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**STUDY ON EPIDEMIOLOGY OF BOVINE TUBERCULOSIS AND ITS PUBLIC HEALTH
SIGNIFICANCE IN JIMMA TOWN AND ITS SURROUNDINGS, SOUTH WEST
ETHIOPIA**

MVSc. Thesis



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BISHOFTU, ETHIOPIA

**STUDY ON EPIDEMIOLOGY OF BOVINE TUBERCULOSIS AND ITS PUBLIC HEALTH
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ETHIOPIA**



A Thesis submitted to School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Public health.

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June, 2017

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As member of the examining board of the final MVSc. open defense, we certify that we have read and evaluated the thesis prepared by: **Wubit Tafese** Titled: “**Study on Epidemiology of Bovine Tuberculosis and its Public Health Significance in Jimma Town and its Surroundings, South West Ethiopia**” and recommended that it be accepted as fulfilling the thesis requirement for the degree of Master in Veterinary Public health.

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DEDICATION

This thesis manuscript is dedicated to my families, instructors and all persons those who are behind my success.

STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) 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 thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS

AFB	Acid fast bacilli
BCG	Bacille Calmett Guérin
BCS	Body Condition Scoring
bp	Base pair
BTB	Bovine tuberculosis
CDC	Centers for disease control
CI	Confidence interval
CIDT	Comparative intradermal tuberculin test
DNA	Deoxy ribonucleic acid
DR	Direct repeat
ELISA	Enzyme linked immunosorbent assay
FAO	Food and agricultural organization
HIV	Human immunodeficiency virus
HPC	Hexadecyl pyridinium chloride
IFN- γ	Gamma interferon
LJ	Lowenstein-Jensen
LJG	Lowenstein-Jensen glycerol
LJP	Lowenstein-Jensen pyruvate
Masl	Meter above sea level
mPCR	Multiplex polymerase chain reaction
MTBC	<i>Mycobacterium tuberculosis</i> complex
OIE	Office international des epizooties
OR	Odds ratio
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PPD	purified protein derivatives
PPD-A	Avium purified protein derivatives
PPD-B	Bovine purified protein derivatives
rRNA	Ribosomal ribonucleic acid

SE	Standard error
TAE	Tris-acetate- ethylenediaminetetra acetic acid
TB	Tuberculosis
WHO	World health organization
ZN	Ziehl-Neelsen

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ABSTRACT

*A cross sectional study was conducted on 468 Cattle, selected using systematic random sampling technique, in Jimma abattoir, South west Ethiopia from December 2016 to May 2016 to investigate the prevalence of bovine tuberculosis (BTB), identifying the risk factors as well as identify its causative agent and to assesses public health awareness towards BTB. The methods used were postmortem examination, bacteriological culturing, molecular characterization using region of difference (RD4) deletion typing and questionnaire survey. The overall prevalence of BTB was 7.5 % (95% CI: 5.08-9.87) on the basis of detailed postmortem examination. Multivariable logistic regression analysis identified age; body condition, and breed to be statistically significant in explaining BTB prevalence. The older cattle were forty five one times (OR=45.13; 95%CI; 10.20-199.52) more likely to have TB-like lesion than the younger ones. Poor body conditioned cattle nineteen times (OR = 19.24; 95% CI; 3.86-95.72) more likely to have tuberculous lesion than the cattle with good body condition. The cross breeds were twelve times (OR =12.36; 95%CI; 3.15-48.39) more likely to developed TB lesions as compared to local breeds. The tuberculous lesions were found most frequently distributed in thoracic cavity lymph nodes (60%), which suggest respiratory route as the most likely mode of infection in the study area. From 35 tissue samples cultured, 12 (38.28%) were culture positive and 4 of them were positive on acid fast staining. However, using RD4 deletion typing only one isolate obtained from right bronchial lymph node was confirmed to be *M. bovis*. Analysis of the awareness of cattle owner, attendants and abattoir worker toward BTB was found to be low (27.5%) and the result also revealed the presence of unsafe practices like, co-residing in the same house with animals, consumption of raw animal products which indicates the potential risk for zoonotic transmission of BTB. In conclusion, the present study confirmed moderately low prevalence of BTB, in the study area and existence of potential risk factors for zoonotic transmission for the community warrants the need to design and implement feasible control strategies in the study area.*

Keywords: *Awareness, Bovine tuberculosis, Jimma, Molecular characterization Public health*

1. INTRODUCTION

Tuberculosis (TB) is recognized as one of the most important threats to human and animal health causing mortality, morbidity and economic losses (Malama *et al.*, 2013; Pal, 2013). It causes death in each year among millions of people as a result it ranked as globally a second leading disease from the infectious disease next to AIDS (Zeweld, 2014; Birhanu *et al.*, 2015). TB is a leading killer among adults in the most economically productive age groups and people living with HIV (Lopez *et al.*, 2006).

Bovine tuberculosis (BTB) is a chronic infectious disease predominantly caused by *Mycobacterium bovis*. This disease is widely distributed throughout the world. BTB can have an impact on the national and international economy, affects the ecosystem via transmission to wildlife and is of public health concern due to it is among the principal zoonotic diseases. It is primarily of economic importance as it can have a considerable direct effect on milk and meat production and animal reproduction (Bemrew *et al.*, 2015).

BTB has been recognized from 176 countries as one of the important bovine diseases causing great economic loss (Awah-Ndukum *et al.*, 2013). 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, the disease is present virtually on the whole continent; however, little accurate information on its distribution and prevalence is available. It is a chronic, generally respiratory disease, which is clinically difficult to diagnose (Bemrew *et al.*, 2015). *M. bovis* is most frequently isolated from domesticated cattle (Grange *et al.*, 1996; Smith *et al.*, 2006), although recent studies indicated that *M. tuberculosis* has been isolated from cattle and *M. bovis* from humans infected with BTB and TB respectively. The members of MTBC are characterized by 99.9% or greater similarity at nucleotide level, and are virtually identical at 16s rRNA sequence but they vary in host specificity (Brosch *et al.*, 2002).

Bovine tuberculosis is a contagious disease, which can affect broad range of mammalian hosts including human being, cattle, deer, pigs, domestic cats, wild carnivores and omnivores, rarely equids or sheep. Organisms are excreted in the exhaled air, in sputum, feaces (from both intestinal lesions and swallowed sputum from pulmonary lesions), milk, urine, vaginal and uterine discharges, and discharges from open peripheral lymph nodes of infected animals (Radostits *et al.*, 2007). Thus, in cattle the main route of infection transmission: aerosol, close contact between animals (Neill *et al.*, 1991) and ingestion of contaminated products (Ameni *et al.*, 2007; Cleaveland *et al.*, 2007). Bovine tuberculosis diseased animal loses 10 to 25% of their productive efficiency; direct losses due to the infection become evident by decrease in 10 to 18% milk and 15% reduction in meat production (Radostits *et al.*, 1994).

In developing countries like Ethiopia, the low standard of living and socio-economic situation for both animals and humans are more contributing in TB transmission between human to human and human to cattle or vice versa (Ameni *et al.*, 2010b). Human infection due to *M. bovis* is thought to be mainly through drinking of contaminated or unpasteurized raw milk and under cooked meat (Ameni *et al.*, 2007). Currently, human TB of animal origin caused by *M. bovis* is becoming increasingly evident in developing countries (Russel, 2003; Mamo *et al.*, 2013a). As humans and animals are sharing the same micro environment and dwelling premises, especially in rural areas, and susceptibility of AIDS patients to tuberculosis (Shitaye *et al.*, 2007). It is estimated that *M. bovis* causes 10 to 15% human cases of tuberculosis in countries where pasteurization of milk is rare and bovine tuberculosis is common (Ashford *et al.*, 2001; Berg *et al.*, 2015).

Ethiopia is one of the African countries where tuberculosis is wide spread in both humans and cattle and the endemic nature of tuberculosis in humans and cattle has long been documented. BTB is an endemic disease of cattle in Ethiopia with prevalence of 1.1%-

24.7% in abattoir and 3.5–50% in cross breed farms (Shitaye *et al.*, 2007; Berg *et al.*, 2009; Biffa *et al.*, 2009; Regassa *et al.*, 2010).

Detection of BTB in Ethiopia is carried out most commonly on the basis of tuberculin skin testing, abattoir meat inspection and very rarely on bacteriological techniques. BTB in cattle remains to be a great concern due to the susceptibility of humans to the disease Shitaye *et al.*, (2007). No recognizable control programs are implemented except the routine abattoir inspection that involves whole or partial condemnation of infected carcasses for the purpose of protecting consumers' health. Even then, routine meat inspection procedures with qualified professionals are only practiced in a handful of municipal and export abattoirs throughout the country. It is estimated that more than half of slaughtered animals each year are illegally processed in backyard system without undergoing proper inspection, thus posing a great health risk to the consumers. This is further exacerbated by the low sensitivity of routine meat inspection to detect carcasses with tuberculosis lesions, and subsequently infected meat can get approved for human consumption (Demelash *et al.*, 2009).

Despite, the large number of cattle population in Jimma zone (CSA, 2016), the status of the disease is not well established except few reports. In addition, no attempt was carried out on isolation and molecular characterization of the causative agent in the study area. Therefore, this study was designed with the following objectives:

- To assess the prevalence of bovine tuberculosis based on postmortem examination of cattle slaughtered at Jimma town municipal abattoir
- To isolate and identify *Mycobacterial* species causing bovine TB from slaughtered cattle, using bacteriological culture and molecular tool (RD4 deletion typing) in the study area
- To assesses public health awareness towards BTB and potential risk factors for zoonotic BTB

2. LITERATURE REVIEW

2.1. Taxonomy of Mycobacteria

The genus *Mycobacterium* is classified under the Order Actinomycetales and Family *Mycobacteriaceae* (Quinn *et al.*, 1999). The genus includes a number of species, some being strict pathogens including to the man and animals, some are opportunistic while others are essentially saprophytic (Thoen, 1984). *Mycobacterium tuberculosis* complex (MTBC) has seven approved members and these are *M. tuberculosis*, *M. bovis*, *M. bovis BCG*, *M. africanum*, *M. microti*, *M. tuberculosis sbsp. canetti* and *M. bovis sbsp. caprae* (Hermans *et al.*, 1991). Among these the following cause human tuberculosis i.e. *M. bovis*, *M. tuberculosis*, *M. africanum* and *M. canetti* (Higgins *et al.*, 2011; Bilal *et al.*, 2010). Members of the MTBC are extremely similar genetically having at least 99.9% similarity on the nucleotide level and an identical 16rRNA sequence (Quinn *et al.*, 1999; Brosch *et al.*, 2002; Marie-France *et al.*, 2009).

2.2. General characteristics of Mycobacteria

Mycobacteria are non-motile, non-spore forming, pleomorphic bacilli or coccobacilli. In tissues they appear as rods, which may be straight, curved or in the form of clubs, measuring 1.0-4.0 μm in length and 0.2-0.3 μm in width. They occur singly, in pairs or as small bundles. On laboratory media they may appear as cocci or rods measuring 6-8 μm (Quinn *et al.*, 1999). The mycobacteria most closely resemble the other mycolic acid producing bacteria, *Corynebacterium*, *Nocardia* and *Rhodococcus*. (Songer and Karen, 2005; Quinn *et al.*, 2011). They are similar to other bacteria in many aspects except for a unique cell wall that is made up of four parts. The first part is a peptidoglycan layer that is similar to that found on other bacterial species. The second layer contains arabinogalactan, which is made up of branched macromolecules of arabinose and galactose (Jovan *et al.*, 2011). The third layer, which contributes to the thickness of mycobacterial cell walls, is made of mycolic acids that are long branched chains of fatty

acids with differing 50 and 30 carbon atom lengths. The mycolic acids are responsible for the acid fast staining reaction of mycobacteria cells (Thoen and Bloom, 1995). The fourth, outer, layer consists of a mixture of lipids and related compounds such as trehalose-containing glycolipids and peptidoglycolipids called mycosides (Chukwu *et al.*, 2013). Mycobacteria when stained are acid fast as they resist decolorizing with strong acid/alcohol solutions (Carter and wise, 2004). The formation of characteristics cords is the distinguishing features of pathogenic mycobacteria (Grange, 1995).

Mycobacterium species grows on medium containing serum, potato and egg. The most commonly used media are Lowenstein-Jensen (LJ) that contains egg, glycerol / pyruvate, asparagines, mineral salt and malachite green and stone brink"s medium. *M. bovis* grows more slowly than *M. tuberculosis*, which needs more than 8 weeks to appear on primary culture. The optimal growth temperature is 37 °c (Quinn *et al.*, 1999; Simons *et al.*, 2011).

2.3. Pathogenesis

The entrance of tubercle bacilli to animal body through respiratory, alimentary, genital, cutaneous and congenital routs (Neill *et al.*, 1994). After infection, the bacteria may localize in tissues related to the route of infection and associated lymph nodes. Miliary TB represents the most sever course of the disease with haematogenous spreading as a result of lysis of macrophages that release bacteria in to the blood from the primary foci and secondary seeding to various tissues (Andersen, 1997; Songer and Karen, 2005).

