



**ASSESSMENT OF BOVINE TUBERCULOSIS IN DAIRY FARMS AND ITS PUBLIC
HEALTH IMPORTANCE IN AND AROUND ADIGRAT DISTRICT**

By

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DEDICATION

This thesis manuscript is dedicated to my beloved wife Alemineh Hadgu and my lovely son Nataniem Ataklti Hadush for their love, ceaseless support, esteem, unlimited moral patience and encouragement

ASSESSMENT OF BOVINE TUBERCULOSIS IN DAIRY FARMS AND RISK FACTORS TO PUBLIC HEALTH IN AND AROUND ADIGRAT DISTRICT

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
AOR	Adjusted odds ratio
BCG	Bacillus Calmette-Guèrin
BTB	Bovine tuberculosis
CMI	Cell Mediated Immune
C	Cytosine

CI	Confidence Interval
CSA	Central Statistics Authority
CIDT	Comparative Intradermal Tuberculin Test
COR	Curd odds ratio
DNA	Deoxyribonucleic Acid
DR	Direct Repeat
FSAI	Food safety authority of Ireland
G	Guanine
Th	T-helper cell
HIV	Humane Immune Deficiency Virus
IFN- γ	Interferon- Gamma
MTBC	Mycobacterium Tuberculosis Complex
NTM	Non-tuberculous <i>Mycobacteria</i>
OIE	Office of International Epizootics
PA	Peasant Association
PPD	Purified Protein Derivative
RD	Regions of Difference
ROC	Receiver Operating Characteristic Curve
rRNA	Ribosomal Ribonucleic Acid
WHO	World Health Organization

ABSTRACT

A cross sectional study was conducted from September 2014 to June 2015 on 384 cattle from 68 dairy farms in and around Adigrat District, North East Ethiopia, to determine the prevalence and assessment of bovine tuberculosis (BTB) and its public health significance, comparative intradermal tuberculin test(CIDT) and microbiological tests were used in the diagnosis of BTB in

dairy animals where as questionnaire survey was conducted on 120 household members in order to observe potential risk factors responsible for the occurrence of the disease in human subjects. The CIDT was performed in 212 cross breed and 172 local breed dairy cattle. The individual animal and herd level tuberculosis prevalence were 11.72% (45/384) and 36.76% (25/68) at cut-off > 4 mm, respectively. Exotic breed (OR= 3.09, 95% , CI: 1.22-7.93), intensive management system (OR= 2.64, 95% , CI: 1.01-6.92), poor body condition (OR= 3.14, 95%, CI; 1.09-9.06), large herd size (OR= 3.29, 95%, CI: 1.04-6.25) and coughing symptoms and presence of BTB (OR= 56.42, 95%, CI: 19.54-136.31) were the major risk factors significantly associated with the occurrence of tuberculosis in cattle. Of the 120 respondents only 23 (19.17%) have recognized or have heard about zoonotic importance of BTB and 19 (15.83%) were aware of BTB which affect animals. The microbiological test of milk culture revealed that only 13.04% (3/23) were grow on Lowenstein Jensen medium pyruvate but there were no growth on LJ medium glycerinated. Based on the finding awareness creation and test and slaughter policy should be introduced to the study district and to the region at large to decrease the public health problem and production loss.

Keywords: Bovine tuberculosis; comparative intradermal tuberculin; microbiological test; questionnaire survey; Adigrat

1. INTRODUCTION

Globally there is growing demand for livestock products, milk and meat. Livestock revolution and the livestock production is changing from a subsistence activity to a global food activity (Fitzhugh and Delgado, 2000). On the other hand, the population of Ethiopia has increased dramatically in the last two decades, from approximately 55 million people in 1992 to a current estimate of around 85 million (Fitzhugh and Delgado, 2000). Increased population size has led to an inexorable increase in demand for food, putting pressure on the agricultural sector in which 85% of the work force is employed. Ethiopia has the largest livestock population in Africa, including an estimated, 52 million heads of cattle (Amanfu, 2006), that contributes to the livelihoods of 60–70% of the population (Ameni *et al.*, 2006). The vast majority of the cattle are indigenous zebu (*Bos indicus*) managed under traditional husbandry systems (grazing in the field) in rural areas. However, in recent years due to the effort of government of Ethiopia to increase the productivity of local breeds and to minimize the field grazing introduction high exotic breed and modern dairy farm practices is increasing the number of dairy cattle of highly productive exotic (*Bos taurus*, mainly Holstein-Friesian) and cross breeds has been on the rise, particularly in urban and peri-urban areas in response to the increasing demand for milk products and the Ethiopian government's effort to improve productivity in the livestock sector (Sisay *et al.*, 2013). The population of dairy cows accounts for 6.3 million animals (around 12% of the total cattle population) and the estimated total national milk production per year is 2.6 billion litres (Asseged *et al.*, 2000) of which the urban and peri-urban dairy farmers produce 2%. In a country such as Ethiopia, where livestock are extremely important for people's livelihood, animal diseases can be a real threat to animal productivity and thus negatively impact on the agricultural sector and economic development.

While the distribution and the quantity of the diseases appear to be diverse according to the type of prevailing animal production systems and agro ecological zones, but the prevalence of various contagious diseases are among the major socioeconomic drawbacks of the country's cattle production. Furthermore, the genetic improvement is becoming a growing concern being integrated with animal intensification, that there is introduction of diseases of various etiologies in several dairy farms concurrent with importation of exotic breeds. Tuberculosis becomes a serious problem in cattle where intensive dairying is established, particularly when European breeds are introduced (Asseged *et al.*, 2000).

