal Ministry of Health Ethiopia, Addis Ababa, Ethiopia.

FMOH (2015). HSDP IV Annual Performance Report. FMOH.

Garedew L.; Mihret A.; Mamo G.; Abebe T.; Firdessa R.; (2013): Strain diversity of mycobacteria isolated from pulmonary tuberculosis patients at Debre Birhan Hospital, Ethiopia. *Int J Tuberc Lung Dis,* **17** (8):1076-81

Garner G.; Saville P and Fediaevsky A.; (2004): Manual for the recognition of exotic disease of livestock. A reference guide for animal healthy staff (on line). Food and Agriculture Organization of UN. At: http www.speitans Accessed on October 9, 2007.

Garnier T.; Eiglmeier K.; Camus JC.; Medina N.; Mansoor H.; Pryor M.; Duthoy S.; Grondin S.; Lacroix C.; Monsempe C.; Simon S.; Harris B.; Atkin R.; Doggett J, Mayes R, Keating L, Wheeler PR, Parkhill J, Barrell BG, Cole ST, Gordon SV, Hewinson RG., (2003): The complete genome sequence of *M. bovis*. Proc Natl Acad Sci U S A; **100** (13):7877-82.

Gavier-Widen D.; Chambers M.A.; Palmer N.; Newell D.G.; and Hewinson R.G.; (2001): Pathology of natural Mycobacterium bovis infection in European badgers (Meles meles) and its relationship with bacterial excretion. The Veterinary record **148***:* 299-304.

Getahun M.; Ameni G.; Kebede A.; Yaregal Z.; Hailu E.; Medihn G.; Demssie D.; Girmachew F.; Fiseha Y.; Meaza A.; Dirse N.; Agonafir M.; Dana F.; Tsegaye F.; Alebachew Z.; Abebe A.; Kebede A. and Lemma E.; (2015): Molecular typing and drug sensitivity testing of *MTB*isolated by a community-based survey in Ethiopia. BMC Public Health **15**: 751.

Gilbert M.; Mitchell A.; Bourn D.; Mawdsley J.; Clifton-Hadley R.; Wint W.; (2005): Cattle movements and BTB in Great Britain. Nature **435:** 491-496.

Glickman MS.; Jacobs WR., Jr.; (2001): Microbial pathogenesis of *MTB*: dawn of a discipline. *Cell*, *104*: 477-485

Godreuil S.; Jeziorski E.; Banuls AL; Fraisse T; Van de Perre P; Boschiroli ML.; (2010): Intrafamilial Cluster of PTB Due to *M. bovis* of the African 1 Clonal Complex. Journal of Clinical Microbiology **48** (12): 4680-4683.

Good M.; (2006): BTB eradication in Ireland. Irish Veterinary Journal **59:** 154 - 162.

Goodchild,T.; Clifton-Hadley R.; (2006): The fall and rise of BTB in Great Britain, In: Thoen C.O.; Steele, J.H.; Gilsdorf M.J.; (eds.), M. bovis infection in animals and humans. Blackwell Publishing Ltd, Iowa, USA. 100 - 116.

Gordon S.V. Bottai D.; Simeone R.; Stinear T.P. and Brosch R.; (2009): Pathogenicity in the tubercle bacillus: molecular and evolutionary determinants. BioEssays **31**, 378-388

Grange J. M.; (2001): Mycobacterium bovis infection in human beings. TB, **81** (1-2):71- 77.

Groenen P. M.; Bunschoten A.E.; van Soolingen D. and van Embden J.D.; (1993): Nature of DNA polymorphism in the direct repeat cluster of *MTB*; application for strain differenciation by a novel typing method. Mol. Microbiol. **10***: 1057-1065.*

Gumi B.; Schelling E.; Berg S.; Firdessa R.; Erenso G.; Mekonnen W.; Hailu E.; Melese E.; Hussein J.; Aseffa A.; and Zinsstag J.; (2012): Zoonotic transmission of TB between pastoralists and their livestock in South-East Ethiopia. EcoHealth **9:** 139-149.

Gumi B.; Schellin E .; Firdessa R.; Aseffa A.; Tschopp R.;Yamuah L.;Young D.; Zinsstag J.; (2011). Prevalence of bovine tuberculosis in pastoral cattle herds in the Oromia region, southern Ethiopia. Tropical animal health and production. 43. 1081-7. 10.1007/s11250-010-9777

Haile-Mariam S.; (1975): A brief analysis of the activities of the meat inspection and quarantine division. Ministry of Agriculture (MoA), Addis Ababa, Ethiopia.

Halderman M.; (2005): The political economy of pro-poor livestock policy-making in Ethiopia.

Haydon D.T.; Cleaveland S.; Taylor L.H. and Laurenson M.K.; (2002): Identifying reservoirs of infection: a conceptual and practical challenge. Emerging Infectious Diseases **8***:* 1468-1

Hiko A. and Agga G.E.; (2011): First-time detection of mycobacterium species from goats in Ethiopia. Tropical animal health and production **43***:* 133-139.

Hirsh C. and Zee C.; (2000): Mycobacteria species; the agent of animal TB. Veterinary Microbiology. Blackwell Science, pp158-164.

Hlokwe TM.; van Helden P. and Michel A.; (2013): Evaluation of the discriminatory power of variable number of Tandem Repeat Typing of *M. bovis* isolates from Southern Africa. Transboundary and Emerging Diseases, **60**: 111-120

Hope JC. and Villarreal-Ramos B.; (2008): BTB and the development of new vaccines. Comp Immunol Microbiol Infect Dis, **31**: 77-100.