A primary lesion or focus of infection is established following the interaction of the host and the agent at the site of entry within 8 weeks of bacterial entrance (Radostits *et al.*, 1994; Songer and Karen, 2005). The *Mycobacterium* is then taken by the alveolar macrophages to the circulation and establishes in the lymph nodes. Cellular responses attempting to control the disease results in the accumulation of large number of

phagocytes and lead to the formation of a macroscopic lesion referred as tubercle (Thoen and Bloom, 1995).

Mycobacterium bovis eludes the bacteriocidal activities of macrophages by escaping from fused phagolysosomes into nonfused vacuoles in the cytoplasm, this due to its cell wall lipids (mycosides, phospholipids, and sulpholipids) (Songer and Karen, 2005). In addition to these survival mechanisms, an important aspect of pathogenicity of mycobacteria is their ability to subvert the protective immune response (Grange, 1995). A characteristic feature of virulent strains of mycobacteria is that they form cords when they grow in a liquid culture media whereas the avirulent strains develop as clumps (Thoen and Bloom, 1995; Ereqat *et al.*, 2013).

2.4. Immunity against Mycobacterial Infection

Although both humoral and cell mediated immune responses can be induced to mycobacterial infection, the cell mediated immunity is generally accepted to have the most significant role in protection (Neill *et al.*, 1994). The macrophages have a central role in processing and subsequent presenting of mycobacterial antigens to antigen specific T lymphocytes (Fentahun and Luke, 2012).

2.5. Epidemiology of Mycobacterium bovis Infections

Mycobacterium bovis combines one of the widest host ranges of all pathogens with a complex epidemiological pattern, which involves interaction of infection among human beings, domestic animals and wild animals (Grange, 1995; Mamo *et al.*, 2012; Gemechu *et al.*, 2013). However, only little is done particularly in developing countries on the epidemiology of this organism and the epidemiological requirements for its control (Ali, 2006).

2.5.1. Source of infection and mode of transmission

The main reservoir of *M. bovis* is cattle, which can transmit the infection to many mammalian species including man (Acha and Szytre, 2001; Tadayon *et al.*, 2013). Organisms leave the host in respiratory discharges, faeces, milk, urine, semen and genital discharges. These body excretions may contaminate grazing pasture, drinking water, feed, water and feed troughs or fomites, which may act as source of infection to other animals (Ameni *et al.*, 2001; Russel, 2003).

Inhalation of *M. bovis* bacilli is the most common route of infection with only a small number of mycobacteria required to cause an infection and spread of the infection can happen between animals when that are confined together in the same air space, such as during housing over the winter period (Neill *et al.*, 1994; Sakamoto, 2012). A secondary source of infection is the ingestion of contaminated milk or contaminated pasture and water, though environmental contamination is not believed to be a significant source of infection for bovine TB. Infection of the reproductive system can lead to genital transmission of the bacilli but this is a particularly rare event as is congenital infection (Mathema *et al.*, 2006; Nahar *et al.*, 2011).

Mycobacterium bovis can infect human primarily by the ingestion of unpasteurized dairy products, inhalation of aerosols and through breaks in the skin. The possibility of transmission of this Mycobacterium to humans from infected animals could be high in areas where there is close contact between human and animals (O'Reilly and Daborn, 1995). Main routes of infection by which tubercle bacilli gain entrance into the host are respiratory and alimentary tracks (O'Reilly and Daborn, 1995; Ameni *et al.*, 2001; Russel, 2003). Experimental studies indicated that infection of cattle by aerosol requires fewer than 10 organisms whereas approximately 107 organisms are necessary to establish infection by the oral route (Quinn *et al.*, 2011). 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).

Mycobacterium bovis caused as much as 25% of cases of human tuberculosis in developed country in the late 19th and early 20th centuries where as in the late 20th centuries, only 1%–2% of human TB cases in developed countries are caused by *M. bovis* which usually affects persons who acquired the infection locally before the implementation of control measures or in developing countries where control measures have not been implemented (O'Reilly and Daborn, 1995).

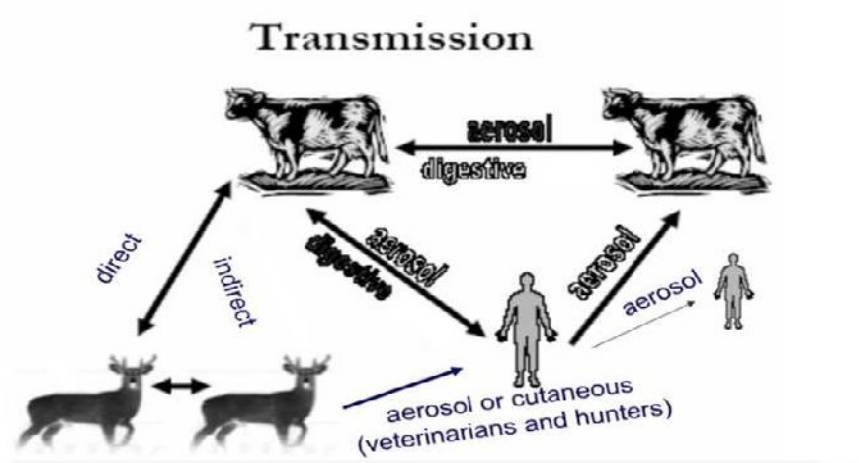


Figure1: Cycle of *M. bovis* transmission between cattle and human

Source: (Grange and Collins, 1987)



Figure 2: A farmer directly discharging tobacco juice into the oral cavity of cattle, a common practice in central Ethiopia.

Source :(Ameni *et al.*, 2011).

2.5.2. Risk factors in Animal

The likelihood of *M. bovis* infection is influenced by factors which are associated to host, environment and the pathogen itself (Regassa, 2005; Ameni *et al.*, 2011).

Environment

As bovine tuberculosis is a disease of intensification, housing predisposes to the disease, as high stocking intensity and a large number of animals on a farm so that the disease is more common and serious where these forms of husbandry are practiced. The closer the animals are in contact the greater is the chance that the disease will be transmitted. In spite of the low overall incidence in countries where cattle are at pasture all the year round, individual herds with 60-70% morbidity may be encountered (Regassa, 2005; Katale *et al.*, 2013).

Agent

The causative organism is moderately resistant to heat, desiccation, and many disinfectants. It is readily destroyed by direct sunlight unless it is in a moist environment. In warm, moist, protected positions, it may remain viable for weeks (Corner *et al.*, 1990; Srivastava *et al.*, 2008).

Host Range

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 (including lions, tigers, leopards, cheetahs and lynx) and several species of rodents. Little is known about the susceptibility of birds to *M. bovis*, although they are generally thought to be resistant. Experimental infections have recently been reported in pigeons after oral or intratracheal inoculation and in crows after intraperitoneal inoculation. Some avian species, including mallard ducks, appear to be resistant to experimental infection (Thoen, 1984; Biffa *et al.*, 2009; Silaigwana *et al.*, 2012; Tadayon *et al.*, 2013). Zebu (*Bos indicus*) type cattle are thought to be much more resistant to tuberculosis than European cattle, and the effects on these cattle are much less severe but under intensive feedlot conditions a morbidity rate of 60% and a depression of weight gain can be experienced in tuberculous Zebu cattle (Regassa, 2005).

2.5.3. Risk factors in human

Milk consumption increase the demand for milk was increasing at estimated rate of 2.5% per year over the period of 1970-1988 in sub Saharan Africa (Nwanta *et al.*, 2010). This rise demand for milk consumption will be met by increasing number of productive animals and intensifying animal production (Regassa, 2005).

Feeding habit

Consumption of raw or soured milk is mainly practiced in some parts of the world. Approximately 90% of the total volume of milk produced in sub Saharan Africa is consumed fresh or soured and only a very small proportion follows official marketing channels (Tamiru *et al.*, 2013). It is known that consumption of milk contaminated by *M. bovis* is regarded as the principal mode of TB transmission from animals to humans (Acha and Szytres, 2001).

Close physical contact

Close physical contact between humans and potentially infected animals is present in some communities, especially in developing region (Samuel, 2010). For example, in many African countries cattle are an integral part of human social life; they represent wealth and are at the center of many events and therefore gatherings. In addition, with 65% of Africa, 70% of Asian, and 26% of Latin America and Caribbean population working in agriculture, a significant proportion of the population of these regions may be at risk for bovine TB (Cosivi *et al.*, 1998).

HIV infection

In many developing countries, TB is the most frequent opportunistic disease associated with HIV infection (Cosivi *et al.*, 1998). HIV Seroprevalence rates greater than 60% have been found in TB patients in various African countries (WHO, 1997; WHO, 2006). Persons infected with both pathogens have annual risk of progression to active TB of 5 to 15% depending on their level of immune-suppression; approximately 10% of non HIV infected persons newly infected with TB become ill at some time during their live. In the remaining 90% effective host defense prevent progression from infection to disease (Cosivi *et al.*, 1998).

Difficulty of implementing control mechanism

Bovine tuberculosis can be eliminated from a country/region by implementing the test and slaughter policy. However, because of financial constraints, scarcity of trained man power as well as the under estimation of the importance of BTB by national governments and donor agencies, control measures are not applied or applied inadequately in most developing countries (Cosivi *et al.*, 1998).

2.5.4. Distribution

Although BTB was once found worldwide, control programs have eliminated or nearly eliminated this disease from domesticated animals in many countries (Regassa, 2005). Nations currently classified as tuberculosis free include Australia, Iceland, Denmark, Sweden, Norway, Finland, Austria, Switzerland, Luxembourg, Latvia, Slovakia, Lithuania, Estonia, the Czech Republic, Canada, Singapore, Jamaica, Barbados and Israel (Ali, 2006). Eradication programs are in progress in other European countries, Japan, New Zealand, the United States, Mexico, and some countries of Central and South America. Bovine tuberculosis is still widespread in Africa, parts of Asia and some Middle Eastern countries (Ashford *et al.*, 2001).

2.5.5. Status of bovine tuberculosis in Ethiopia

The knowledge of molecular epidemiology in the field of tuberculosis have been used to provide novel information about the spread of tubercle bacilli in outbreaks, to track the transmission dynamics of tuberculosis in the population and to distinguish exogenous reinfection from endogenous reactivation. In addition molecular epidemiology is also being used to identify the source of contamination, to determine the risk factors TB transmissions in a community, to investigate drug resistance pattern and to track the

geographic distribution and spread of clones of mycobacteria species or strains of public health importance (Mamo *et al.*, 2013a). According to the World Health Organization (WHO) Ethiopia is the tenth in the world and third in Africa among the 22 high-burden TB countries that accounts for 81% of estimated cases and among the 27 MDR TB countries in the world (WHO, 2015). The prevalence of BTB in Ethiopia is high and molecular typing of *M. bovis* has also indicated the existence of unique strains of tuberculosis (Zeru *et al.*, 2013).

Concerning the molecular epidemiology of bovine tuberculosis in livestock of Ethiopia, isolation and molecular characterization of the causative agent of BTB has been carried out in the last decade mainly in cattle and a number of isolates has been reported from different regions of the country and the first study was carried out by Ameni *et al.* (2007).

Berg and his colleagues has established important information in term of the molecular epidemiology of BTB and geographic distribution the causative agents of BTB in cattle of Ethiopia; out of the 32,779 Post mortem examination 135 isolates were characterized using molecular methods, of which 58 were *M. bovis*, 8 were *M. tuberculosis*, 53 were nontuberculous mycobacteria and 16 were not identified (Berg *et al.*, 2009).

The studies in Ethiopia revealed a higher prevalence of BTB in cattle kept indoors compared to free grazing animals and a higher susceptibility to *M. bovis* infection of exotic Holstein Bos Taurus cattle compared to local zebu cattle (Bilal *et al.*, 2010). A number of studies confirmed that *M. tuberculosis* of different strains as a causative agents of tuberculosis in cattle and other domestic animals particularly in cattle grazing in the field under semi-intensive and/or extensive husbandry systems in central and southeast Ethiopia (Berg *et al.*, 2009; Ameni *et al.*, 2010a; Gumi *et al.*, 2012). Ameni and his colleagues revealed that 27% of the isolate from grazing cattle in central Ethiopia were *M. tuberculosis* and the potential route of transmission from human-to-cattle of *M. tuberculosis* was suggested to be the common local practice of farmers spitting chewed tobacco directly into mouths of the cattle (Ameni *et al.*, 2010a) (Figure 2).