Bovine tuberculosis (BTB) caused by *Mycobacterium bovis*, is a chronic and contagious disease of cattle and other domestic and wild animals (Sisay *et al.*, 2013). BTB is prevalent worldwide but prevalence data is scarce in most developing countries due to lack of available data as there were only limited data on the disease. Several studies conducted since 2006 have confirmed that BTB is endemic in Ethiopia with prevalence varying from 0.8% to around 10% in extensive rural farming systems (Asseged *et al.*, 2000) while higher prevalence rates have been reported from regions in Ethiopia where intensive husbandry systems are more common (Zinsstag *et al.*, 2006) causing a high morbidity, BTB can also be a financial burden to farmers owning infected cattle, it has been suggested that cattle with BTB have a reduced productivity affecting milk yield, carcass value (Meisinger, 1970) and reduced draught power in traditional farming system (Tschopp *et al.*, 2010).

Bovine tuberculosis of cattle remains to be a great concern due to the susceptibility of humans to the disease caused by *M. bovis* and there is increasing evidence that *M. bovis* infections may be much more significant than generally considered. In Sub-Saharan Africa, nearly 2 million tuberculosis cases in humans occur each year; yet it is unknown what role BTB plays in the rising epidemic of tuberculosis fostered by HIV/AIDS (Zinsstag *et al.*, 2006).

A varying portion of pulmonary tuberculosis cases are considered to occur, however, almost all cases of the non-pulmonary type of tuberculosis in humans has been caused due to BTB in the human population mainly takes place through drinking of raw milk and occurs in the extra-pulmonary form in the cervical lymphadenitis form in particular. Recently (Kidane *et al.*, 2000) indicated that *M. bovis* is found to be a cause for tuberculous lymphadenitis in 17.1% of 29 human tuberculosis cases in Ethiopia.

1.1. Scientific Justifications

One of the main strategies to control Bovine tuberculosis (BTB) is test and slaughter of the positive animal's, unfortunately the case detection rates remain low in the study districts. Thus, it needs intervention to detect the disease and find with some control measures and control of Bovine tuberculosis (BTB). Ailments and production losses in intensive dairy and fattening farms are increasing and the habit of consumption of raw foods of animal origin. Available studies on tuberculosis are few and even do not provide detail epidemiological information in every districts of Tigray region and the prevalence of the disease has not been well investigated and there is a lack of information on the epidemiology and zoonotic significance of *M. bovis* in Tigray region in particular. The circumstances that promote the transmission of tuberculosis among different species of animals as well as between animals and human beings are still vague to the livestock owning society (Sisay *et al.*, 2013).

Bovine tuberculosis (BTB) has also zoonotic potential (Grange, 2001), mainly through consumption of unpasteurized milk products and its prevalence in Ethiopian, cattle can therefore be a contributing factor to the human burden of TB in Ethiopia that currently is ranked as the 7th highest in the world (WHO, 2011). Even though the disease is known endemic in Adigrat, documented information regarding the disease is unavailable

Therefore, the objectives of this paper were

- To test for BTB in small holder dairy cows of Adigrat district
- To determine the potential risk factors responsible for the occurrence of BTB in dairy farm owners and their family members.

2. LITERATURE REVIEW

2.1 Tuberculosis

Tuberculosis is an infectious disease with distinctive clinical and pathological features. Tuberculosis occurs in humans and many animal species including species of animals used for production of food (milk or meat) for human consumption (cattle, sheep, goats and deer). The principal microorganism associated with human tuberculosis is *M. tuberculosis*. *M. bovis* is the causative agent of tuberculosis in animals used for production of food and accounts for a relatively small proportion of human cases. Infection with these microorganisms is chronic and the infected human host may remain entirely asymptomatic or may have mild to moderate illness that does not come to medical attention for long periods. In a proportion of human or animal hosts infected with these microorganisms, the infection may ultimately progress to severe systemic illness. Pulmonary disease is the classical feature and ultimately the disease may progress to death of the host if untreated. The classical pathological feature of the disease in humans is the caseating granuloma. This is an organized aggregation of macrophages surrounding an area of caseous necrosis (Food Safety Authority of Ireland (FSAI), 2008).

2.2 Microbiology

The genus *Mycobacterium* comprises more than 80 species. Many species of mycobacteria occur in the environment and are rarely associated with disease in humans or animals. A number of species of mycobacteria are important pathogens of animals or humans. Human tuberculosis is chiefly associated with infection with the species *M. tuberculosis*, although *M. africanum* is also important in some regions. Tuberculosis in bovines and many other animal species is primarily associated with infection with *M. bovis*. *M. tuberculosis*, *M. bovis* and *M. africanum* together with *M. microti* (associated with infection of rodents) form a very closely related phylogenetic group and may be referred to collectively as the *M. tuberculosis* complex (MTBC). Human infection with members of the MTBC produces an indistinguishable clinical picture and the individual species cannot be distinguished from each other based on microscopic examination of stained tissues or other clinical specimens. Determination of which species is responsible for infection in a particular case normally requires culture of the microorganism in the laboratory.

Culture of the infecting microorganism remains the gold standard for diagnosis of infection with the MTBC; however, the process may take weeks, as the microorganisms grow slowly *in vitro* (OIE, 2009).