Homolka S.; Post E. and Oberhauser B.; (2008): High genetic diversity among *Mycobacterium tuberculosis* complex strains from Sierra Leone, BMC Microbiol. **8:** 103.

Houben R. M. and Dodd P. J.; (2016): The Global Burden of Latent TB Infection: A Re-estimation Using Mathematical Modelling. PLoS Med. 13:

Huard RC., Fabre M., de HP, Lazzarini LC., van SD. and Cousins D. (2006): Novel genetic Polymorphisms that further delineate the phylogeny of the *MT*C. *J Bacteriol*, **188**: 4271-4287

Huard RC.; Fabre M.; de HP.; Lazzarini LC.; van SD. and Cousins D.; (2006): Novel genetic polymorphisms that further delineate the phylogeny of the *MT*C. *J Bacteriol*, **188**: 4271-4287

Hughes M.S.; Neill S.D.; Rogers M.S.; (1996): Vaccination of the badger (*Meles meles*) against *M. bovis*. Veterinary Microbiology **51:** 363-379.

Idigbe EO.; Anyiwo CE.; Onwujekwe DI.; (1986): Human pulmonary infections with bovine and atypical mycobacteria in Lagos, Nigeria. The Journal of Tropical Medicine and Hygiene **89** (3):143-148.

Idrisu A. and Schnurrenberger P.; (1977): Public-health significance of BTB in 4 northern states of nigeria-myco-bacteriologic study. *Nigerian Medical Journal* **7:** 384-387.

ITIS (2017): Integrated Taxonomic Information System on-line database.

Iwnetu, R., Van Den Hombergh, J., Woldeamanuel, Y., Asfaw, M. and Gebrekirstos, C., (2009): Comparative Performance of Diagnostic Tests for Mycobacterial Lymphadenitis in a High-Burden Country. Scand J of Infect Dis, **41**: 462-468.

Jebara K.B.; Caceres P.; Berlingieri F. and Weber-Vintzel L.; (2012): Ten years' work on the World Organisation for Animal Health (OIE) Worldwide Animal Disease Notification System. Preventive Veterinary Medicine **107:** 149-159.

Jiwa S.; Kazwala R.; Aboud A.; and Kalaye W.; (1997): Bovine tuberculosis in the Lake Victoria zone of Tanzania and its possible consequences for human health in the HIV/AIDS era. Veterinary research communications **21:** 533-539.

Johnson L.; Dean, G.; Rhodes S.; Hewinson G.; Vordermeier M. and Wangoo A.; (2007): Low-dose *M. bovis* infection in cattle results in pathology indistinguishable from that of high-dose infection. TB **87:** 71-76.

Johnson M.M.; Waller E.A. and Leventhal J.P.; (2008): Nontuberculous mycobacterial pulmonary disease. Current Opinion in Pulmonary Medicine**14:** 203-210.

Jong B.; de Hill P.; Brookes R.; (2006):*M. africanum* elicits an attenuated T cell response to early-secreted antigenic target, 6 kDa in patients with tuberculosis and their household contacts, J. Infect. Dis. **193**; 1279-1286.

Kallenius G.; Koivula T.; Ghebremichael S.; (1999): Evolution and clonal traits of *MTC* in Guinea-Bissau, J. Clin. Microbiol. **37**; 3872-3878.

Kamerbeek J.; Schouls L.; Kolk A.; van Agterveld M.; van Soolingen D.; Kuijper S.; Bunschoten A.; Molhuizen H.; Shaw R.; Goyal M. and van Embden J.; (1997): Simultaneous detection and strain differentiation of *MTB* for diagnosis and epidemiology. J. Clin. Microbiol. **35**: 907-914.

Kaneene J.B.; Pfeiffer D.; (2006): Epidemiology of *M. bovis*, In: C. O. Thoen; Steele, J.H.; Gilsdorf, M.J. (eds.), *M. bovis* infection in animals and humans Blackwell Publishing Ltd., Iowa, USA. 34 - 48.

Kassa G.M.; (2014): Molecular Epidemiology and Transmission patterns of *MT*C in Afar Pastoral Communities and their livestock in Ethiopia. (University of Oslo Oslo, Norway).

Katale BZ; Mbugi EV.; Karimuribo ED.; Keyyu JD.; Kendall S.; Kibiki GS.; Godfrey-Faussett P.; Michel AL.; Kazwala RR.; van Helden P.; Matee MI.; (2013): Prevalence and risk factors for infection of BTB in indigenous cattle in the Serengeti ecosystem, Tanzania. BMC Veterinary Research, **9**:267.

Katia V.; Marzia P.; Daaniela M.; Francesco I.; Maria C and Elena B.; (2008): Comparison of histological technique for the diagnosis of BTB in the framework of eradication programs. Journal of Veterinary Diagnostic Investigation. **20:** 164-169.

Katial K.; Hershey P.; Seth T.; Belisle J.; Brannan S. and Spancer J.; (2001): Cellmediatedimmune response to TB antigens: Comparison of skin testing and in whole-bloodculture. Cl Diagn Lab Immuno, **8**: 329-345.

Kazwala R.; Daborn C.; Sharp J.; Kambarage D.; Jiwa S. and Mbembati N.; (2001): Isolation of Mycobacterium bovis from human cases of cervical adenitis in Tanzania: a cause for concern? The International Journal of Tuberculosis and Lung Disease **5,** 87-91.

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

Kidane D.; Olobo J.O.; Habte A.; Negesse Y.; Aseffs A.; Abate G.; Yassin M.A.; Betreda K.; Harboe M.; (2002): Identification of the causative organism of TB lymphadenitis in Ethiopia by PCR. Journal of Clinical Microbiology **40:** 4230–4234.