Table 1: The molecular based studies of Bovine tuberculosis in some part of Ethiopia

	Title	Molecular techniques used	Authors
1	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	mPCR	Shitaye <i>et al.</i> (2006)
2	Molecular typing of <i>Mycobacterium bovis</i> isolated from tuberculosis lesions of cattle in north eastern Ethiopia	RD4 deletion; PCR; Spoligotyping	Ameni <i>et al.</i> (2010b)
3	Molecular characterization of <i>Mycobacterium bovis</i> isolates from Ethiopian cattle	RD4 deletion; Accuprobe gene probe method; PCR; Spoligotyping	Biffa <i>et al.</i> (2010b)
4	Conventional and Molecular Epidemiology of Bovine Tuberculosis in Dairy Farms in Addis Ababa City, the Capital of Ethiopia.	RD4 deletion; mPCR; Spoligotyping; VNTR Analysis	Tsegaye <i>et al.</i> (2010)
5	Mycobacteria and zoonoses among pastoralists and their livestock in South-East Ethiopia	Genus typing; RD4 and RD9 deletion; 16S rDNA sequencing;	Gumi <i>et al.</i> (2012)

6	Prevalence study on bovine tuberculosis and molecular characterization of its causative agents in cattle slaughtered at Addis Ababa municipal abattoir, Central Ethiopia	Spoligotyping PCR; RD4 deletion; Spoligotyping	Mekibeb <i>et al.</i> (2013)
7	Gross and Molecular Characterization of <i>Mycobacterium tuberculosis</i> Complex in Mekelle Town Municipal Abattoir, Northern Ethiopia	mPCR; Spoligotyping	Zeru <i>et al.</i> (2013)
8	Epidemiology of mycobacterial infections in cattle in two districts of Western Tigray Zone, northern Ethiopia	mPCR	Romha <i>et al.</i> (2013)
9	Cultural and molecular detection of zoonotic tuberculosis and its public health impacts in selected districts of Tigray region, Ethiopia	mPCR; RD4 deletion; Spoligotyping	Zeweld (2014)
10	Molecular Epidemiology of <i>Mycobacterium Tuberculosis</i> Complex at Nekemte Municipality Abattoir, Western Ethiopia	RD deletion; mPCR	Woyessa <i>et al.</i> (2014)
11	Epidemiology of bovine tuberculosis in Butajira southern Ethiopia: A cross sectional abattoir based study	mPCR	Nemomsa <i>et al.</i> (2014)
12	Strain Diversity of <i>Mycobacterium tuberculosis</i> Isolates from	m-PCR; Deletion typing; Spoligotyping	Belay <i>et al.</i> (2014)

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13	Identification and Characterization of <i>Mycobacterium Tuberculosis</i> Isolates from Cattle Owners in North Western and North Eastern Parts of Rural Ethiopia	Deletion typing; Spoligotyping; SNPs typing	Mengistu <i>et al.</i> (2015)
14	Molecular Epidemiology Of Bovine Tuberculosis In Cattle And Its	<i>multiplex PCR, RD deletion typing and spoligotyping</i>	Alemu <i>et al.</i> (2015)

2.6. Diagnosis

The identification of the closely related members of the MTBC has remained a challenging task in diagnostic laboratories (David *et al.*, 1978; Van Soolingen *et al.*, 1997; Niemann *et al.*, 2000). A panel of classical tests based on microbiological features such as growth rate and phenotypic and biochemical characteristics has conventionally been utilized to distinguish members of M TBC (David *et al.*, 1978). However, these tests are slow, cumbersome,unreliable and time consuming. The high degree of variability among these tests warrants the development of molecular biological tools for identification of MTBC members. In this regard, multiple gene targets have been used to detect and differentiate genetically identical species such as *M. tuberculosis* and *M. bovis* (David *et al.*, 1978).

A presumptive diagnosis of TB in cattle and other susceptible species is often made on history, clinical findings, tuberculin skin tests and/or necropsy findings. Invitro lymphocyte assays including an interferon gamma assay and enzyme linked immune-

sorbent assays have been developed for the detection of the disease in cattle and so other animals exposed to *M. bovis* (Samuel, 2010; Nahar *et al.*, 2011; Sharifipour *et al.*, 2014).

2.6.1. Clinical examination

Due to the chronic nature of the disease and the multiplicity of signs caused by the variable localization of the infection, TB is difficult to diagnose on clinical examination (Radostits *et al.*, 1994; Tsegaye *et al.*, 2010). Enlarged superficial lymph nodes provide a useful diagnostic sign when lungs are extensively involved; there is commonly an intermittent cough. The principal sign of TB is commonly chronic wasting or emaciation that occurs despite good nutrition and care (Thoen and bloom, 1995; Smith *et al.*, 2006).

2.6.2. Tuberculin skin test

It is a screening test, results are not considered absolute proof that an animal or herd has BTB. Therefore, it is used to identify animals and herds that need to undergo further testing for BTB. The primary diagnostic test for TB in both humans and cattle is Tuberculin skin test, which may be performed using bovine tuberculin alone or as a comparative test using avian and bovine tuberculins (Angus, 1978; Radostits *et al.*, 1994; OIE, 2008). Tuberculins are crude antigen preparations derived from heat-killed cultures of mycobacteria and contain mixtures of proteins, polypeptides, nucleic acids, and substantial amounts of polysaccharides (Angus, 1978). The tuberculin test is usually performed on the shaved mid-neck, but the test can also be performed in the caudal fold of the tail. 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 (OIE, 2009).

2.6.3. Postmortem examination

Postmortem examinations should be supported by a histological examination of samples stained with haematoxylin and eosin (OIE, 2009). Typically, lesions caused by *M. bovis* in cattle are described as having a center of caseous necrosis with some calcification and a boundary of lymphocytes, neutrophils and epitheloid cells. Some of epitheloid cells may fuse together and form multinucleated giant cells (Ulrichs and Kaufmann, 2006; Palmer, 2007; Russell, 2007; Gil *et al.*, 2010; Ramakrishnan, 2012). An outer border fibrous of connective tissue is usually present, giving the lesion a focal appearance and providing encapsulation to some extent, which may limit the mycobacterial growth (Cosma *et al.*, 2004; Volkman *et al.*, 2004; Gil *et al.*, 2010) and spread of infection. Since the lesions are not conclusive, it is necessary to demonstrate the etiological agent using Ziehl-Neelsen stain (ZN) (Thoen and Bloom, 1995).

2.6.4. Bacteriological

Mycobacterium bovis can be demonstrated microscopically on direct smears from clinical samples and on prepared tissue materials (Quinn *et al.*, 1999). The presumptive diagnosis of mycobacteriosis can be made if the tissue has characteristic histological lesions (caseous necrosis, mineralization, epitheloid cells, multinucleated giant cells and macrophages). As lesions are often paucibacillary, the presence of acid-fast organisms in histological sections may not be detected, although *M. bovis* can be isolated in culture (OIE, 2004; Shitaye *et al.*, 2006; Jovan *et al.*, 2011; Deressa *et al.*, 2013).

2.6.5. Differential staining

Final confirmatory diagnosis of BTB depends on isolation and identification of the bacteria, but preliminary examination of stained smears from lesions, sputum, milk, urine, pleural and peritoneal fluids, uterine discharges and feces is very important

(Radostits *et al.*, 2007). In the smear, the organism appear red rods against a blue background (Ziehl Nielsen staining), while in the fluorochrome procedures, the acid fast organisms appear as fluorescent rods, yellow to orange (WHO, 1997; OIE, 2009).

2.6.6. Culture

To process specimens for culture, the tissue is first homogenized using a mortar and pestle (Woyessa *et al.*, 2014), stomacher or blender, followed by decontamination with detergent (such as 0.375 – 0.75% hexadecylpyridiniumchloride [HPC]), an alkali (2–4% sodium hydroxide) or an acid (5% oxalic acid) (OIE, 2009). The alkali or acid mixture is shaken for 10–15 minutes at room temperature and then neutralized (Regassa, 2005). Neutralization is not required when using HPC. The suspension is centrifuged, the supernatant is discarded, and the sediment is used for culture and microscopic examination (Quinn *et al.*, 1999). For primary isolation, the sediment is usually inoculated on to a set of solid egg based media, such as Lowenstein Jensen, Coletsos base or Stone brinks; these media should contain either pyruvate or pyruvate and glycerol. An agar based medium such as Middle brook 7H10 or 7H11 or blood based agar medium may also be used (Ali, 2006; Mengistu *et al.*, 2015). Cultures are incubated for a minimum of 8 weeks (and preferably for 10–12 weeks) at 37°C with or without CO₂. The media should be in tightly closed tubes to avoid desiccation. Slopes are examined for macroscopic growth at intervals during the incubation period. When growth is visible, smears are prepared and stained by the Ziehl Neelsen technique. Growth of *M. bovis* generally occurs within 3–6 weeks of incubation depending on the media used (Silaigwana *et al.*, 2012).

Liquid culture systems are used routinely in some hospital and veterinary laboratories; in these systems growth is measured by radiometric or fluorometric means (Bilal *et al.*, 2010). If gross contamination of culture media occurs, the culture process should be repeated using the retained inocula with an alternative decontaminating agent (Zerdo *et al.*, 2014). Characteristic growth patterns and colonial morphology can provide a

presumptive diagnosis of *M. bovis*; however every isolate needs to be confirmed. It is necessary to distinguish *M. bovis* from the other members of the tuberculosis complex, i.e. *M. tuberculosis* (the primary cause of tuberculosis in humans), *M. africanum* (occupies an intermediate phenotypic position between *M. tuberculosis* and *M. bovis*), *M. microti* (the ‘vole bacillus’, a rarely encountered organism), *M. pinnipedii* and *M. caprae* (Hlokwe *et al.*, 2013).

2.6.7. Serological diagnostic methods

Besides the classical intradermal tuberculin test, a number of blood tests have been used (Haagsma, 1993). Due to the cost and the more complex nature of laboratory-based assays, they are usually used as auxiliary tests to maximize the detection of infected animals (parallel testing), or to confirm or negate the results of an intradermal skin test (serial testing). The serological diagnostic tests are gamma-interferon assay, lymphocyte proliferation assay and ELISA. Both the gamma-interferon assay and lymphocyte proliferation assay are measured cellular immunity, while the ELISA measures humoralimmunity (Jovan *et al.*, 2011).

Gamma interferon assay/Bovigam

In this test, the release of a lymphokine gamma interferon (IFN- γ) is measured in a whole blood culture system. The assay is based on the release of IFN- γ from sensitized lymphocytes during a 16-24 hours incubation period with specific antigen (PPDtuberculin) (Wood *et al.*, 1990). The test makes use of the comparison of IFN- γ production following stimulation with avian and bovine PPD (Asimwe, 2008). Because of the IFN- γ test capability of detecting early infections, the use of both tests in parallel allows detection of a greater number of infected animals before they become a source of infection for other animals as well as a source of contamination of the environment (Gormley *et al.*, 2006). In animals that are difficult or dangerous to handle, such as excitable cattle or other bovidae, the advantage of the IFN- γ test over the skin test is that

the animals need be captured only once. The test is available as commercial kits for bovine species and primates (OIE, 2009).

Lymphocyte proliferation assay

This type of *in-vitro* assay compares the reactivity of peripheral blood lymphocytes to tuberculin PPD (PPD-B) and (PPD-A). The lymphocyte proliferation assay can be performed on whole blood (Buddle *et al.*, 2001) or purified lymphocytes from peripheral blood samples (Griffin *et al.*, 1994). Results are usually analyzed as the value obtained in response to PPD - B minus the value obtained in response to PPD-A, the B-A value must then be above a cut-off point. The assay has scientific value, but is not used for routine diagnosis because the test is time-consuming and the logistics and laboratory execution are complicated (it requires long incubation times and the use of radio-active nucleotides). As with the IFN- γ test, the lymphocyte proliferation assay should be performed shortly after blood is collected (OIE, 2009).

Enzyme linked immunosorbent assay (ELISA)

The ELISA appears to be the most suitable of the antibody detection tests and can be a complement, rather than an alternative, to tests based on cellular immunity. It may be helpful in anergic cattle. An advantage of the ELISA is its simplicity, but sensitivity is limited mostly because of the late and irregular development of the humoral immune response in cattle during the course of the disease. Specificity is also poor in cattle when complex antigens such as tuberculin or *M. bovis* culture filtrates are used. However, a comparison of antibody levels to PPD-B and PPD-A has been shown to be useful in increasing specificity in the ELISA (OIE, 2009).

2.6.7. Molecular techniques for diagnosis of bovine tuberculosis

Studies relied on bacteriological methods to differentiate *M. tuberculosis* from *M. bovis*. However, molecular typing of *M. tuberculosis* complex isolates provides a rapid means for discriminating members of the *M. tuberculosis* complex, a process which can often be difficult when using classical bacteriological methods. The identification of *M. bovis* is critical for determining the impact of zoonotic transmission of infection to humans, as it gives impetus to the adoption of public health measures such as the pasteurization of milk, cooking of meat, and control of tuberculosis in domestic animals. Genetic typing of *Mycobacterium tuberculosis* complex strains is now commonly used in the molecular epidemiology of tuberculosis (Barnes and Cave, 2003). Molecular techniques are used to track specific strains of pathogens and to determine more precisely the distribution of infectious diseases in populations, providing opportunities for more effective interventions (Genewein *et al.*, 1993).