2.3. Taxonomy

Mycobacterium belongs to the Kingdom of Bacteria; Phylum of Actinobacteria; Order of Actinomycetales; Family of *Mycobacteriaceae* (Seifert, 1996; Quinn *et al.*, 2004). They are grouped in the suprageneric rank of actinomycetes that, usually, have a high content (61 – 71%) of guanine plus cytosine (G+C) in the genomic deoxyribonucleic acid (DNA), and a high lipid content in the wall, probably the highest among all bacteria (Palomino *et al.*, 2007). The *Mycobacteria* comprise more than 80 species, within the complex of related and poorly studied organisms (Rainy *et al.*, 1995). Most of them live and replicate freely in natural ecosystems and seldom, if ever, cause disease. Only a few *Mycobacteria* become successful pathogen of higher vertebrates, preferentially inhabiting the intracellular environment of mononuclear phagocytes. The host-dependent *Mycobacteria* that cannot replicate in the environment are *M. leprae*, *M. lepraemurium*, *M. aviumsubsp.paratuberculosis*, and the members of the *M. tuberculosis* complex (Palomino *et al.*, 2007).

Mycobacterium comprised within the *M. tuberculosis* complex and generically called the tubercle bacilli, the various etiologic agent of tuberculosis have distinct hosts, zoonotic potential and reservoirs (Vincent *et al.*, 1992; Palomino *et al.*, 2007). The *M. tuberculosis* complex which includes *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, *M. canetti*, *M. bovis subsp. caprae* and *M. pinnipedii* (Tauroet *et al.*, 1996; Haddad *et al.*, 2004). *M. tuberculosis*, and the regional variants or subtypes *M. africanum* and *M. canettii* are primarily pathogenic in humans (Palomino *et al.*, 2007). *M. bovis* and *M. microti* are the causative agents of TB in animals, and can be transmitted to humans. Some particular strains isolated from goats and seals have been named *M. caprae* and *M. pinnipedi*, although sometimes they are identified as *M. bovis* subspecies or variants. It could be expected that the major evolutive shifts involved in adaptation to different hosts would have entailed significant microbiological differentiation (Niemann *et al.*, 2000; Mostowy *et al.*, 2005).

Mycobacteria not identified as tuberculosis or leprosy complex, have been addressed by a variety of nomenclature including; ‘atypical *Mycobacteria*’, ‘*Mycobacteria* other than tubercle’ bacilli (MOTT), ‘environmental *Mycobacteria*’ or ‘non-tuberculous *Mycobacteria*’ (NTM) (Wolinsky, 1979). *Mycobacterium* species other than the MTBC that cause TB like diseases in man and animals are commonly called ‘atypical mycobacterium’ (Quinn *et al.*, 2004). Atypical mycobacterium are not pathogenic to man and animals except in certain situation such as direct inoculation into wound or introduction in to immune compromised hosts due to immune suppressive therapy or due to HIV infection (Thoen *et al.*, 2006); however, they are important during diagnosis as they sensitize man /animals to tuberculin test (Carter and Chengappa, 1991).

2.4. Morphology and Staining

Mycobacteria are thin rods of varying length (0.2-0.6 by 1.0-10.0µm) and sometimes branching filamentous, non-motile, non-spore forming, aerobic and oxidative (Tauro *et al.*, 1996; Quinn *et al.*, 2004). *M. tuberculosis* is straight or slightly curved rod, where as *M. bovis* is usually straighter, stouter and shorter (Gupte, 2006). All *Mycobacteria* are acid fast and share a characteristics cell wall, thicker than many other bacteria, which is hydrophobic, waxy and rich in mycolic acid/mycolates (Palomino *et al.*, 2007).

The *Mycobacteria* surface lipids also have a potent biologic activity and are thought to play a crucial role in pathogenesis (Glickman and Jacobs, 2001). As all *Mycobacterium* species, *M. bovis* has an unusual cell wall surface structure characterized by the dominant presence of mycolic acids and a wide array of lipids (60%) (Sherma and Adalakha, 1996). This waxy lipid envelope confers an extreme hydrophobicity, resistance to injury, including that of many antibiotics, and distinctive immunological properties which renders the bacteria acid- and alcohol-fast and also a feature that can be exploited to identify *Mycobacteria* via the Ziehl-Neelsen staining technique. It probably also contributes to the slow growth rate of some species by restricting the uptake of nutrient (Sherma and Adalakha, 1996; Palomino *et al.*, 2007).

Mycobacteria do not have an additional membrane in the outer layers of the cell wall that is found in Gram-negative bacteria. They are structurally more closely related to Gram-positive bacteria. However, *Mycobacteria* do not fit into the Gram-positive category as the molecules

attached to the cell wall are distinctively lipids rather than proteins or polysaccharides (Palomino *et al.*, 2007). Usually the Ziehl-Neelsen technique of staining is employed for identification of acid-fast bacteria including *Mycobacteria*. Moreover, *Mycobacteria* can also be stained with fluorescent dyes like auramine rhodamine (Bibelstein and Hirsh, 1999).

2.5. Growth and Cultural Characteristics

Unlike *M. leprae* and *M. lepraemurium*, bacteria within the *M. tuberculosis complex* are able to reproduce *in vitro*, the formers are uncultivable and require the intracellular milieu for survival and propagation (Palomino *et al.*, 2007). According to WHO (1998), tubercle bacilli can be cultivated on many different media like egg-based media, agar-based media and liquid media but, the only media which allow abundant growth of tubercle bacilli are egg-enriched media containing glycerol/pyruvate and asparagines, and agar or liquid medium supplemented with serum or bovine albumin. *M. bovis* is a slow growing, facultative intracellular, aerobic and gram-positive bacterium with a dysgonic colony shape when cultured on Löwenstein-Jensen medium (Kubica *et al.*, 2006). *M. bovis* can be identified on the basis of specific biochemical and metabolic properties. E.g., *M. bovis* requires pyruvate as a growth supplement, is negative for niacin accumulation and nitrate reduction, shows microaerophilic growth on Lebek medium and is generally resistant to pyrazinamide. In contrast, *M. tuberculosis* does not require pyruvate as a growth supplement, is positive for niacin accumulation and nitrate reduction, shows aerophilic growth on Lebek medium, and is usually not mono-resistant to pyrazinamide (Cole, 2002; Kubica *et al.*, 2006).