Kinfe G.; Eshetu L.; (1987): Isolation of *M. bovis* from milk and tissues: Implication for public health and animal production. In: Proceeding of the first National Livestock Improvement Conference, I A R, Addis Ababa, Ethiopia.

Kiros T.; (1998): Epidemiology and zoonotic importance of BTB in selected sites of Eastern Shoa Ethiopia, Faculties of Veterinary Medicine, Addis Ababa University and Freie Universitaet Berlin. (MSC thesis).

Kremer K.; Benschoten A.; Schouls L.; van Soolingen D. and van Embden J.; (2002): Spoligotyping: a PCR based method to simultaneusly detect and type MTC bacteria.Bilthoven, the Netherlands, National Institue of Public Health and the Environment.

Kubica T.; Agzamova R., Wright A., Rakishev G., Rusch-Gerdes S. and Niemann S.; (2006): M. bovis isolates with MTB specific characteristics. Emerg Infect Dis, **12**: 763-765.

Ledru S.; Cauchoix B.; Yameogo M.; (1996): Impact of short-course therapy on tuberculosis drug resistance in South-West Burkina Faso. Tuber Lung Dis. **77:** 429-436.

Lee E. and Holzman R.S.; (2002): Evolution and current use of the tuberculin test. Clin Infect Dis, **34**: 365–370.

Liebana E.; Johnson L.; Gough J.; Durr P.; Jahans K.; Clifton-Hadley R.; Spencer Y.; Hewinson R.G.; Downs S.H.; (2008): Pathology of naturally occurring BTB in England and Wales. The Veterinary Journal **176**: 354-360.

Lilenbaum W. and Fonseca L.; (2005): The use of ELISA as a complementary tool for bovine tuberculosis in Brazil. Department of Microbiology, Brazil, pp256-261

Lowenstein E.; (1933): *Ann. Inst. Pasteur.*; **50**:161.

Luciani F.; Francis A. R. and Tanaka M. M.; (2008): Interpreting genotype cluster sizes of *MTB*isolates typed with *IS6110* and spoligotyping. Infect.Gen. Evol. **8**: 182-190

Lyashchenko K.P.; Greenwald R.; Esfandiari J.; Olsen J.H.; Ball R.; (2006): TB in elephants: antibody responses to defined antigens of MTB, potential for early diagnosis, and monitoring of treatment. Clin Vaccine Immunol **13:** 722–732.

Magdalena J.; Vachee A.; Supply P. and Locht C.; (1998): Identification of a new DNA region specific for members of *MTC.Clin Microbiol.* **36**: 937-943

Malama S.T.; Johansen B. J.; Muma B.; Mwanza S.; Djonne B. and Godfroid J.; (2013): Isolation and molecular characterization of*M. bovis* from Kafue lechwe (Kobus leche kafuensis)from Zambia, Tropical Animal Health and Production, **46:** no. 1, pp. 153–157.

Mamo G.; Fekadu A.; Yalelet W.; Nesredin H.; Mengistu L.; Getachew T. Gir;may M.; Gunnar B. and Ameni G.; (2013): BTB and its associated risk factors in pastoral and agro-pastoral cattle herds of Afar Region, Northeast Ethiopia. *J. Vet. Med. Anim. Health*, **5** (6): 171-179.

Mamo G.; Bayleyegn G.; Sisay Tessema T.; Legesse M.; Medhin G.; Bjune G. Abebe F.; and Ameni G.; (2011): Pathology of camel TB and molecular characterization of its causative agents in pastoral regions of Ethiopia. PloS One **6*:*** e1586.

Mathews F.; Macdonald D.W.; Taylor G. M.; Gelling M.; Norman R.A.; Honess P.E.; Foster R.; Gower C.M.; Varley S.; and Harris A.; (2006): BTB (M. bovis) in British farmland wildlife: the importance to agriculture. Proceedings Biological Sciences / the Royal Society **273:** 357-365.

Mawak J.; Gomwalk N.; Bello C. and Kandakai-Olukemi Y.; (2006): Human pulmonary infections with bovine and environment (atypical) mycobacteria in Jos, Nigeria. Ghana Med J, **40**: 132-136.

Mazars E.; Lesjean S.; Banuls A.; Gilbert M.; Vincent V.; Gicquel B.; Tibayrenc M.; Locht C. and Supply P.; (2001): High-resolution minisatellite-based typing as a portable approach to Global analysis of *MTB* molecular epidemiology. Proc. Natl.Acad. Sci.USA.**98**: 1901-6.

Mazurek G. H.; Cave M. D.; D, E. K.; Wallace R. J.; Bates J. H. and Crawford J. T.; (1991): Chromosomal DNA fingerprinting patterns produced with *IS6110* as strain specific markers for epidemiological study of TB. J Clin Microbiol **29**: 2030-2033.

Mekibeb A.; Fulasa T.T.; Firdessa R.; Hailu E.; (2013): Prevalence study on BTB and molecular characterization of its causative agents in cattle slaughtered at Addis Ababa municipal abattoir, Central Ethiopia. Trop. Anim. Health Prod. **45:** 763–769.

Menzies F. and Neill S.; (2000): Cattle-to-cattle transmission of BTB. The Veterinary Journal **160:** 92-106.

Michel A.L.; Muller B. and van Helden P.D.; (2010): M. bovis at the animal-human interface: a problem, or not? Veterinary microbiology **140:** 371-381.

Michel A.L.; Venter L.; Espie I.W. and Coetzee M.L.; (2003): MTB infections in eight species at the National Zoological Gardens of South Africa, 1991-2001. Journal of zoo and wildlife medicine: **34:** 364-370.