Multiplex PCR

Molecular technique differentiates MTBC from *M. avium*, *M. intracellulae*, and other mycobacterial species. Heat killed AFB positive sample DNA was used as source of DNA template. The PCR targets the sequence of the genus *Mycobacterium*, within the 16S rRNA gene (G1,G2) sequences, within the hyper variable region of 16S rRNA, that is known to be specific to *M. intracellulae* (MYCINT-F) and *M. avium* (MYCAV-R), and the MTB70 gene specific for MTBC (TB-A, TB-1B) (Mamo *et al.*, 2013b).

RD deletion typing

Primers directed against the RD4, RD9, and RD10 loci are used to generate a deletion profile that would allow species identification of the isolates (Cadmus *et al.*, 2006). Mycobacterial genomic DNA will be obtained by heat killing, the isolates at 80 °C for 60 min and stored at -20 °C until it will be subjected to PCR. Isolates will be confirmed as

M. bovis by deletion typing of the RD4 region according to a PCR protocol (Brosch *et al.*, 2002).

Spoligotyping

Another molecular typing method for MTBC is the PCR based on spacer oligonucleotide typing (Spoligotyping). This method was proposed as an alternative to hybridization based fingerprinting methods for diagnosis and epidemiology of tuberculosis (Aranaz *et al.*, 1996; Kamerbeek, *et al.*, 1997; Haddad *et al.*, 2001; Njanpop-Lafourcade *et al.*, 2001; Cadmus *et al.*, 2006; Diguimbaye-Djaïbe *et al.*, 2006). Furthermore, the international designation of spoligotype patterns (Brudey *et al.*, 2006) has facilitated the comparison of results from different countries and helps elucidate the distribution and spread of strains. Assuming that spoligotype spacers can only be lost and not regained, phylogenetic relationships between strains can be suggested (Van Embden *et al.*, 2000; Smith *et al.*, 2006). A number of MTBC strain families are readily identifiable through Spoligotyping (Vitol *et al.*, 2006; Rahim *et al.*, 2007; Streicher *et al.*, 2007). Spoligotyping is based on the variability of spacer sequences interspersed with repeat sequences in the polymorphic chromosomal direct repeat (DR) locus. This locus contains multiple, well-conserved 36-bp long direct repeats (DR) (Hermans *et al.*, 1991). Strains vary in the number of DRs and in the presence or absence of particular spacers and *M. bovis* characteristically lacks spacers 39 to 43 in the spoligotype system (Kamerbeek *et al.*, 1997). Spoligotyping is not only useful for differentiation of *M. bovis* strains but can also be used to distinguish these from *M. tuberculosis*, a distinction which is often difficult to make by using conventional bacteriological techniques (Wayne, 1984).

2.7. Zoonotic Importance of Bovine Tuberculosis

Ethiopia is among the 22 high TB-burden countries that accounts for 81% of estimated cases the tenth in the world and third in Africa and 27 MDR TB countries (WHO, 2015). Human tuberculosis of animal origin is an important public health concern in developing

countries. Due to poor experimental controls, *M. bovis* was initially believed not to be a disease of man but this was later proved to be inaccurate (Thoen and Bloom, 1995; Rodwell *et al.*, 2008). The consumption of unpasteurized milk from infected cattle is the primary route of *M. bovis* infection in man and is associated with non-pulmonary TB, particularly in children (Radostits *et al.*, 2007). Cervical lymphadenitis and lupus vulgaris (chronic skin TB) are the most common presentations of a non-pulmonary *M. bovis* infection (Romha *et al.*, 2013). Pulmonary TB due to *M. bovis* is clinically, radiologically and pathologically identical to one caused by *M. tuberculosis* but it is uncommon and usually associated with animal handlers and abattoir workers (Elias *et al.*, 2008; Tigre *et al.*, 2012).

Human TB caused by *M. bovis* is unusual in countries in the developed world, due to the implementation of eradication programs for domesticated animals, accounting for <1% of TB infections (Pal *et al.*, 2014; Romha *et al.*, 2014). In the developing world, *M. bovis* is responsible for 5-10% of human TB cases but this varies between countries (Nwanta *et al.*, 2010; Parmar *et al.*, 2014). Limited laboratory facilities, in most developing countries, means that bacteriological diagnosis of TB infection tends to be carried out by acid fast bacillus smear examination only, so under diagnosis of *M. bovis* infection may be occurring (Bekele and Belay, 2011). Due to the effectiveness of the WHO recommended TB treatment regime against human *M. bovis* infections, an argument can be made that diagnosis is not necessary from a case management and therapy viewpoint (Michel *et al.*, 2014; Sisay *et al.*, 2014).

The current increasing incidence of tuberculosis in humans, particularly in immunocompromized persons, has given a renewed interest in the zoonotic importance of *M. bovis*, especially in developing countries (Pal, 2007). The role of meat and milk in the transmission of the disease remain significant. Moreover, zoonotic infection of BTB among dairy farm workers has been reported (Hassanian *et al.*, 2009). Due to the grave consequences of *M. bovis* infection on animal and human health, it is necessary to introduce rigorous control measures to reduce

the risk of the disease in human and animal populations (Saidu *et al.*, 2015). The institution of proper food hygiene practices and stronger inter sectoral collaboration between the medical and veterinary professions is vital to the control of the disease (Nwanta *et al.*, 2010; Tamiru *et al.*, 2013; Zeweld, 2014).

Animal and human health is inextricably interwoven and food animals, especially cattle serve as a reservoir of diseases of public health importance (Tschopp *et al.*, 2011; Müller *et al.*, 2013; Pal *et al.*, 2014). The safety of food of animal origin with regard to infection by *M. bovis* is worth giving consideration, taking into cognizance the current tuberculosis crisis ravaging the world. Though animals with tuberculosis pose some risk to humans, this risk is extremely remote in developed countries due to introduction of milk pasteurization and effective bovine tuberculosis control programmes (Shitaye *et al.*, 2007; Munyeme *et al.*, 2010).

In contrast, spread from animals to humans in developing countries remains a very real danger, mostly from infected milk. This seems to be a danger, which is being entirely ignored (Gemechu *et al.*, 2013; Michel, 2014). The animal and public health consequences of *M. bovis* are grave. Disease surveillance programmes in animals and humans should be considered a priority, especially in areas where risk factors are present (Biru *et al.*, 2014). Other recommendations made by the WHO (2005) in its memorandum on zoonotic tuberculosis include: Training of personnel at all levels of control programmes and the urgent need for further research on the diagnosis and control, immunological, epidemiological and socioeconomic aspects of the disease. International cooperation in all aspects of zoonotic tuberculosis remains essential in the fight against this disease (Nwanta *et al.*, 2010).

2.8. Control and Prevention

Due to, the risk of infection to the human population; loss in productivity due to infected animals; and animal market restrictions set by countries with advanced eradication programmes, need the efforts attempting BTB eradication. The priority will be given to these points vary depending up on the specific question to the country (Cousins, 2001).

Though, in developing countries bovine TB remains a major animal health problem, mainly because these countries cannot shoulder the financial burden required to implement a control programme and compensate for slaughtered animals. Limited access to education, poor information networks and lack of disease surveillance are other factors that limit the implementation of any such programme. And also due to the disease prevails in wide geographic distribution and has significant economic impact to the livestock sector and creates a zoonotic risk to the exposed human population, where control program is lacking or not implemented (Ayele *et al.*, 2004). But In industrialized countries, control and eradication of BTB has been successfully carried out by regular testing and removal of infected animals under mandatory national bovine TB programmes, such programmes have been successful in many European Union member states and in seven central European countries between 1953 and 1980 (Pavlik *et al.*, 2002) and pasteurization of animal products have greatly reduced the economic and public health significance of BTB.

Due to the cattle movement and where the natural reservoirs of the disease in wild animals pose a serious risk of transmission to domestic livestock, as a result the eradication programme of the BTB makes difficult or unsuccessful. In most African countries, controlling free movement of animals within a country is difficult and movement between countries cannot be regulated, primarily due to a lack of border controls. In addition, spread of bovine TB amongst wildlife in game parks in Africa is increasingly being recognized as a serious problem, with consequences for domestic animals (OIE, 2009).

Bacille Calmette Guérin (BCG) is an attenuated strain produced by continuous subculture of a wild-type *M. bovis* isolate from cattle, has played a crucial role in controlling human TB, particularly in children. However, its use for BTB is less effective. This has shown variable efficacy in cattle trials, which may be attributable to various factors including vaccine formulation, route of vaccination, and the degree of exposure to environmental mycobacteria (skinner *et al.*, 2001). In infected countries where there is no test and slaughter control scheme, BCG vaccination may be used to reduce the spread of infection in cattle (Daborn and Grange, 1993; OIE, 2009); however, there is no solid knowledge of long-term reduction in prevalence and safety for human beings and the environment. Before embarking on a vaccination programme, the vaccination schedule must be optimized for local conditions. Typical dosage would be from 10⁴ to 10⁶ colony-forming units given subcutaneously (OIE, 2009). Development and production of an effective vaccine with appropriate methods and strategies for delivery could therefore contribute to BTB control in Africa.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted from December 2016 to May 2017 in Jimma town municipal abattoir. Jimma zone is one of the thirteen zones of Oromia regional state which geographically lies at southwestern part of Ethiopia. Jimma Town is the capital of the zone that is 345 km far away from Addis Ababa, capital city of Ethiopia. It covers a total surface area of 19+, 305.5 km².

The zone bordered in Northwest by Illubabor, in East by Wellega and in West by Shewa zones as well as in south by Southern Nations and Nationalities People's Regional state. In general, topographical features elevation varies from 1000 to 3360 masl with average maximum and minimum temperatures in range of 25–30°C and 7–12°C, respectively. Annual rainfall of the zone is one of the highest in the country reaching up to 1200–2800 mm per year (Yared *et al.*, 2014). According to the reports of CSA, (2016) the livestock population of Jimma zone is composed of 2,006,467 bovine, 894,719 ovine, 248,049 caprine, 85,192 horses, 52,663 donkeys, 33,093 mules and 1,337,182 chickens.

The Jimma town municipal abattoir: - The abattoir which is administered under Jimma town municipality is the only source of inspected beef for about 169,446 inhabitants (CSA, 2013).

Even though the Municipal abattoir has recently built, there is no water supply for it and the rooms including the facilities were not functional yet. In addition to these although it is fenced; the place used to dispose condemned carcasses was not secured since it was easily accessed by hyenas, dogs and other animals. The overall hygiene and the internal

facilities including the drainage were not good in the municipal abattoir. The minimum and maximum numbers of cattle slaughtered per day during the study period were about 40 and 200 heads of cattle. Although one veterinarian and one assistant meat inspectors were delivered services both during ante mortem and post mortem examination very less attention was given for post mortem examination during the study period in such a ways the population is endanger of meat born zoonosis and sanitation problems.