In addition, tubercle bacilli may also be grown on chick embryos and in tissue culture (Gupte, 2006). To date, the most frequently used media for isolation of *M. bovis* are Löwenstein- Jensen (LJ) and Ogawa- medium (both containing eggs phosphate, magnesium) and the former contains asparagines (Seifert, 1996).

In the laboratory, an atmosphere of 5 to 10% carbon dioxide favors culture growth, at least during the early stage of incubation. On the other hand, *M. bovis* is microaerophilic, i.e. it grows preferentially at a reduced oxygen tension. *M. tuberculosis* is mesophile and neutrophile as its multiplication is restricted to conditions offered by warm-blooded animals: about 37⁰C and a

neutral PH. The temperature and hydrogen ion concentration ranges, in which the bacillus is able to multiply, are relatively narrow (Palomino *et al.*, 2007). All the members of the *Mycobacteria* complex are slow growers (Seifert, 1996). Therefore, the inoculated media may have to be incubated at 37⁰C up to 8 to 12 weeks (Quinn *et al.*, 2004).

2.6. Evolution of *Mycobacterium Tuberculosis* Complex

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

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

The genome of *Mycobacteria* has a high GC (GC 61% ~ 71%) content, and its polymorphism is very limited compared to its genome size (4.4 Mb). But some regions are highly polymorphic, either by a variation in number and /or position or by a variation in primary structure (Haddad *et al.*, 2004). Strikingly, the genome sequence of *M. bovis* is greater than 99.95% identical to that of *M. tuberculosis*, but deletion of genetic information has led to a reduced genome size (Garnier *et al.*, 2003). Moreover, *M. bovis* does not have any new genetic material when compared with genome of *M. tuberculosis*. Thus, the genome difference between *M. tuberculosis* and *M. bovis* is attributed to DNA deletion in *M. bovis* and hence deletion more than 2000 single nucleotide polymorphism have been found (Thoen and Barletta, 2006).

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

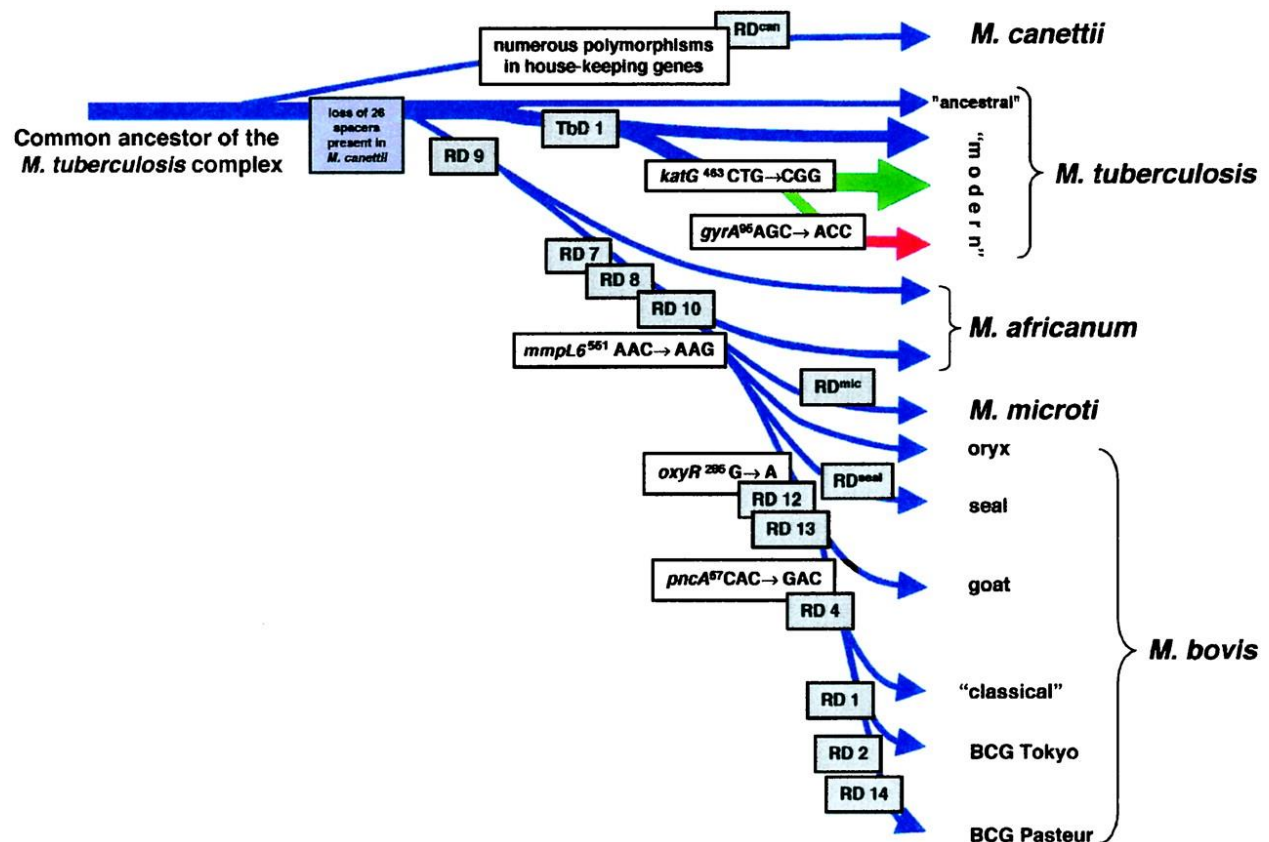


Figure1: Evolutionary scheme of the members of the *M. tuberculosis* complex, Brosch *et al.* (2002)