Mihret A.; Bekele Y.; Loxton A. G.; Jordan A. M.; Yamuah L.; Aseffa A.; Howe R. and Walzl G.; (2012): Diversity of *MTB* isolates from new PTB cases in Addis Ababa, Ethiopia. Tuberc. Res. Treat. **2012**: 892079.

Mihreteab B. and Indris B.; (2011): Evaluation of Routine Meat Inspection Procedure to Detect BTB Suggestive Lesions in Jimma Municipal Abattoir, South West Ethiopia. Global Veterinaria, **6**: 172-179.

Molhuizen H.; Shaw R.; Goyal M.; van Embden J.D.; (1997): Simultaneous detection and straindifferentiation of MTB for diagnosis and epidemiology, J Clin Microbiol, **35**: 907–914.

Montali R. J.; Mikota S. K. and Cheng L. I..; (2001): *MTB* in zoo and wildlife species. Revue Scientifique et Technique–OIE **20:** 291-303

Mostowy S.; and Behr M. A.; (2005): The origin and evolution of *MTB*, Clin. Chest. Med**.** **26**: 207-216.

Mostowy S.; Gordon S.V.; Inwald J.; Martin C.; Warren R.; Remer K.; (2005); revisiting the evolution of *M.bovis*.J Bacteriol **187**:6386–6395.

Mostrom P., Gordon M., Sola C., Ridell M. and Rastogi N. (2002): Methods used in the molecular epidemiology of TB. *Clin Microbiol Infect*, **8**: 694-704.

Mposhy M.; Binemo-Madi C. and Mudakikwa B.; (1983): Incidence of BTB and its relation to the health of the population of North Kivu (Zaire). Revue D'élevage etDe Médecine Vétérinaire Des Pays Tropicaux **36:** 15.

Muller B.; Hilty M.; Berg S.; Garcia-Pelayo M.C.; Dale J.; Boschiroli M.L.; Cadmus S.; Ngandolo B.N.; Godreuil S.; Diguimbaye-Djaibe C.; (2009): African 1, an epidemiologically important clonal complex of M. bovis dominant in Mali, Nigeria, Cameroon, and Chad. Journal of Bacteriology **191:** 1951-1960.

Munyeme M.; Muma J.B.; Samui K.L.; Skjerve E.; Nambota A.M.; Phiri I.G.; Rigouts L. and Tryland M.; (2009): Prevalence of BTB and animal level risk factors for indigenous cattle under different grazing strategies in the livestock/wildlife interface areas of Zambia. Tropical Animal Hhealth and Production **41:** 345-352.

Nafeh M.A.; Medhat A.; Abdul-Hameed A.-G.; Ahmad Y.A. Rashwan N.M. and Strickland G.T.; (1992): Tuberculous peritonitis in Egypt: the value of laparoscopy in diagnosis. The American journal of Tropical Medicine and Hygiene **47:** 470-477.

Nega M.; Mazengia H.; Mekonen G.; (2012): Prevalence and zoonotic implications of BTB in Northwest Ethiopia. International Journal of Medicine and Medical, **2**(9):188-192.

Niemann S.; Richter E. and Rusch-Gerdes S.; (2000): Differentiation among members of the *MT*C by molecular and biochemical features: evidence for two Pyrazinamide-susceptible subtypes of M. bovis. J Clin Microbiol, **38**: 152-157.

Niemann S.T.; Kubica F. Bange ; (2004): The species *M. Africanum* in the light of new molecular markers, *J. Clin. Microbiol* **42**:3958-396.

Niemann S.; (2002): Mycobacterium africanum Subtype II Is Associated with Two Distinct Genotypes and Is a Major Cause of Human TB in Kampala, Uganda. J Clin Microbiol **40:** 3398–3405

Niobe-Eyangoh S.N.; Kuaban C.; Sorlin P.; Cunin P.; Thonnon J.; Sola C.; Rastogi N.; Vicent V.; Gutierrez M.C.; (2003): Genetic biodiversity of *MTC* strains from patients with pulmonary tuberculosis in Cameroon. J Clin Microbiol **41:** 2547-2553.

Nuru A.; Mamo G.; Teshome L.; Zewdie A.; Medhin G.; Pieper R.; Ameni G.; (2015). Bovine tuberculosis and its risk factors among dairy cattle herds in and around Bahir Dar City, Northwest Ethiopia. Ethiop. Vet. J. **19**: 27–40.

Nuru A.; Zewdie A.; Mohammed T.; Wondale B.; Teshome L.; Getahun M.; Mamo G.; Medhin G.; Pieper R.; and Ameni G.; (2017). Nontuberculosis mycobacteria are the major causes of TB like lesions in cattle slaughtered at Bahir Dar Abattoir, northwestern Ethiopia. BMC Vet. Res. 13, 237.

Ocepek M.; Pate M.; Zolnir-Dovc M.; and Poljak M.; (2005). Transmission of MTB from human to cattle. J Clin Microbiol **43***:* 3555-3557.

Odubanjo M; Dada-Adegbola H.; (2011): The Microbiological diagnosis of TB in a resource -limited setting: is acid-fast bacilli microscopy alone sufficient? Annals of Ibadan Postgraduate Medicine. **9**:24-29.

Oh P.; Granich R.; Scott J.; Sun B.; Joseph M.; Stringfield C.; and others (2002): Human exposure following *MTB*infection ofmultiple animal species; in a Metropolitan Zoo. Emerging Infectious Diseases **8**, 1290-1293

OIE (2008): Terrestrial Animal Health Code 2008. OIE - World Organisation for Animal Health, Paris, France.

OIE (2009): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2009. World Organisation for Animal Health, Paris, France.