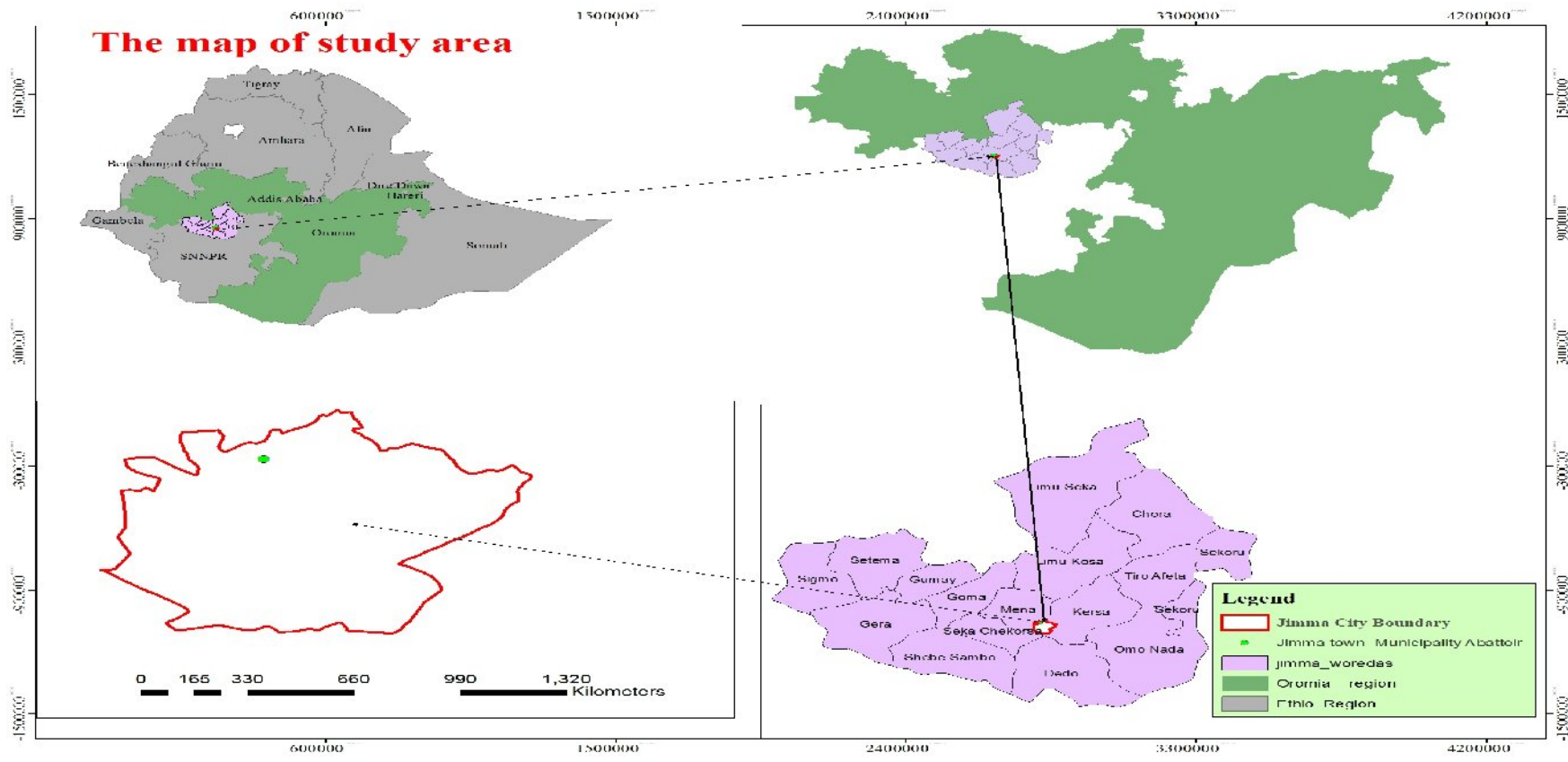


Figure 3: Map of the study area

3.2. Study Population

A total of 468 apparently healthy cattle brought for slaughter, regardless of breed and origin were considered in the study. The cattle sampled in this study were originated from different local markets mainly from Dedo, Mana, Kersa and Jimma, Markets.

Questionnaire survey was administered to abattoir workers, dairy farm workers and farmers coming at the abattoir to sell their cattle.

3.3. Study Design and Sampling Method

A cross sectional study was the design used in this study. The animals were selected by employing systematic random sampling when they were moving in line to the slaughter hall. The selected animals were identified using permanent marker, kept separately and released for slaughter one after the other. The sampling interval was obtained by dividing the total number of animals slaughtered within that day by the estimated daily sample size. Individual heads of cattle to be slaughtered were given identification number for proper recording of ante mortem and post mortem examination results. Host related risk factors like age, breed, body condition and origin were considered.

The study was carried out in every days of the week except Tuesday and Thursday when animals were not slaughtered due to fasting occasions according to Ethiopian Orthodox Christian Faith.

3.4. Sample Size Determination

The total sample size was calculated based on the predetermination of the following parameters: 95% level of confidence, 5% desired level of precision and 5.4% expected prevalence of bovine tuberculosis based on previous study (Tigre *et al.*, 2012) it was calculated to obtain the sample size according to (Thrusfield, 2007).

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

Where n= required sample size

P_{exp} =expected prevalence

d= desired absolute precision of 5%

Accordingly, 78 animals were considered and to increase the precision of the estimate, the minimum sample size was multiplied by 6 and a total of 468 animals were sampled.

The questionnaire survey was administered to 120 selected individuals depending on their occupational closeness to the animals: 65 farmers; 15 abattoir workers; 40 dairy farm attendants.

3.5. Study Methodologies

3.5.1. Ante mortem examinations

Physical examinations of the animals were carried out before they were slaughtered. Body temperature, pulse rate, respiratory rate, condition of superficial lymph nodes and visible mucus membranes were examined and recorded for individual animals to be slaughtered. Breed, body condition and source or origins were also recorded. Age was estimated based on dentition (teeth) as previously described by De-Lahunta and Habel, (1986) and Torell *et al.* (2003) (appendix 5). The body condition of each of the study animals was scored using the guidelines established by Nicholson and Butterworth (1986); and Maurya *et al.* (2009) (Appendix 4). Accordingly, on the basis of observation of anatomical sites such as vertebral column, ribs, and spines, the study animals were classified as poor (score, 1 to 3), medium (4 to 6), or good (greater than 6). The breed and source of the animals (markets) were recorded during ante mortem examination.

3.5.2. Postmortem examinations

Detailed postmortem examination (inspection, palpation and incision) of the carcass, lungs, liver and kidneys together with mesenteric, hepatic lymph nodes and lymph nodes of the head was undertaken in accordance with the method developed by Ethiopian meat inspection and quarantine division of the Ministry of Agriculture (Hailemariam, 1975; Ameni *et al.*, 2007; OIE, 2009). All lymph nodes, livers, kidney and lungs were visualized, palpated, and incised into a size of 2 mm to facilitate the detection of tuberculous lesion from each animal. These include the mandibular, medial retropharyngeal, cranial and caudal mediastinal, left and right bronchial, hepatic, mesenteric lymph nodes as well as The seven lobes of the two lungs, including the left apical, left cardiac, left diaphragmatic, right apical, right

cardiac, right diaphragmatic and right accessory lobes were investigated. The animal was classified as suspected tuberculous lesion when tuberculous lesion was found, and if not as non-lesioned.

Pathological lesion scoring was undertaken as developed by Ameni *et al.* (2006). The severity of gross lesions in individual lymph nodes and other organs were scored as follows; 0= no gross lesion, 1= small lesion at one focus, 2= small lesions at more than one focus and 3= extensive necrosis. While for organs, the pathological scoring was scored separately as follows: 0 = no visible lesions; 1 = no gross lesions but lesions apparent on slicing of the lobe; 2 = fewer than five gross lesions; 3 = more than five gross lesions; 4 = gross coalescing lesions. The scores for the individual lobes were added up to calculate the lung score.

The cut surfaces were examined under bright light for the presence of abscess, cheesy mass, and tubercles (Corner *et al.*, 1990). In the presence of suspected tuberculosis lesion, tissue samples were collected under aseptic conditions by carefully removing from the carcass and placed in to 50 ml capacity universal bottles containing 5 ml sterile PBS. The samples were transported under cold chain by ice box with packed ice to the Jimma University Mycobacteriology Research Laboratory for culture and further processing and kept at -20⁰c refrigerator.

3.5.3. *Culturing and acid fast staining of mycobacteria*

Specimen processing and culturing for mycobacteria was carried out in accordance with the guidelines of the Office International des Epizooties (OIE, 2010). In the laboratory, individual animal tissue specimens were, recorded in the sample receptions area (general room), then set foot in to culture room (negative pressure room), sectioned using sterile blades in sterile Petri dishes to obtain fine pieces and then homogenized with a mortar and pestle by adding sterile PBS. The homogenate

was decontaminated using equal volume of 4% NaOH for 15 min and then centrifuged at 3,000 rpm for 15 min. The supernatant was discarded, while the sediment was neutralized with 10 % (1N) HCl using phenol as an indicator. Neutralization was considered to be achieved when the color of the solution was changed from purple to yellow. Then 0.1 ml of the suspension from each sample was inoculated on to a duplicate set of Löwenstein-Jensen (LJ) slants; one supplemented with 0.4% sodium pyruvate (LJ pyruvate) and the other with glycerol (standard LJ). The Cultures were incubated slanted at 37°C for 1 week and in upright position for the rest 8-12 weeks. The media was in tightly closed tubes to avoid desiccation and slopes were examined for macroscopic growth at intervals during the incubation period for the presence of any mycobacterial colonies (OIE, 2009). All reagents and medium were prepared at the separate room, reagent preparation room (positive pressure room) (appendixes VI, VII, VIII and IX).

Whenever, colonies were seen, ZN staining was performed to confirm the presence of Acid fast bacilli (Quinn *et al.*, 1999; OIE, 2009) (Appendix 10). In parallel to the ZN staining, Positive colonies were preserved with freezing media, and some portion of the colonies were heat killed in water bath maintained at 80 °C heat for 45 minutes, by mixing two loops full of colonies in 200µl distilled water (Brosch *et al.*, 2002; Cadmus *et al.*, 2006). The frozen and heat killed isolates were stored at -20 °C for future Mycobacteriology and further molecular typing analysis was performed at Akililu Lemma Institute of Pathobiology according to the standard developed by Cadmus *et al.* (2006), Hewinson *et al.* (2006) and WHO (2012).

3.5.4. Region of difference (RD) deletion typing

Heat killed isolates from tissue samples were subjected for RD4 deletion typing was performed according to the procedure described by Cadmus *et al.* (2006). RD4 is

12.7 kb genetic segment that is deleted from *M. bovis* BCG strain, but present in *M. microti*, *M. africanum* and *M. tuberculosis* (Gordon *et al.*, 1999). RD4intF 5'-ACA CGC TGG CGA AGT ATA GC-3', RD4flankF5'- CTC GTC GAA GGC CAC TAA AG-3' and RD4flankR 5'-AAG GCG AAC AGA TTC AGC AT-3' were used to check for the presence of RD4 locus.

Within each PCR tube 7µl RNase free water (Qiagen), 10 µl of HotStarTaq Master Mix, 0.3µl of each of the three primers and 2.1µl of DNA template making the total final volume of 20µl. *M. tuberculosis* H37Rv and *M. bovis* SB0134 were used as positive control, and RNase free water (Qiagen) was used as a negative control. The mixture was heated in a Thermal Cycler (VWR, International Ltd. Portsmouth, UK) using an initial denaturation 95°C for 15 minutes and then subjected to 35 cycles for denaturation 95°C for 1 minute, annealing 55°C for 1 minute, Extension 72°C for 1 minute and final extension step 72°C for 10 minutes to complete the cycle. The product was electrophoresed in 1.5% agarose gel in 1x TAE running buffer; Ethidium bromide at a ratio of 1:10, 100 bp DNA ladder and orange 6 x loading dye were used in gel electrophoresis. The gel was visualized using Syngene Bio Imaging System (Syoptics Group). Interpretation of the result was based on the detection of bands of different sizes. The presence of RD4 (RD4 is intact in *M. tuberculosis*, *M. africanum*) gives a product size of 335 bp (RD4 intF + RD4flankR), and its absence (*M. bovis*) gives a product size of 446 bp (RD4flankF + RD4flankR).

3.6. Questionnaire survey

Structured questionnaire was administered on the respondents (farmers, abattoir workers and dairy farm workers) to assess awareness towards BTB and the potential risk of zoonotic transmission of bovine tuberculosis.

3.7. Data Management and Analysis

For each individual animal examined, information relevant to epidemiological investigation like age, breed, BCS and market source in were recorded on a data sheet. Presence or absences of tuberculous lesions, lessoned organs or tissues with their pathology score were all recorded on database established in Microsoft® Excel for Windows 2010.

Descriptive statistics were used to estimate prevalence of carcass with tuberculous lesion across the individual factors, frequency of anatomical location and severity of the lesion. Chi-squared (χ^2)-test and logistic regression were used to investigate possible associations and the strength of association between the prevalence and the explanatory variables (age, breed, and origin of the animals) respectively, p-value < 0.05 was considered statistically significant. In cases of estimating the effect of different risk factors in terms of odds ratio (OR), to assess the strength of association of different factors with the prevalence of BTB, the variables which had significant association were being identified on the basis of 95% confidence interval. All statistical analysis was carried out using STATA version 13.

4. RESULTS

4.1. Prevalence of Bovine Tuberculosis

Based on detailed post mortem examination of 468 cattle, an overall prevalence of BTB was 7.5% (95% CI, 5.08-9.87). From 468 slaughtered cattle, 93.59% were local breed and only 30 (6.41%) were cross breed. The prevalence of tuberculos lesion (TB lesion) and local breed and cross breed cattle was statistically significant ($\chi^2=59.55$, $p=0.000$). In univariate analysis, the difference in prevalence of TB lesion was statistically significant ($p=0.000$) among the different age group, body condition scores and market source of the animal (Table 2).

Table 2: Association of different risk factors with gross pathological lesion of bovine tuberculosis in Jimma abattoir, south west Ethiopia

Variables	No. of cattle examined	Positive% (95 CI)	χ^2 Value	P-value
Age (year)				
<=5	105	2.85 (0.59-8.12)		
>5-<=8	306	2.94(1.35-5.50)	101.36	0.00
>8	57	40.35(27.56-54.17)		
BCS				
Good	183	1.64(0.34-4.71)		
Medium	162	6.17(3.00-11.05)	28.67	0.000
Poor	123	17.88(0.50-6.96)		
Breeds				
Local	438	5.02 (3.17-7.50)		
Cross	30	43.33(25.46-62.57)	59.55	0.000
Markets				
Jimma	30	16.66(5.64-34.72)		
Dedo	229	10.91(7.19-15.69)		
Kersa	47	8.51(2.36-20.37)		
Mana	162	0.61(0.02-3.39)	18.66	0.000
Overall	468	7.5 (5.08-9.87)		

Similarly, in logistic regression analysis prevalence of TB lesion was statistically significant between age, body condition and breed (Table 3). Older cattle were forty five times more likely to have tuberculous lesion compared with the younger age group (OR=45.13; 95% CI: 10.20-199.52). The cross breed cattle slaughtered in the abattoir were twelve times more likely to develop tuberculous lesion than the local breeds (OR =12.36; 95% CI: 3.15-48.39) (Table 3).