2.7. Transmission

Infection of the mammary gland may occur and may occasionally result in tuberculous mastitis leading to contamination of milk within the mammary gland. Shedding of *M. bovis* in oral/respiratory secretions and in feces may occur earlier in the course of infection and before a clinical diagnosis of tuberculosis is suspected. Expressed milk may become contaminated with

M. bovis from feces or secretions. In the past, the principal route of human infection with *M. bovis* in the general population is via ingestion of raw cow's milk contaminated with *M. bovis*, rather than by inhalation (OIE, 2009).

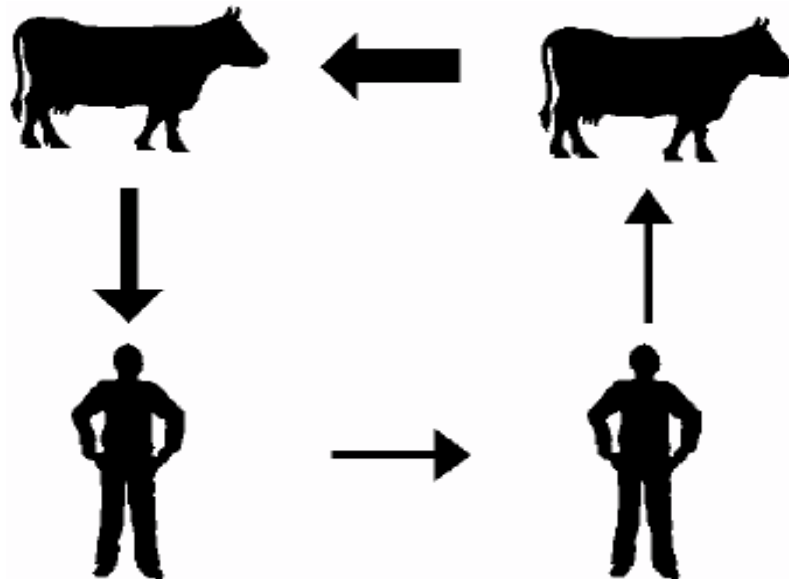


Figure 2: Cycle of *M. bovis* transmission between cattle and humans. The thickness of arrows suggests level of probability. Source: Anaelom *et al.* (2010).

There are two principal concerns with respect to the potential transfer of *M. bovis* via milk. (OIE, 2009). Through the consumption of unpasteurized milk on the farm represents a hazard in relation to *M. bovis* and consumption of dairy products made from unpasteurized milk represents a hazard in relation to *M. bovis* to a potentially wider population. The most common dairy product made from unpasteurized milk is cheese. However, the effect of the cheese production process on the viability of *M. bovis* is not well defined. Validated laboratory methods for the detection of viable *M. bovis* in milk or dairy products are not routinely available. Therefore, there is no practical way to assure that cheese made from unpasteurized milk can be considered “free of *M. bovis*”.

Bovine TB can be transmitted in several ways by direct contact, contact with the excreta of an infected animal, or inhalation of aerosols, depending on the species involved though the possible routes of infection that include the respiratory, alimentary, congenital, cutaneous, venereal,

percutaneous routes and via the teat canal with different degree of importance between species (Menzies and Neill, 2000; Phillips *et al*, 2003).

2.8. Pathogenesis and Immunology

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

Mycobacterial infection triggers a Th1-induced cell mediated immune response (CMI) which leads to release of cytokines of such as tumor necrosis factor- α , Interleukin-12 (IL-12) and interferon gamma (IFN- γ). This pathway is essential to activate macrophages (Orme and cooper, 1999). Depending on the balance of cytokines involved, three outcomes are possible: 1) macrophages kill and eliminate the bacteria, 2) the bacteria lies dormant (latency), 3) the bacteria cannot be contained by the immune system and the disease develops to active TB (Welsh *et al.*, 2005).

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

The initial CMI response is followed later in time by a humoral antibody response, which is caused by a shift of Th1 to Th2 cell activation (Dlugovitzky *et al.*, 2000). A state of anergy may occur in advanced stage of the disease and a CMI response is no more detected. Initial pathological changes are associated with the onset of CMI response (Cosivi *et al.*, 1998). CMI response can be affected by the animal’s nutritional state (e.g. deficiency in energy, protein and

micro nutrients), by stress or concurrent diseases, which lead to a reduction of the host resistance (Pollock and Neill, 2002).

2.9. Virulence Factor

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

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

2.10. Source of Infection

2.10.1. Cattle

Infected cattle are the main source of infection for other cattle. Organisms are excreted in the exhaled air, in sputum, feces (from both intestinal lesions and swallowed sputum from pulmonary lesions), milk, urine, vaginal and uterine discharges, and discharges from open peripheral lymph nodes. Animals with gross lesions that communicate with airways, skin, or intestinal lumen are obvious disseminators of infection. Cattle in the early stages of the disease,

before any lesions are visible, may also excrete viable mycobacteria in nasal and tracheal mucus. In experimentally infected cattle excretion of the organism commences about 90 days after infection (OIE, 2009).