OIE (2012): BTB, in Manual of diagnostic tests and vaccines for terrestrial animals, tahm/2.04.07_BOVINE_TB.pdf [Accessed on July 11, 2014].

OIE (2016): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. OfficeInternationale des Epizooties (OIE), Paris, France.

Oloya J.; Opuda-Asibo J.; Kazwala R.; Demelash A.B.; Skjerve E.; Lund A.; Johansen T.B. and Djonne B.; (2008): Mycobacteria causing human cervical lymphadenitis in pastoral communities in the Karamoja region of Uganda. Epidemiology and Infection **136***:* 636-643.

Oloya J.; Kazwala R.; Lund A.; Opuda-Asibo J.; Demelash B.; Skjerve E.; Johansen T.B. and Djonne B.; (2007): Characterisation of mycobacteria isolated from slaughter cattle in pastoral regions of Uganda. BMC microbiology *7*, 95.

Omer M.: Skjerve E.: Woldehiwet Z. and Holstad G.; (2001): A cross-sectional study of bovine tuberculosis in dairy farms in Asmara, Eritrea. Tropical Animal Health and Production **33***:* 295-303.

O'Reilly L.M. and Daborn C.J.; (1995): The epidemiology of M. bovis infections in animals and man: AReview. Tubercle and lung diseaseSuppl **1***:* 1-46.

Pal M.; Zenebe N and Rahman MT.; (2014): Growing significance of M. bovisin human, health. Microbes and Health, **3**:29-34.

Palmer M.V.: Thacker T.C.: Waters W.R.: Gortazar C. and Corner L.A.: (2012): M. bovis: a Model Pathogen at the Interface of Livestock, Wildlife, and Humans. Veterinary Medicine International, **23**: 6205.

Palomino J.C.: Leão S.C. and Ritacco V.: (2007): Tuberculosis 2007: From basic science to patient care, Palomino JC, Leão SC, Ritacco V(eds), 1st edn, Bourcillier Kamps, Belgium, pp 53-680.

Patterson J. and Grooms D.; (2000): Diagnosis of bovine tuberculosis: Gross necropsy, histopathology and acid fast staining. Michigan State University Extension, 35: 1-2.

Pavlik I.; (2006a): Control and eradication of BTB in Central Europe, In: Thoen, C.O.; Steele, J.H.; Gilsdorf, M.J. (eds.), *M. bovis* infection in anaimals and humans. Blackwell Publishing Ltd, Iowa, USA. 136 - 148.

Pavlik I.; (2006b): The experience of new European Union Member States concerning the control of BTB. Veterinary Microbiology **112**: 221-230.

Pavlik I.; Yayo Ayele W.; Parmova I.; Melicharek I.; Hanylikova M.; Svejnochova M.; Koermendz B.; Nagz G.; Cvetnic Y. and Katalinic Jankovic V.; (2003): MTB in animal and human populations in six Central European countries during 1990-1999. Vet. Med. **48** (4): 83–89

Philips C.J.C.; Foster C.R.W.; Morris P.A.; Teverson R.; (2003): The transmission of *M. bovis* infection to cattle. Research in Veterinary Science **74**: 1-15.

Pinsky BA and Banaei N. (2008): Multiplex real-time PCR assay for rapid identification of MTBC members to the species level. Journal of Clinical Microbiology, **46**: 2241-2246.

Pollock J.M. and Neill S.D.; (2002): M. bovis infection and tuberculosis in cattle. Veterinary journal **163***:* 115-127.

Prasad H.K.; Singhal A. Mishra A. Shah N.P.; Katoch V.M.; (2005): BTB in India: potential basis for zoonosis. TB (Edinb.). **85:** 421–428. Quinn P. and Markey B. (2003): Concise review of Veterinary Microbiology, UK: Blackwell Publishing, pp34-37.

Quinn P. and Markey B.; (2003): Concise review of Veterinary Microbiology, UK: Blackwell Publishing, pp 34-37.

Radostits O.; Blood D. and Gray C.; (2007): Veterinary Medicine, a Text Book of the Diseases of cattle, sheep, Pigs, Goats and Houses. 8thed, London: Ballier Tindals, pp 830-838.

Radunz B.; (2006): Surveillance and risk management during the latter stages of eradication: experiences from Australia. Veterinary Microbiology**112***:* 283-290.

Reed M. B.; Pichler V. K.; McIntosh F.; Mattia A.; Fallow A.; Masala S.; Domenech P.; Zwerling A.; Thibert L.; Menzies D.; Schwartzman K. and Behr M. A.; (2009): Major *MTB* lineages associate with patient country of origin. *J. Clin. Microbiol.* **47**: 1119-1128.

Regassa A.; Tassew A.; Amenu K.; Megersa B.; Abunna F.; Mekibib B.; Marcotty T. and Ameni G.; (2010): A cross-sectional study on BTB in Hawassa town and its surroundings, Southern Ethiopia. Tropical Animal Health and Production **42***:* 915-920.

Regassa A.; Tassew A.; Amenu K.; Megersa B.; Abunna F.; Mekibib B.; Macrotty T. and Ameni G.; (2009). A cross-sectional study on bovine tuberculosis in Hawassa town and its surroundings, Southern Ethiopia. Tropical Animal Health and Production, 42: 915-920.

Regassa A.; Medhin G. and Ameni G.; (2008): Bovine tuberculosis is more prevalent in cattle owned by farmers with active tuberculosis in central Ethiopia. *The Veterinary Journal* **178**: 119-125.

Regassa A.; (2005): Study on *M. bovis* in animals and human in and around Fiche, North Shewa Zone, Ethiopia. M.Sc. Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre-Zeit, Ethiopia.