Table 3: Logistic regression analysis of bovine tuberculous lesion with various host related risk factors

Variables	No. of cattle examined	No. of positive (%)	Crude OR (95%CI)	Adjusted OR (95% CI)
Age				
(year)				
<=5	105	3(2.85)	Ref	
>5-<=8	306	9 (2.94)	1.03 (0.27-3.87)	3.34 (0.74-14.97)
>8	57	23(40.35)	23.03(6.49-81.42)*	45.13(10.20-199.52)*
BCS				
Good	183	3 (1.64)	Ref	
Mediu m	162	10 (6.17)	3.95(1.06-14.60)	3.45(0.67-17.69)
Poor	123	22 (17.88)	13.07(3.81-44.74)*	19.24(3.86-95.72)*
Breeds				
Local	438	22 (5.02)	Ref	
Cross	30	13 (43.33)	14.46(6.24-33.48)*	12.36(3.15-48.39)*
Markets				
Dedo	229	25(10.91)	Ref	
Mana	162	1(0.61)	0.05(0.01-0.38)	0.07(0.01-0.61)
Jimma town	30	5(16.66)	1.63(0.57-4.64)	0.65(0.11-3.70)
Kersa	47	4(8.51)	0.75(0.25-2.29)	1.26(0.27-5.90)

* Statistically significant

4.2. Distribution of Gross Pathological Lesions

Gross lesions were observed in the lymph nodes and lung of the slaughtered cattle. The majority of the lesions were typical of tuberculous lesions characterized by central round, oval, or irregular, often coalescing areas of caseous necrosis and mineralization (calcification) (Figure 4). Whenever gross lesions suggestive of TB were detected in any of the tissue, the tissue was classified as having lesions.

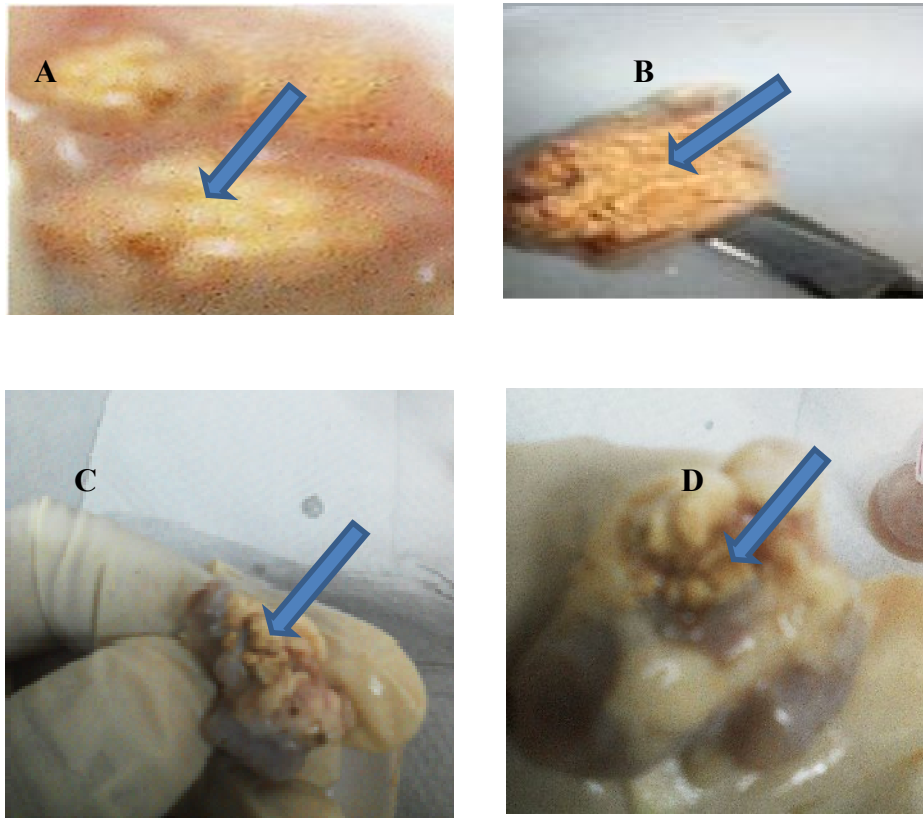


Figure 4: Typical TB lesions of cattle slaughtered in Jimma town municipal abattoir Caseous necrosis and granulomatous lesions from bronchial lymph nodes (A, B, C, D).

The frequency and distribution of lesions according to organ level and anatomical site is indicated in (Table 4). The majorities (60%) of the gross lesions were observed in lymph nodes of thoracic cavity followed by mesenteric (25.7%) and head lymph nodes (14.3 %).

Table 4: Distribution of lesions in different anatomical sites with their respective frequency of occurrence

Anatomical site	Organ / lymph nodes affected	Frequency (%)	Total (%)
Head	Retropharyngeal LN	3 (8.57%)	6(17.14)
	Mandibular LN	2 (5.71%)	
Thorax	Mediastinal LN	6 (17.14%)	21(60)
	Bronchial LN	11 (31.43%)	
	Lung	4 (11.43%)	
Abdomen	Mesenteric LN	9 (25.71%)	9 (25.71%)
Total		35 (100%)	35 (100%)

LN=Lymph nodes

Proportion of lymph nodes and lungs of TB suspected 35 cattle with visible lesions. The pathological scoring showed that the mandibular lymph nodes were most severely affected (2.5 ± 0.35) followed by mesenteric lymph nodes (2.44 ± 0.65), whereas the mediastinal lymph nodes were least affected (1.17 ± 0.15) (Table 5, Figure 5).

Table 5: Mean pathological scoring of lesion from different lymph nodes and lungs

Tissue	Number Examined	Number of positive (%)	Mean ± SE
Retropharyngeal LN	468	3 (0.64)	1.33 ± 0.27
Mandibular LN	468	2 (0.43)	2.5 ± 0.35
Mediastinal LN	468	6 (1.28)	1.17 ± 0.15
Bronchial LN	468	11(2.35)	1.64 ± 0.19
Lung	468	4 (0.85)	1.5 ± 0.25
Mesenteric LN	468	9 (1.92)	2.44 ± 0.65

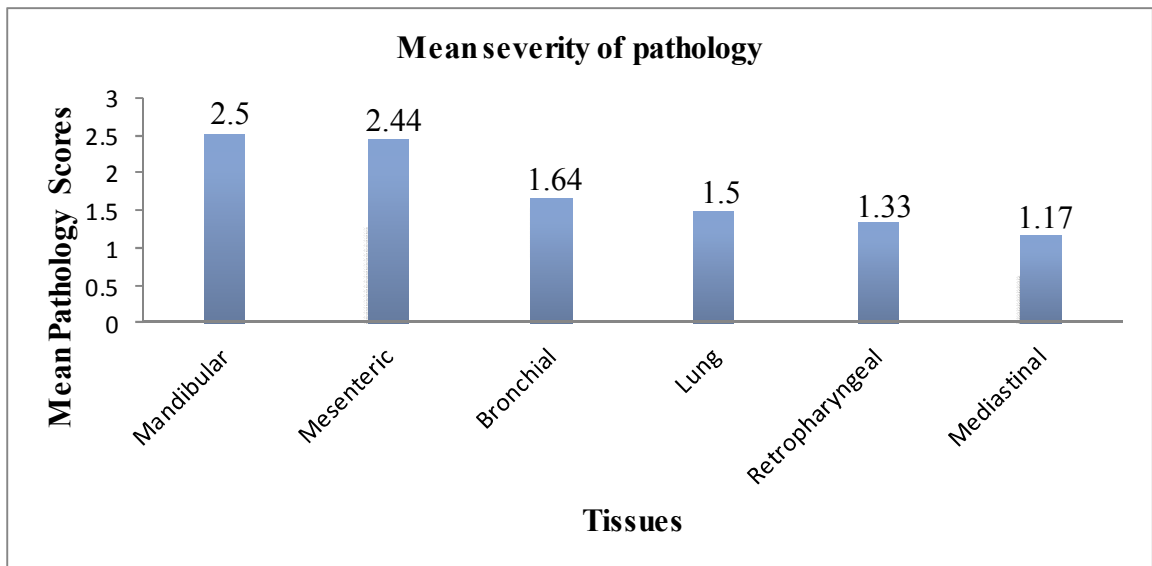


Figure 5: Mean severity of lesion in different lymph nodes and lungs

4.3. Mycobacteriological Culture and Acid Fast Stain Results

From 35 suspected BTB lesions growth was observed in 12 (34.28%) tissue samples on LJ culture medium. From this growth, colonies collected at a higher frequency from glycerol supplemented LJ medium, 6/12 (50%) than pyruvate supplemented LJ medium, 4/12 (33.33%) and the remained 16.67 % (2/12) from both LJ medium. From twelve grossly visible colonies, four (4) of them were found to be AFB positive.

4.4. RD4 deletion typing result

RD4 deletion typing was performed on the six isolates (4 AFB positive and 2 AFB negative) to identify the species of the bacteria. Only one isolate was confirmed to be *M. bovis* indicating 446bp band size. The sample was obtained from right bronchial lymph node, while the rest 5 did not show any signal (Figure 6) suggesting that they do not belong to *Mycobacterium tuberculosis* complex.

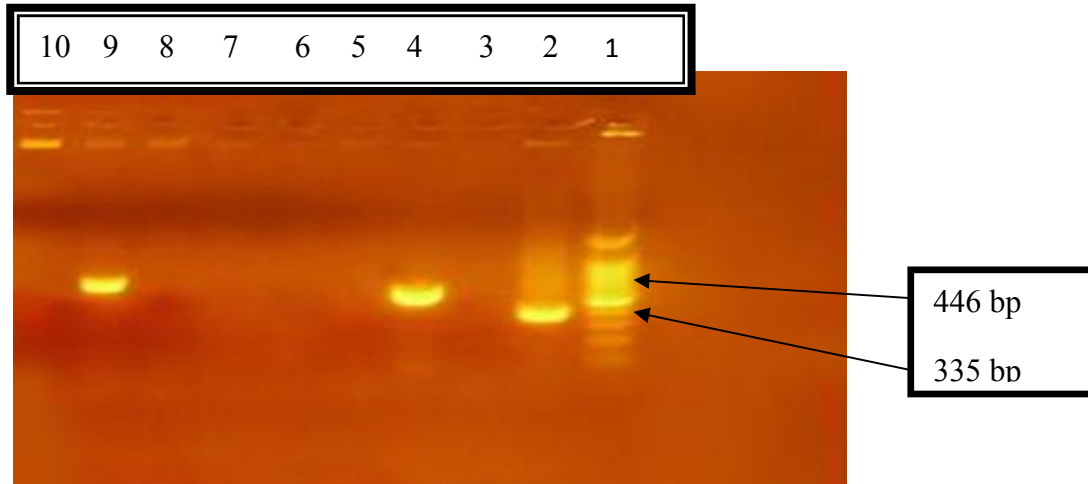


Figure 6: Electrophoretic separation of PCR products by RD4 deletion typing of mycobacteria isolates.

Lane 1- Ladder (100bp), Lane 2- *M. tuberculosis H37Rv* positive control, Lane 3- RNase free water (negative control), Lane 4-*M. bovis SB0134* positive control, Lane 9 positive for *M. bovis* while lane 5-8 and lane10 were negative for *M. bovis*.

4.5. Assessment of knowledge and practice towards BTB

According to this survey, majority of the respondents (72.5%) had no awareness about bovine tuberculosis. Among the respondents, 10% dairy farm workers and 7.7% farmers stated that they had coworkers and/or families suffering from human tuberculosis (Table 6).

Table 6: Awareness of the respondents about the disease and its means of transmission

Knowledge assessed	Category of the Responders			Total (%)
	Dairy farm workers (N=40)	Abattoir workers (N=15)	Farmers (N=65)	
Heard of about bovine TB	14 (35%)	2 (13.33%)	17 (26.15%)	33(27.5)
Had noticed respiratory problems in their cattle	10 (25%)	-	23 (35.38%)	33 (31.42)
Knew that cattle transmit bovine TB to humans	10 (25%)	3(20%)	16 (24.61%)	29(24.6)
Knew that milk is a source of infection	4 (10%)	2 (13.33%)	8 (12.30%)	14(11.6)
Knew that meat is a source of infection	2 (5%)	1(6.66%)	3(4.61%)	6(5)
Knew that humans transmit TB to cattle	0(0%)	0(0%)	0(0%)	0(0)
Had tuberculosis patient in their family or farm worker	4(10%)	0(0%)	5(7.7%)	9(8.6)

N=Total number of respondents from each category

Sixty percent (60%) and 40% of the respondents drink raw milk and eat raw meat respectively. Moreover, 44.7% of the respondent used the same watering point with animals and a lesser proportion (29.5%) of the respondents shared the same house with animals (Table 7).