2.10.2. Wildlife reservoirs

A large number of wildlife and feral species are naturally infected with *M. bovis* (FSAI, 2008). While most wildlife and feral animals are unimportant as sources for infection to cattle, in some areas of the world certain wildlife species appear to be a significant maintenance host and disease of cattle reservoir for infection in cattle. This reservoir escapes traditional test and slaughter control programs and results in regions where the disease remains endemic in cattle herds (FSAI, 2008).

In areas of south-west England and the Republic of Ireland infected badgers (*Meles meles*) are significant in the epidemiology of the disease in cattle and infection of cattle is believed to be from badger urine contamination of pastures (FSAI, 2008). Badgers have also been found to make nocturnal visits to farm buildings and cattle troughs to feed during which they defecate and urinate directly onto the cattle feed (Radostits *et al.*, 2006).

In New Zealand infection occurs in the brush-tail possum (*Trichosurus vulpecula*) and produces lesions in peripheral lymph nodes with discharging sinuses. Much of New Zealand's residual problem with bovine tuberculosis is in cattle running on the pasture-bush margin where there is ample opportunity for cattle-possum contact. Infection to cattle is believed to occur when curious cattle sniff moribund possums. (OIE, 2009). Mule deer (*Odocoileus hemionus*), white tailed deer (*O. virginianus*), elk (*Cervus elaphus canadensis*) and bison (*Bison bison*) in North America and red deer in Great Britain and Ireland can all act as maintenance hosts and in some regions spread infection to cattle through comingling or sharing of winter feed resulting in foci of herd infections. Buffaloes (*Syncerus caffer*) in South Africa (FSAI, 2008). and water buffaloes (*Bubalis bubalis*) in Australia can also act as maintenance hosts in these countries (OIE, 2009).

2.11. Zoonotic Importance

2.11.1. Natural history of tuberculosis in animals

The natural history of zoonotic tuberculosis has been best studied in cattle, although the progression and outcome of infections are probably similar in most species of animal used for food production in the world. As with human infection, access of *M.bovis* to the tissues is followed by an initial macrophage response that is not, however, sufficient to prevent proliferation of the microorganism. Dissemination of the mycobacterium to local and regional lymph nodes may be followed in rare cases by blood borne spread to other organs. In animals with clinical manifestations of tuberculosis, the respiratory tract and draining lymph nodes are the principal foci of disease. Clinical manifestations and pathological lesions may also be observed in other organs (liver, spleen, kidney, mammary gland and bone marrow) and their associated lymph nodes, particularly in advanced disease (FSAI, 2008).

The route of infection in most animals is via the respiratory tract. Less commonly, *M. bovis* may also gain entry via the pharynx or gastro intestinal tract. The principal source of infection is shedding of *M. bovis* by infected animals. *M. bovis* is excreted intermittently throughout all stages of the disease and in particular during its advanced stages, when pulmonary lesions discharge *M. bovis* into the bronchi and the upper respiratory tract in considerable numbers. Exhalation of the bacillus follows. Likewise, after infective sputum is swallowed, *M. bovis* is excreted in the feces and, with some reduction in numbers, persists in the excreta and in the contaminated slurry and environment for 330 days and longer (OIE, 2009).

In animals, as in humans, pre-clinical infection may be recognized by use of the tuberculin test. This test is based on detection of the specific immunological response to MTBC infection. The test involves intradermal injection of protein antigens derived from *M. bovis* (purified protein derivative, PPD) and inspection three days later for evidence of a local inflammatory reaction at the site of injection. In cattle, PPD is administered in parallel with administration at an adjacent site of protein derived from another species of mycobacterium that is commonly present in the environment (*viz. M.avium*). In cattle, interpretation of the tuberculin test is based on measurement of any alteration in skin fold thickness at the site of administration of *M. bovis* PPD

and at the site of administration of the comparator antigen, three days after administration of PPD (Radostits *et al.*, 2006).

Comparison of any increase in the skin fold thickness at the *M. bovis* PPD site with that at the site of administration of the *M. avium* antigen, relative to the initial measurements, is the basis of interpretation. A positive reaction is indicative of infection with *M. bovis*. Animals with a positive tuberculin test are referred to as “reactors”. The tuberculin test is valuable in the control of zoonotic tuberculosis because early recognition of preclinical infection in animals intended for food production and early removal of infected animals from the herd eliminates a future source of infection for other animals and for humans (OIE, 2009).

2.11.2. Zoonotic importance of bovine tuberculosis

Approximately 85 per cent of cattle and 82 per cent of human populations in Africa live in areas where BTB is either partly controlled or not controlled at all (Cosivis *et al.*, 1998).

The current increasing incidence of tuberculosis in humans, particularly in immune compromised humans, has given a renewed interest in the zoonotic importance of *M. bovis*, especially in developing countries and the ease and frequency of the spread of tuberculosis from animals to humans in an uncontrolled environment makes this important zoonosis (OIE, 2009).

M. bovis can be responsible for 10 to 15% of human tuberculosis with higher rates in children in some areas. Infection in humans occurs largely through consumption of infected raw milk and raw milk products by children but spread can also occur by inhalation. Transmission to humans can be significantly reduced by pasteurization of milk but only complete eradication of the disease can protect the farmer and his family. Transmission from cattle to humans in developed countries is an unlikely event now a days but still occurs and resurgence of the disease in association with wildlife reservoirs has resulted in a spillover into human populations. The widespread occurrence of tuberculosis in exotic animals maintained in captivity adds to the public health importance of these infections (Radostits *et al.*, 2006).