Renwick A.R.; White P.C. and Bengis R.G.; (2007): BTB in southern African wildlife: a multi-species host-pathogen system. *Epidemiology and infection* **135***:* 529-540.

Rigouts L.; Maregeya B.; Traore H.; Collart J.P.; Fissette K. and Portaels F.; (1996): Use of DNA restriction fragment typing in the differentiation of *MT*C isolates from animals and humans in Burundi. Tubercle and lung disease**77***:* 264-268.

Rodriguez-Campos S.; Schurch A.C.; Dale J.; Lohan A.J.; Cunha M.V.; Botelho A.; De Cruz K.; Boschiroli M.L.; Boniotti M.B.; Pacciarini M.; (2012): European 2--a clonal complex of M. bovis dominant in the Iberian Peninsula. Infection, genetics and evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases**12***:* 866-872.

Russell D.; (2003): Highlighting the parallels between human and BTB. Journal of Veterinary Medicine Education, **30**: 140-142. Doi: Org/10.3138 /jvme. 30.2.140

Schelling E.; Diguimbaye C.; Daoud S.; Daugla D.; Bidjeh K.; Tanner M. and Zinsstag J.; (2000): Zoonoses in nomadic populations of Chad—Preliminary results obtained in humans and livestock. In Proceedings of the Ninth Conference of the International Society of Veterinary Epidemiology and Economics.

Sharma R. and Gupta V.; (2011): Spoligotyping for the detection of *MT*C bacteria. Asian J of Biochem, **6**: 29-37

Sharma S.K.; Mohan A.; Sharma A. and Mitra D.K.; (2005): Miliary TB: new insights into ``an old disease. Lancet Infect Dis, **5**: 415–430.

Shitaye J.; Tsegaye W. and Pavlik I.; (2007): BTB infection in animal and human populations in Ethiopia: A Review. Veterinary Medicine-PRAHA-**52**: 317.

Shitaye J.; Getahun B.; Alemayehu T.; Skoric M.; Treml F.; Fictum P.; Vrbas V. and Pavlik I.; (2006): A prevalence study of bovine tuberculosis by using abattoir meat inspection and tuberculin skin testing data, histopathological and IS6110 PCR examination of tissues with tuberculous lesions in cattle in Ethiopia. *Veterinaries medicine-PRAHA-* **51***:* 512.

Sibhat B.; Asmare K.; Demissie K.; Ayelet G.; Mamo G.; Ameni G.; (2017): BTB in Ethiopia: A systematic review and meta-analysis. Preventive Veterinary Medicine, **147**:149-157.

Siddiqi S. and Rusch-Gerdes S.; (2006): MGITTM Procedure Manual for BACTECTM MGIT 960TM TB System: Mycobacteria Growth Indicator Tube (MGIT) Culture and Drug Susceptibility Demonstration Projects. Foundation for Innovative New Diagnostics. 2006. 1-52.

Skuce R.A.; Allen A.R.; McDowell S.W. and Branch B.; (2011): BTB: A review of cattle-to-cattle transmission, risk factors and susceptibility. (Agri-food and Biosciences Institute, Belfast).

Small P.M.; Hopewell P.C.; Singh S.P.; (1994): The epidemiology of TB in San Francisco. A population-based study using conventional and molecular methods, TheNew England Journal of Medicine.**330**: 1703–1709.

Smith N.H.; Berg S.; Dale J.; Allen A.; Rodriguez S.; Romero B.; Matos F.; Ghebremichael S.; Karoui C. and Donati C.; (2011): European 1: a globally important clonal complex of M. bovis. Infection, Genetics and Evolution**11***:* 1340-1351.

Smith G.C.; Bennett R.; Wilkinson D.; Cooke R..; (2007): A cost-benefit analysis of culling badgers to control BTB. The VeterinaryJournal **173**: 302-310.

Smith N.H.; Gordon S.V.; de la Rua-Domenech R.; Clifton-Hadley R.S. and Hewinson R.G.; (2006a): Bottlenecks and broomsticks: the molecular evolution of M. bovis. Nature Reviews. Microbiology**4***:* 670-681.

Smith N.H.; Kremer K.; Inwald J.; Dale J.; Driscoll J.R..; Gordon S.V.; van Soolingen D., Hewinson, R.G., and Smith, J.M.;. (2006b): Ecotypes of the *MT*C. Journal of Theoretical Biology**239***:* 220-225.

Smith R.M.; Drobniewski F.; Gibson A.; Montague J.D.; Logan M.N.; Hunt D.; Hewinson G.; Salmon; R.L. and O'Neill B.; (2004): Mycobacterium bovis infection,

Sreevatsan S.; Pan X.; Stockbauer K.E.; Connell N.D.; Kreiswirth B.N.; Whittam T.S.; Musser J.M.; (1997): Restricted structural gene polymorphism in the *MT*C indicates evolutionarily recent globaldissemination. Proceedings of the National Academy of Sciences USA 94, 9869-9874.

Stata Corp (2011): Release 12. Statistical Software. College Station, TX: Stata Corp LP.

Steingart KR. Ng V.; Henry M.; Hopewell PC; Ramsay A. and Cunningham; (2006): Sputum processing methods to improve the sensitivity of smear microscopy for TB: a systematic review. Lancet Infect Dis, **6**: 664-674.

Sternberg S.; Bernodt K.; Holmstrom A. and Roken B.; (2002): Survey of tuberculin testing in Swedish zoos. *Journal of zoo and wildlife medicine* : official publication of the American Association of Zoo Veterinarians **33***:* 378-380.