Table 7: Selected practices of the respondents

Selected Practices	Category of the Responders			Total (%)
	Dairy farm workers (N=40)	Abattoir workers (N=15)	Farmers (N=65)	
Drink raw milk	25 (62.5%)	6 (40%)	41 (63.1%)	72 (60)
Eat raw meat	10 (25%)	7 (46.67%)	31 (47.69%)	48 (40)
Use the same watering point with their animals	0 (0%)	0(0%)	47 (72.30%)	47 (44.7)
Share the same house with their animals	0(0%)	0 (0%)	31(47.69%)	31 (29.5)
Mix their cattle with other cattle	0 (0%)	0 (0%)	38(58.46%)	38 (36.2)

N=Total number of respondents from each category

5. DISCUSSION

In the present study, the overall prevalence of BTB was 7.5% (95% CI, 5.08-9.87) which is comparably in agreement with the previous findings in different parts of the country; 5.84% in Hawassa abattoirs (Tekle *et al.*,2016); 5.8% in western Tigray Zone (Romha *et al.* 2013),6.4%; in Mekelle town municipal abattoir (Zeru *et al.*,2013); 6.79% in Adama municipal abattoir (Tegegne, 2014); 7.96% in Wolayta, Southern Ethiopia (Regassa ,1999); 8.8% in Hawassa municipal abattoir (Biffa *et al.*,2009) and 9% by Nemomsa *et al.* (2014) in Butajira abattoir.

However, the prevalence of this study was lower than previous studies carried out by other authors; 24.7% (Biffa *et al.* 2009) in Adama municipal abattoir, 11.50% by Abdurohaman (2009) in Butajira and 11% by Mamo *et al.* (2013a) in Afar. This lower prevalence recorded in the present study could be due to the fact that animals slaughtered in the abattoirs were mainly local breeds (Zebu) (438 out of 468) which are relatively resistant to BTB (Ameni *et al.*, 2007). On the other hand, the finding of this study was higher than 4.2% Biffa *et al.*, (2010a) in Yabello municipal abattoir, 4.5% Teklu *et al.*, (2004) in Hosaana abattoir, 5.16% Ameni and Wudie, (2003) in Adama Municipality abattoir, 2.7% Mihreteab and Indris (2011) in Jimma abattoir, 5.1% Gudeta (2008) in Nekemte Municipality abattoir, 5% Desta (2008) in Kombolch ELFORA abattoir and 5.4% Tigre *et al.*, (2012) in Jimma abattoir. This higher prevalence specially from the previous finding of Jimma may be due to the expansion of dairy farm and increased the number of exotic/cross breed cattle and most importantly the dairy cattle were brought from central Ethiopia where BTB is prevalent.

The prevalence of BTB showed a statistically significant difference among the age groups of cattle ($p=0.000$). This result is in agreement with the reports of Gebremedhin *et al.* (2014) in Dilla Municipal Abattoir, Nemomsa *et al.* (2014) and

Hussein (2006) in Butajira abattoir. The prevalence of TB lesions was highest (40.35%) in the older cattle compared to the young (2.85 %) and adult, (2.94 %) counterparts; this indicated as the age of the cattle increased the prevalence also increased. The older cattle were forty five times more likely to have the gross pathological lesions (OR = 45.13; 95 %CI: 10.20-199.52)) than the younger cattle. The results of the current research also agreed with findings of Barwinnek and Taylor (1996), Ameni *et al.* (2007), Regassa *et al.* (2010) and Biffa *et al.* (2011) who argued that as the age of the cattle increases the probability of acquiring TB infection also increases. The reason can be declining of protective capability in aging animals, have weaker immune system (O'Reilly and Daborn, 1995) due to the fact that stresses, malnutrition and immunosuppression increase with age and also due to the chronic nature of the disease (Humblet *et al.*, 2009).

There is a statistically significant difference in the prevalence of the disease ($P = 0.000$) between the animals with different body conditions, the prevalence being the highest in poor body condition (17.88 %) as compared to medium (6.17%) and good body conditioned animals (1.64 %). The poor body conditioned cattle were (OR = 19.24; 95% CI: 3.86-95.72) nineteen times more likely to have the BTB compared to the good body conditioned cattle. This is inline with study reported by Nemomsa (2014). This could be related to the weak protective immune response in poor body conditioned animals as compared to good one that may result extensive lesions and wasting of the body condition as well as the chronic nature of the disease. The present result is consistent with previous reports which indicated that animals with good body condition have relatively good immunological response to the infectious agent than animals with medium body condition (Radostits *et al.*, 1994; Radostatit *et al* 2007).

In this study, there was statistically significant difference between breed of cattle and tuberculous lesion occurrence ($P=0.000$). The prevalence of the BTB was higher in cross breed cattle 43.33% compared to the local one (5.02 %). The cross breeds

(OR=12.36; 95%CI; 3.15-48.39) were twelve times more likely to develop BTB lesions as compared to local breeds. Similarly, difference among breeds in susceptibility to tuberculosis has been documented in Ethiopia (Ameni *et al.*, 2006) and elsewhere by several authors (Bonsu *et al.*, 2000; Omer *et al.*, 2001; Kazwala *et al.*, 2001). This could be due to the fact that different breeds show difference in susceptibility to BTB infections (Radostits *et al.*, 1994; O'Reilly and Daborn, 1995; Ameni and Roger, 1998; Kiros, 1998; Biffa *et al.*, 2009; Zeru *et al.*, 2013) which showed the difference in susceptibility of BTB infection between breeds could also be related to differences in management (Elias *et al.*, 2008): those genetically improved cattle are more prone to BTB infection than local breeds since they may suffer more severely from deficient housing and malnutrition.

In this study, gross tuberculous lesions were found most frequently in the lymph nodes of thoracic cavity 60%. This report is lower than the results of previous studies which reported greater than 84% TB lesions occurrence in the respiratory system (Neill *et al.*, 1994; Collins, 1996; Whipple *et al.*, 1996; Teklu *et al.*, 2004). Similarly, Tamiru *et al.*, (2013) Tegegne (2014) respectively reported about 70 % and 67.7%, TB lesions in lungs and associated lymph nodes. However, this report is higher than the report of Miliano-suazo *et al.* (2000) (49.2%), Regassa *et al.* (2010) (50%) and Alemu *et al.*, 2015) (13.2%). The confinement of most of the TB lesion to thoracic cavity in this study suggest that the animals acquire the infection mainly through the respiratory route (Goodchild and Clifton-Hadley, 2001; Phillips *et al.*, 2003; Ameni and Wudie, 2003; Teklu *et al.*, 2004; Regassa *et al.*, 2010; Tigre *et al.* 2012).

In this study, the growth rate of mycobacteria was 34.28% which is lower than the reported of Ameni *et al.* (2007), 56% and (47%) of Ameni *et al.* (2010b). The lower growth rate in this study could be due to the fact that *M. bovis* grows poorly on

standard LJ medium (Amanfu, 2006; Cleaveland *et al.*, 2007). In addition, the presence of caseous and/or calcified lesions that have no or very few viable mycobacteria; miscategorization of non- tuberculous lesions as tuberculous lesions (Teklu *et al.*, 2004) might result in poor isolation rate (Pritchard, 1988; Diguimbaye-Djaibe, 2006). The current isolation rate, is higher than the previous report (23.6%) by Araujo *et al.* (2005), (32%); Shimeles (2008), and (31.4%) Woyessa *et al.* (2014). But this report concurs with finding by Müller *et al.* (2008) who reported 35% mycobacterial growth rate.

In this study, RD4 deletion typing of the six isolates; one isolate was confirmed to be *M. bovis* indicating product size of 446bp. This finding was similar with the previous findings of (Ameni *et al.*, 2007; Biffa *et al.*, 2010; Tekle *et al.*, 2016). However, we were not able to do further strain identification of the isolate due to shortage of resource (consumables).

In the present questionnaire survey, 27.5% of them heard of BTB but they did not know about the disease. This proportion of respondent is lower than the findings by Tamiru *et al.*, (2013) who reported 80.7% of respondents to be aware of BTB with low level knowledge about zoonotic importance. Our result was comparable with the study on assessment of the knowledge of cattle owners about BTB in Gambela (22%) by Alemu *et al.*, 2015) and 38.3% from Wuchale Jida district by Ameni *et al.*, (2003).

In this study, among 24.16% (29/120) respondents who had knowledge about zoonotic importance of BTB, only 11.66 % and 5% of them aware about the transmission of BTB through consumption of raw milk and uncooked meat respectively. Ameni and his co-workers (2007) have indicated that lack of understanding regarding the zoonotic importance of BTB, food consumption habit

and poor sanitary measures is the potential risk of BTB to public health. The proportion of BTB contributes to total tuberculosis cases in humans depends on the prevalence of the disease in cattle, consumer habits, socio-economic conditions, level of food hygiene (Ashford *et al.*, 2001) and medical prophylaxis measures in practice (Tigre *et al.*, 2011).

In the present study, 60% of the respondents consume unpasteurized or raw milk. This report is higher than the previous findings (52.1%) by Ameni *et al.*, (2003) and 45 % by Alemu *et al.*, (2015). Regassa *et al.*, (2007) stated that cattle owners who consumed raw milk were at greater risk of having active tuberculosis than those who consumed boiled milk. However, the current report on proportion of respondents with habit consuming raw milk was lower than previous report from the same study area (Jimma twon) by Tigre *et al.*, (2011) which was 85.7%. This indicates some improvement in awareness level of the respondents. In this study, none of the respondents were aware about the transmission of the disease from human to cattle which is inline with the report of Alemu *et al.*, (2015).

In this study, 29.52% the respondents and close to 48% of the farmers, share the same house with their animals. This practice can facilitate transmission of mycobacterium from animal to human or vice versa. Bogale (1999) also indicated that conditions such as habits of consuming raw milk, keeping cattle in close proximity to the owner house and using cow dung for plastering wall or floor and as source of energy for cooking do exacerbate the chance of spread of tuberculosis as zoonosis in Ethiopia.

6. CONCLUSION AND RECOMMENDATIONS

The result of the percent study has shown moderately low prevalence of BTB 7.5%. There was statistically significant variation in the prevalence of TB lesion among different age, body condition scores and breed category. The prevalence was higher in cross breed cattle, older and animals with poor body condition. The majority of the TB lesions reported in this study were confined to the thoracic cavity suggesting respiratory route as major means of BTB transmission among the cattle population. However, mandibular and mesenteric lymph nodes were most severely affected by TB lesion. Gross pathological lesion, bacteriological culture, ZN staining and the molecular identification of *M. bovis* indicated the occurrence of BTB in apparently healthy cattle in the study area.

The deep rooted habits of consumption of raw animal product, sharing of the same microenvironment with their livestock, and lack of awareness regarding BTB and its routes of transmission in the study area and isolation of *M. bovis* in their cattle population is a threat to livestock production and potential risk to public health.

On the basis of findings of the present study, the following points are recommended:

- Further epidemiological investigation should be carried out to elucidate the epidemiological information on circulating strains of *M. bovis* as well as their zoonotic role.
- A proper postmortem meat inspection should be carried out in abattoirs with due emphasis in the thoracic cavity before approving beef for the retail markets to reduce the public health risk.
- Public health awareness campaigns should be carried out to raise communities' awareness about the public health risk of BTB and its means of transmission

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8. APPENDICES

Appendix I: Questionnaire to interview abattoir workers

Date _____ Name _____
Region _____ Zone _____ Wereda _____
Kebele _____ Age _____ Sex _____

1. Marital status: a, Married b, Single c, Divorced
2. Educational status:
 - a. Illiterate b. Basic writing & reading c. Primary (Grade 1 to 6)
 - d. Junior secondary (grade 7 to 8) e. Secondary (Grade 9 to 12)
 - f. Diploma g. Degree and above
3. How long do you work in dairy farm?
 - A < 6 month b, 1-5 years c, 6-10 years e, >10 years
4. Species of animals commonly slaughtered in the abattoir
 - a. Cattle b. Sheep c. Goat
5. Which breed of cattle commonly slaughtered
 - b. Local b. Cross c. Exotic
6. Source of cattle to be slaughtered?
7. Number of cattle slaughtered in a day.....
8. Did the technician perform ante mortem examination?
 - a. Yes b. No
9. Have you ever heard /do you know bovine tuberculosis?

a. Yes b. No

10. What type of lesion you has been encountered?

11. In which organ of the animal body the lesion commonly found?

a. Liver b. Lung c. Intestine d. Lymph nodes e. Others (specify).....