2.12. Bovine Tuberculosis in Ethiopia

In Ethiopia, tuberculosis and leprosy have been recognized as major public health problems since the 1950s. Tuberculosis is the most frequent cause of hospital admission (9.4% of all cases according to the report of (Sisay *et al.*, 2013) admitted to hospital) and the leading cause of hospital deaths. In the year 2005, the HIV prevalence among adult TB patients has been determined to be 11% (Sisay *et al.*, 2013).

The individual animal prevalence (7.3%) reported by (Sisay *et al.*, 2013), and (Omer *et al.*, 2001) who recorded 11%, 14.2% and in central Ethiopia, southern Ethiopia, respectively. Asseged *et al.*, (2000) reported a similar animal prevalence in and around Addis Ababa, the capital of Ethiopia. As herd size increased, this report indicating that a corresponding increase in the prevalence of bovine TB, 4.6%, 6.4% and 10.5% for small, medium and large herd size, respectively (Asseged *et al.*, 2000) also indicated that bovine TB is a disease of overcrowding. Thus, when the number of animals in a herd increases, the transmission of the bacillus is promoted. Animals with no grazing are at a higher risk of infection than those kept on free grazing and mixed grazing. The closer the animals are packed together, the greater the chance of transmitting the disease (Asseged *et al.*, 2000). The prevalence of bovine TB is higher in Holstein, Cross [HFxZebu] and Begait cattle than pure Zebu breed. Fewer reactor animals have been recorded in the younger age groups (3.5%) and reactivity to the CIDT test increased with age, up to six years of age adult (9.1%) (Sisay *et al.*, 2013), after which it declined old (6.8%). It is possible that the infection may not become established in young animals but, as they get older, their chance of acquiring infection also increases, due to the increased time of exposure. Infection of cattle with *M. bovis* constitutes a human health hazard as well as an animal welfare problem. Furthermore, the economic implications in terms of trade restrictions and productivity losses have direct and indirect implications for human health and the food supply (Awah-Ndukum *et al.*, 2012).

A study conducting by (Shitaye *et al.*, 2007) using the comparative intradermal tuberculin test (CIDT) showed that the prevalence of BTB in the central Ethiopia 2.7%. The disease is assumed to be more prevalent in dairy cattle kept under intensive management system than in extensive due to closer confinement, longer life spans and greater productivity stress (Shitaye *et al.*, 2007).

Comparable study has been reported by (Ashenafi *et al.*, 2013) who described prevalence rates of 3.5% (18/514) in Assela and 3.8% (12/320) in Bodji district.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in dairy farms of Adigrat town located 960 km away from Addis Ababa located in the north east direction, 14° 16' N and 39° 29' E, altitude with a range of 2460-2970 m above sea level. The minimum and maximum temperatures are 9.28°C and 21.91°C, respectively. The area receives a bimodal rainfall of 400 mm minimum and 600 mm maximum (CSA, 2010). The district share boundaries with Hawzien in the south, Enticho in the west, Gulomahda in the north, and SaesiTsaedaemba in the East parts (Tyhra and Kwadwo, 2011). A total population for this district of 122,827 of whom 58,398 were men and 64,429 were women, live stock are main components as main factors for the livelihood of the community to undertake agricultural activities and also as source of income. The livestock population of the district includes 51,514 cattle, 60,040 sheep, 30,050 goats and 67,769 poultry (chickens) respectively (CSA, 2010).



Figure 3: Map of Tigray region showing the selected District (Study site).

Source: Tyhra and Kwadwo, (2011).

3.2. Study Design

A cross-sectional study design was conducted from September 2014 to June 2015 to detect the prevalence of bovine tuberculosis in selected peasant associations (PAs) of Adigrate district and to identify potential risk factors associated with BTB and zoonotic importance. The study was conducted in eight randomly selected PAs. CIDT test for the dairy cattles and questionnaire survey to the dairy owners and their family members were administered.

3.3. Sampling Method

First the list of 20 peasant associations of the Adigrat district and their corresponding animal (dairy cows) were obtained from the Ganta Afeshum Agricultural and rural development Bureau. Out of 20 PAs, 40% of the PAs (8) were selected purposely based on their accessibility (non-probability; purposive sampling). There were about 3621 dairy cows in the eight selected PAs of which around 10.6% (proportionalized) were included in this study.

3.4. Sample Size Determination

Previously there was no recorded data on the prevalence of tuberculosis in the study area. Therefore, the average expected prevalence rate was assumed to be 50% for the areas within 95% level of Confidence (CL) at 5% desired level of absolute precision. Hence, the formula by (Thrustfield, 2005) was used to calculate sample size (n).

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

Where n= sample size, d= desire absolute precision (0.05), p exp= expected prevalence 50%, thus the desired sample size for Pex = 0.5 is n = 384. These numbers of cattle's were obtained proportionally from all randomly selected farms.

3.5. Study Subjects

Selected proportionality out of 3621 dairy cows. A total of 384 dairy cattle of which 212 exotic (Holstein) and 172 local breeds (zebu) dairy cows were considered in this study.

3.6. Study Methodology

3.6.1. Comparative intradermal tuberculin test

Comparative intradermal tuberculin test (CIDT) was used mainly to differentiate between animals infected with *M. bovis* and those sensitized to tuberculin due to exposure to other mycobacterium or related genera. Two sites at the middle of the neck were shaved and cleaned 12 cm apart on the same side of the neck, the areas was examined for the presence of any gross lesions. The skin fold thickness at the two sites were measured by caliper and recorded. Each animal is then injected 0.1 ml (25,000 IU) avian PPD (Avituber, symbiotic corporation, France) and 0.1 ml (25,000 IU) bovine PPD (Bovituber, symbiotic corporation, France) intradermal using insulin syringe at the anterior and posterior parts respectively (OIE, 2010).