Sulieman M.S. and Hamid M.E.; (2002): Identification of acid-fast bacteria from caseous lesions in cattle in Sudan. Journal of veterinary medicine, **49**: 415-418.

Supply P.; Allix C.; Lesjean S.; Cardoso-Oelemann M.; Rüsch-Gerdes S.; Willery E.; Savine E.; de Haas P.; van Deutekom H. and Roring S.; (2006): Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. Journal of clinical microbiology **44:** 4498-4510.

Supply R.P.; Lesjean S.; Savine E.; Kremer K.; Soolingen D.V.; Locht C.; (2001): Automated high throughput genotyping for study of global epidemiology of Mycobacterium tuberculosis based on mycobacterial interspersed repetitive units. *J Clin Microbiol*, **39:**3563-3571.

Tamiru F.; Hailemariam M. and Terfa W. (2013): Preliminary study on prevalence of BTB in cattle owned by tuberculosis positive and negative farmers and assessment of zoonotic awareness in Ambo and Toke Kutaye districts, Ethiopia. *J VetMed AH*, **5:** 288-295.

Tan D.H.S.; Upshur R.E.G.; Ford N.; (2003): Global plagues and the Global Fund: Challenges in the fight against HIV, TB and Malaria. *BMC* InternationalHealth and Human Rights **3**: 2.

Taracha E.; Bishop A; Musoke A.; Hill S and Gilbert S.; (2003): Heterologous priming boosting immunization of cattle with MTB 85aindces antigen specifics T-cell response. Infection and Immunity, **71**: 6906-6914. Doi: 10.1128/IAI.71 .**12**.6906-6914.

Taylor G. M.; Stewart G. R.; Cooke M.; Chaplin S.; Ladva S.; Kirkup J.; Palmer S. and Young D. B.; (2003): Koch's bacillus - a look at the first isolate of *MTB* from a modern perspective. Microbiology **149**: 3213- 3220.

Teklul A.; Asseged B.; Yimer E.; Gebeyehu M. and Woldesenbet Z.; (2004): TB lesions not detected by routine abattoir inspection: the experience of the Hossana municipal abattoir, southern Ethiopia. Revue Scientifique etTechnique (IOE) **23:** 957-964.

Terefe D.; (2014): Gross pathological lesions of BTB and efficiency of meat Inspection procedure to detect infected cattle in Adama municipal abattoir. Journal of Veterinary Medicine and Animal Health, **6**: 48-53. Doi:10.5897/JVMAH12.015,

Thoen C.O.; LoBue P.A. and de Kantor I.; (2010): Why has zoonotic TB not received much attention? The international Journal of TB and Lung Disease**14***:* 1073-1074.

Thoen C.O.; LoBue P.A.; Enarson D.A.; Kaneene J.B.; and de Kantor I.N.; (2009): Tuberculosis: a re-emerging disease of animals and humans. *Veterinaria Italiana* **45**: 135 - 181.

Thoen C.O.and LoBue P.A.; (2007): *M. bovis* tuberculosis: forgotten, but not gone. The Lancet **369**: 1236-1238.

Thoen C. and Steele J; (1995): Regional and country status reports. M. bovis, 167-345.

Thoen C.O.; Karlson A.G. and Himes E.M.; (1981): Mycobacterial infections in animals. Reviews of Infectious Diseases **3***:* 960-972.

Thrusfield M.; (2005): Veterinary epidemiology, 3rd ed. UK, Black well Science, Oxford, Pp, 182-187.

Tigre W.; Gudeta T. and Regassa F.; (2010): Preliminary study on BTB in Nekemte municipality abattoir, Western Ethiopia. Bull. Anim. Heal. Prod. Afr. **58**: 323–327.

Tschopp R.; Aseffa A.; Schelling E.; Berg S.; Hailu E.; Gadisa E.; Habtamu M., Argaw K. and Zinsstag J.; (2010): BTB at the wildlife-livestock-human interface in Hamer Woreda, South Omo, and Southern Ethiopia. PloS One **5**, e12205.

Tsegaye W.; Aseffa A.; Mache A.; Mengistu Y.; Stefan B. and Ameni G.; (2010): Conventional and Molecular Epidemiology of BTB in Dairy Farms in Addis Ababa City, the Capital of Ethiopia. The Journal of Applied Research in Veterinary Medicine **8***:* 143.

van Soolingen D.; Hoogenboezem T.; De Haas P.E.W.; Hermans P.W.M.; Koedam M.A.; Teppema K.S.; Brennan P.J.; Besra G.S.; Portaels F.; Top J.; Schouls L.M. and Van Embden J.D.A.; (1997): A Novel Pathogenic Taxon of the *MTC*, Canetti: Characterization of an Exceptional Isolate from Africa. International Journal of Systemic Bacteriology **47**, 1236-1245.

Vasanthakumari R.; (2007): Textbook of Microbiology. BI Publications Pvt Ltd. 54, Janpath, New Delhi 110001, pp 238-239.

Vekemans M.; Cartoux M.; Diagbouga S.; Dembele M.; Kone B.; Delafosse A.; Dera A. and Perre P.; (1999): Potential source of human exposure to Mycobacterium bovis in Burkina Faso, in the context of the HIV epidemic. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases **5:** 617-621.

Viana-Niero C.C.; Gutierrez C. Sola.; (2001): Genetic diversity of *Mycobacterium africanum* clinical isolates based on IS6110-restriction fragment length polymorphism analysis, spoligotyping and variable number of tandem DNA repeats, J. Clin. Microbiol. **39**: 57-65.