12. In which age group of the cattle are mostly you found the lesion?

a. <2years b. 2-4 years c. 4-7 years d. > 7 years

13. What do you recommend if you get TB lesion on the meat of slaughtered animal?

14. In which body condition group of animal do you found the TB lesion?

a. Emaciated b. Tin c. Medium d. Fat

15. Do you know that tuberculosis can be transmitted from cattle to man and vice versa?

a. Yes b. No

16. If yes, would you please indicate some ways?

17. Habit of eating meat

a. Raw b. Cooked c. Mixed

18. How do you characterize the nature of raw meat consumption?

a. Increasing b. Decreasing c. No change

19. Habit of eating blood? a. Cooked b. Raw c. Mixed

20. Habit of drinking milk a. Raw b. Boiled c. Mixed

21. Do you have tuberculosis patient in your family or farm worker?

a. Yes b. No

Thank you!

Name of interviewer _____

Signature _____

Appendix II: Questionnaire to interview farmers and dairy farm workers

Date _____ Name _____
Region _____ Zone _____ Wereda _____
Kebele _____ Age _____ Sex _____

1. Marital status, a, Married b, Single c, Divorced
2. Educational status:
 - a. Illiterate b. Basic writing & reading c. Primary (Grade 1 to 6)
 - d. Junior secondary (grade 7 to 8) e. Secondary (Grade 9 to 12)
 - f. Diploma g. Degree and above
3. How long do you work in dairy farm/ abattoir?
 - A < 6 month b, 1-5 years c, 6-10 years e, >10 years
4. Which species of domestic animals do you own?
 - a. Cattle b. Sheep c. Goats d. Horse/mules/donkeys e. Poultry
5. Which breed of cattle do you own/ the farm has?
 - a. Local b. Cross c. Exotic
6. Purpose of keeping cattle for.
 - a. Draft b. Milk for home use c. Milk for sell d. Others
7. How many cattle do you have/ the farm has?
 - a. Less than 10 b. 10-20 c. More than 20
8. Have you noticed respiratory problems in your cattle/ in the cattles of the farm?
 - a. Yes b. No
9. Have you ever heard /do you know bovine tuberculosis?
 - a. Yes b. No
10. How do you manage cattle?
 - a. Free grazing b. Stall feeding
11. Do you practice mouth chew tobacco or local medications to your cattle?
 - a. yes b. No
12. Do you mix your cattle with other cattle? a. Yes b. No
13. If yes where? a. Watering point b. Grazing field c. Market

14. Is there any contact of your cattle with wild animals? a. yes b. No
 If yes which type, in order of priority? 1..... 2
 3.....
15. Do you use the same watering point with animals? a. Yes b. No
16. Do you share the same house with your animals? a. Yes b. No
17. Do you know that tuberculosis can be transmitted from cattle to man and vice versa? a. Yes b. No
18. If yes, would you please indicate some ways?
19. Habit of drinking milk a. Raw b. Boiled c. Mixed
20. Do you boil milk? a. Yes b. No
21. Reason for boiling? a. Fear of milk born disease b. Culture
22. Habit of eating meat? a. Cooked b. Raw c. Mixed
23. Habit of eating blood? a. Cooked b. Raw c. Mixed
24. If you sell milk/milk products, who buys it?
 a. Local people b. Milk collection unit c. Others (specify).....
25. Do you have tuberculosis patient in your family or farm worker?
 a. Yes b. No
26. If yes how many? -----25. If you have TB patient in your family or farm worker, indicate the type of TB?
 a. Pulmonary b. Extra pulmonary c. Do not known
27. How long have you /family/farme worker been sick? a. Less than a year b. More than a year
 c. Others
28. Have you ever taken any treatment? a. yes b. No
29. if yes, a. Traditional b. Drugs

Thank you!

Name of interviewer_____

Signature_____

Appendix III: Sample registering sheet

Code	Anti-mortem data				Postmortem data						Result	
	Markets	Age	Breed	Bo.con.	Lung	Bronchial LN	Midiastinal LN	Retropha. LN	Mesentric LN	Mandibular LN	Culture	AFB

LN=Lymph node, Retropha. = Retropharyngeal, AFB=Acid fast bacilli

Appendix IV: Description of body condition scores (BCS)

Poor

1. Clearly defined bone structure of shoulder, ribs, back, hooks and pins easily visible.
2. Little muscle tissue or fat present.
3. Small amount of muscling in the hindquarters. Fat is present, but not abundant.
4. Space between spinous process is easily seen.
5. Fat begins to cover loin, back and fore ribs. Upper skeletal structures visible. Spinous process is easily identified.





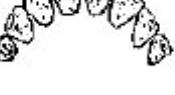
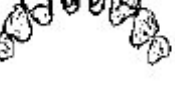
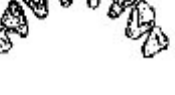
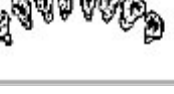
Medium/ Borderline (Optimum)

1. Fore ribs becoming less noticeable. The transverse spinous process can be identified by palpation. Fat and muscle tissue not abundant, but increasing in fullness.
2. Ribs are visible only when the animal has been shrunk. Processes not visible. Each side of the tail head is filled, but not mounded.
3. Ribs not noticeable to the eye. Muscling in hindquarters plump and full. Fat around tail head and covering the fore ribs.

Good/ fat

1. Spinous process can only be felt with firm pressure. Fat cover in abundance on either side of tail head.
2. Animal smooth and blocky appearance; bone structure difficult to identify. Fat cover is abundant.
3. Structures difficult to identify. Fat cover is excessive and mobility may be impaired.

Appendix V: cattle age determination by teeth

Diagram 1. Handy guide to determining the age of cattle by the teeth		
	At birth to 1 month	Two or more of the temporary incisor teeth present. Within first month, entire 8 temporary incisors appear.
	2 years	As a long-yearling, the central pair of temporary incisor teeth or pinchers is replaced by the permanent pinchers. At 2 years, the central permanent incisors attain full development.
	2-1/2 years	Permanent first intermediates, one on each side of the pinchers, are cut. Usually these are fully developed at 3 years.
	3-1/2 years	The second intermediates or laterals are cut. They are on a level with the first intermediates and begin to wear at 4 years.
	4-1/2 years	The corner teeth are replaced. At 5 years the animal usually has the full complement of incisors with the corners fully developed.
	5 to 6 years	The permanent pinchers are leveled, both pairs of intermediates are partially leveled, and the corner incisors show wear.
	7 to 10 years	At 7 or 8 years the pinchers show noticeable wear; at 8 or 9 years the middle pairs show noticeable wear; and at 10 years, the corner teeth show noticeable wear.
	12 years	After the animal passed the 6th year, the arch gradually loses its rounded contour and becomes nearly straight by the 12th year. In the meantime, the teeth gradually become triangular in shape, distinctly separated, and show progressive wearing to stubs. These conditions become more marked with increasing age.

Appendix VI: Löwenstein–Jensen egg based media preparation

LJ Pyruvate (LJP):

- Weigh 37.2g of LJ medium base powder
- Weigh 6.65g of sodium pyruvate powder
- Measure 600 ml of distilled water into a 1000 ml beaker.
- Pour the Lowenstein-Jensen medium base and the pyruvate powder in the sterile distilled water.
- Mix well and bring to boil in a microwave oven with constant agitation until the reagents are completely dissolved.
- Transfer the solution to 1 liter glass flask.
- Autoclave the mixture at 121°C for 15 minutes.
- Let cool to room temperature.

LJ Glycerol (LJG):

- Weigh 37.2g of LJ medium base powder
- Measure 600 ml of distilled water into a 1000 ml beaker.
- Dissolve the Lowenstein-Jensen Medium base in the water.
- Mix well and bring to the boil into a microwave oven with constant agitation until the reagents are completely dissolved.
- Using a single sterile pipette (25ml), measure 12ml of glycerol and add it to the mixture.
- Mix and autoclave the mixture at 121°C for 15 minutes.
- Let cool to room temperature.

Egg Fluid

- Soak 24-25 fresh egg in to detergent for 30 minutes
- wash and dry the egg using gauze
- Soak the egg in to 70% alcohol for 15 minutes
- Break 1000m
- 1 of eggs into a sterile beaker (2000ml)
- Add the fluid eggs to the autoclaved mineral solution.
- Using a Homogenizer, mix until homogeneous.
- Add 20 ml of 2% Malachite Green.
- Stir for at least 10 minutes before dispensing.
- Filter the mixture through sterile sieve/muslin into a sterile round glass flask (2000ml) containing a sterile magnetic bar.
- Add antibiotics: 3.0ml of Polymyxin B (100, 000 iu/ml), 0.75ml of Carbenicillin (0.2g/ml), 15ml of Fungi zone (5mg/ml) and 3.0ml 1% Trimethoprim.
- Mix well and slowly on a magnetic mixer for at least one hour under UV light in a biosafety cabinet (without running the cabinet).
- Decant into two separate sterile Duran glass flasks and close each flask with sterile lids surmounted by a sterile dispenser.

Allocating the medium

- Dispense 8ml into each sterile test tube or 10 ml into each universal tube. Avoid air bubbles.
- Clean the dispenser top with paper tissue impregnate with 70% ethylic alcohol after every set of 10 tubes.
- Transfer the tubes containing the medium into a sterile crate.
- After dispensing the entire medium, range the tubes and lean them to give a slope when placing on an inspissater.
- Tighten screw caps, slant them and coagulate by inspissations at 85°C for 50 min.

- Let cool.

Appendix VII: preparation of 4% NaOH

- ❖ To prepare 100 ml 4% NaOH
 - 4 gram NaOH was Measured
 - Placed in to 100 ml distilled water
 - Swirled to mix
 - Allocated in to 50 ml falcon tube
 - Autoclaved at 121 °c for 15 minutes

Appendix VIII: preparation of 10% HCl

- ❖ To prepare 500 ml 10% HCl
 - Measured 140.4 ml HCl solution and add in to water
 - Measured and add 359.6 ml distilled water to 1000 ml flask
 - Swire to mix
 - Aliquoted desired amounts in to clean glass or polypropylene bottles with caps
 - Labeled the entire bottle with the name of the reagent lot number, date of preparation expiration date and technologist.
 - Autoclaved at 121 °c for 15 minutes
 - Cooled to room temperature stored at 2-8°c
 -

Appendix IX: preparation of PBS (Phosphate Buffered Solution)

- Measured 9.07 gram of KH_2PO_4
- Placed in to 1000 ml distilled water
- Mixed well
- Measured 9.47 gram Na_2HPO_4

- Placed in to 1000 ml distilled
- Swirled to mix
- Both of them were placed in to 5000 ml flask
- Mixed well
- PH was Measured using PH meter
- PH Adjusted to 6.8
- The desired amount was Aliquoted in to clean glass or polypropylene bottles with caps
- The entire bottles were Labeled with the name of the reagent, lot number, date of preparation expiration date and technologist.
- Autoclave at 121 °c for 15 minutes
- Cooled to room temperature, stored at 2-8°c

Appendix X: Ziehl-Neelsen (Acid Fast) Staining

- Label new, clean, unscratched microscope slide at one end with the relevant sample name/number.
- Thoroughly mix the specimen with a pipette (1ml) and place about one drop (or 2-3 loop full) on the slide.
- Using a loop spread the smear over a surface of about 1.5cm x 1cm.
- Allow the smear to air dry completely and kept UV light in the BSC for 30 minutes.
- Heat-fix the slide either by passing it through a flame three to four times with the smear side up.
- Let the slide cool before staining.
- Put the glass slides with the fixed smears on a staining rack (don't stain more than 12 smears at a time).
- Flood the entire slide with basic fuchsine solution.
- Heat the slides slowly until slight steam rises.

- Maintain steaming for 3-5 minutes (do not let slides dry, add basic fuchsin onto the slides if you note too much of evaporation).
- After 5 minutes of basic fuchsin reaction, gently wash off all free stain completely from each slide with distilled water (using forceps, slope the slide one by one to remove the water completely).
- Destain the smear by covering the glass slides with acid-alcohol solution and incubate for a maximum of 3 minutes.
- Wash the slides with distilled water (using forceps, slope the slide one by one to remove the water completely).
- Counter stain the smear by covering the glass slides with methylene blue solution and incubate for 2 minutes.
- Wash the slide thoroughly with distilled water (using forceps, slope the slide one by one to remove the water completely).
- Leave the smear to dry before examination under the microscope.

Thus, identification of AFB is done by examining the stained smears under a microscope using the (100x) oil immersion objective. Mycobacteria and other acid-alcohol resistant bacteria appear pink or red colored, whereas the background and other nonacid-alcohol resistant material will appear blue colored. *M. bovis* will appear as short thick acid-fast rods with cord formation being a characteristic property of strains of the *M. tuberculosis* complex.