Vordermeier H.M.; Chambers M.A.; Cockle P.J.; Whelan A.O.; Simmons, J. and Hewinson R.G.; (2002): Correlation of ESAT-6-specific gamma interferon production with pathology in cattle following M. bovis BCG vaccination against experimental BTB. Infection and Immunity **70:** 3026-3032.

Wedlock D.N.; Skinner M.A.; de Lisle G.W.; Buddle B.M.; (2002): Control of M. bovis infections and the risk to human populations. Microbes and Infection **4**: 471-480.

WHO (1994): Zoonotic TB (*M. bovis*) memorandum from a WHO meeting (with the participation of FAO). *Bulletin of World HealthOrganization, Geneva, Switzerland* **72**: 851–857.

WHO (1998): Laboratory services in TB control Part II : Microscopy. World Health Organization (WHO/TB/98.258), Geneva, Switzerland. 63.

WHO (2008): Global TB Control 2008: Surveillance, planning and financing. World HealthOrganization, Geneva, Swaziland. 295.

WHO (2009): WHO report 2009: Global TB control: epidemiology, strategy, financing World Health Organization, Geneva, Switzerland.

WHO (2013a): Global TB Control. Epidemiology, Strategy and Financing, pp1-341.

WHO (2017): Global TB Report. World Health Organization, Geneva, Switzerland. WHO/HTM/TB/2017.23.

Wirth T.; Hildebrand F.; Allix-Beguec C.; Wolbeling F.; Kubica T. and Kremer K.; (2008): Origin, spread and demography of the MTBC. PLoS Pathog, **4**: e1000160

Woyessa M.; Jibril Y.; Ameni, G. and Duguma R.; (2014): Molecular Epidemiology of *MT*C at Nekemte Municipality Abattoir, Western Ethiopia. Science, Technology and Arts Research Journal**3:** 167-173.

Yimer S. A.; Hailu E.; Derese Y.; Bjune G. A. and Holm-Hansen C.; (2013): Spoligotyping of *MTB*isolates among PTB patients in Amhara Region, Ethiopia. APMIS **121**: 878-885.

Young D B.; Perkins MD.; Duncan K. and Barry CE; (2008): Confronting the scientific Obstacles to global control of TB. Journal of Clinical Investigation, **118**: 1255-1265

Zewdie O.; Mihret A.; Ameni G.; Worku A.; Gemechu T. and Abebe T.; (2016): Molecular typing of mycobacteria isolated from TB lymphadenitis cases in Addis Ababa, Ethiopia. Int. J. Tuberc. Lung Dis. **20**: 1529-1534.

Zeru F.; Romha G.; Berhe G.; Mamo G.; Sisay T.; Ameni G.; (2014): Prevalence of BTB and assessment of cattle owners' awareness on its public health implication in and around Mekelle, Northern Ethiopia. J. Vet. Med. Anim. Health**6**: 159–167.

Zewdie O.; Mihret A.; Ameni G.; Worku A.; Gemechu T. and Abebe T.; (2016): Molecular typing of mycobacteria isolated from TB lymphadenitis cases in Addis Ababa, Ethiopia. Int. J. Tuberc. Lung Dis. **20**: 1529-1534.

Zinsstag J.; Ould Taleb M. and Craig P.S.; (2006): Editorial: health of nomadic pastoralists: new approaches towards equity effectiveness. Tropical Medicine and International Health: TM & IH**11**: 565-568.

Zolnir-Dovc M.; Poljak M.; Erzen D. and Sorli J.; (2003): Molecular epidemiology of TB in Slovenia: results of a one-year (2001) nation-wide study. Scandinavian journal of infectious diseases **35**: 863-868.

8. APPENDICES

Appendix I: list of figures

Appendix Figure 1. Adama municipal abattoir lairage

Appendix Figure 2. Strong bovine tuberculin reactor after 72 hours post tuberculin injection

Figure 3 Tuberculosis lesions in the thoracic cavity of adult cattle at abattoir, in Central Ethiopia September 2015 (arrow shows tuberculosis lesion).

Appendix II: consent

Study Participant Consent Form

Name of the participant:____Age____Sex___ Code____Study site/Health facility_____

I confirm that I, and/or my parent (s) (in individuals under 18 years) have been given adequate information about the research project. I and/or my parent have been requested to provide sputum. The researchers informed me and/or my parent that there is no risk associated with participating in the study or providing the requested samples. I and/or my parent have understood that the results of clinical and laboratory diagnosis will be used for research purposes and the information related to myself/my family will be kept strictly confidential. I and/or my parent well informed that participation in the study is voluntary and I and/or my family can withdraw anytime without giving any reason. Moreover, I am and/or my family fully aware that non-participation in this project will not subject me or my family to any health service denial from this health facility either now or in the times to come. I and/or my parent confirm that all the information provided to me is very clear and has been conveyed by the language that I fully understand. Finally, I and/or my parent agreed to participate in the study, and I and/or my parent signed this informed consent.

Name of participant....................................Signature...............Date……

Name of parent/guardian ______________Signature…………Date……

Name of obtaining consent.........................signature………….Date -----

Name of witness…………………………Signature…………Date……

Appendix III: list of publications

1. Tefera Woldemariam, Gezahegne Mamo Temesgen Mohammed and Gobena Ameni (2016). Prevalence of BTB in beef feedlot of Borena cattle by using comparative intradermal skin test, Adama, Ethiopia. *Ethiopian Veterinary Journal*, **20** (2):17-29
2. Tefera Woldemariam, Gezahegne Mamo, Rahmeto Abebe, Gobena Ameni (2018). Prevalence of BTB in Dairy Cattle by using Comparative Intradermal Skin Test, in Adama, Ethiopia. *Journal of veterinary medicine and animal health.* DOI: 10.5897/JVMAH