3.6.2. Operational definitions

The sites were examined and the skin thickness was measured 72hr. after injection. The interpretation was made in the following ways: When the skin thickness was increased at both sites the difference of increase at bovine (B) and increase at avian (A) site was considered. Thus, when B-A is less than 2 mm, between 2 mm and 4 mm, or 4 mm and above, the animals were considered as negative, doubtful, or positive, respectively (OIE, 2010).

The sampled animals were screened on their habitation and each individual study animal was recorded with its breed, herd size from which animals were sampled (small: 2-5 animals, medium: 6-10 animals and large herd size: above 10 animals), age group (young: [6 months-3years], adult: (3-7 years], old: above 7 years) (Grange, 2001), management intensive and, semi-intensive, and body condition (poor, medium and good). Body condition score for each cattle was estimated according to the standard set by Nicholson and Butterworth (Delarua-Domenech, 2006). Accordingly animals were grouped as good, medium and poor.

3.6.3. Questionnaire survey

The structured questionnaire survey was prepared and administered to 120 households members in Adigrat district that were engaged directly or indirectly in dairy farming activity. The owners

of the farm and attendants of cattle were interviewed about their habit of raw milk consumption and recent history of tuberculosis upon them or their family members.

3.6.4. Specimen collection of milk and processing

About 70 ml of the last few streams of milk from the 4 quarters of CIDT test positive cows were collected into a sterile universal bottle aseptically. The samples were kept in a cool box and transported to laboratory. The milk samples were centrifuged at 3000 rpm for 15 min and the supernatant discarded. The sediments were suspended in 2ml of sterile physiological saline solution and decontaminated with equal volume of sterilized 4% NaOH solution (Quinn *et al.*, 2002).

The suspension of the decontaminated milk sample was concentrated with HCl using phenol red as indicator. Neutralization was achieved when the suspension color changed from purple to yellow. The neutralized suspension from each sample was spread on 2 slants of Lowenstein-Jensen (L-J) media (one enriched with sodium pyruvate and other enriched with glycerol). The cultures were incubated aerobically at 37°C for 8-12 weeks in appropriate positions with weekly observation for growth colonies (Ameni and Wudie, 2003 and Vestal, 1977).

3.7. Data Analysis

The data obtained from the questionnaire survey and CIDT were stored to Microsoft Excel 2007 spreadsheet and analyzed using a STATA Version 11.0. Multivariate logistic regression was used to analyze the data and to identify the risk factors for (CIDT) test. Descriptive and analytic statistics were computed and logistic regression and Chi-square test (χ^2) was employed to see the association of risk factors with that of the disease; the degree of association was computed using Odds ratio (OR) and 95% confidence interval (CI). Crude Odds ratio (COR) and adjusted odds ratio (AOR) were applied to indicate the degree of risk factor association with the disease occurrence signified by 95% confidence intervals.

4. RESULTS

A total of 384 cattle were tested for tuberculosis from the study districts and the results of the comparative intradermal tuberculin test (CIDT) in cattle of the study districts revealed that 11.72% (45/384) were found CIDT positive. Moreover, herd level prevalence of the disease indicated that out of the 68 herds examined 36.76% (25/68) were found to be positive as indicated in the (table 1) below

Table 1; Over all animal and herd level prevalence of BTB

Individual animal level prevalence		Herd level prevalence	
Number of animals tested	Number of animal positive (%)	Number of herd tested	Number of herd positive (%)
384	45 (11.72)	68	25(36.76)

The prevalence of BTB in exotic and local breeds were affected significantly (OR=3.09: 95% CI; 1.22-7.93) exotic breeds were more likely to be positive for CIDT at cut-off greater than or equal to 4mm. Similarly, there was significant difference b/n intensive and semi-intensive production system. Body condition score and the clinical signs of coughing were significant associated with BTB. On the other hand, there were no significance differences of the prevalence of BTB in different age category as shown (Table2)

Table 2; Logistic regression analysis of CIDT positivity and risk factors (N=384)

Variable		No_ of animals	BTB reactors	COR (95% CI)	AOR(95% CI)
Age	young	137	18(13.14)	0.72 (0.35-1.50)	
	adults	199	21 (10.55)	0.95(0.44-2.09)	
Breed	old	48	6(12.50)		
	exotic	212	35(16.51)	3.20(1.54-6.68)	3.09* (1.22-7.93)
Body condition score	local	172	10 (5.81)	0.31(0.15-0.65)	
	poor	75	17 (22.67)	3.43 (0.19-0.89)	3.14* (1.09-9.06)
Management system	medium	131	14(10.69)	1.40 (0.62-0.82)	1.52* (1.36-2.10)
	good	178	14 (7.87)	0.29(0.14-0.63)	
	intensive	320	31 (8.07)	2.61 (1.30-5.25)	2.64* (1.01-6.92)
Herd size	Semi-intensive	64	14 (3.65)		
	small	133	23 (17.29)	4.27 (1.56-11.64)	3.06* (1.74-4.61)
Sign of coughing	medium	107	5(4.67)		
	large	144	17 (11.8)	2.73 (0.97-7.65)	3.29* (1.54-6.25)
	absent	358	19(5.31)		
	present	26	26 (100)	56.62 (22.63-141.71)	56.42* (19.54-136.31)

Note that; * means there is significance difference

Table 3; Logistic regression analysis of CIDT positivity and P-value (N=384)

variable		No of animals	BTB reactors	COR (95% CI)	p-value
age	old	48	6(12.50)	1	0.757
	young	137	18(13.14)	0.72 (0.35-1.50)	
	adults	199	21 (10.55)	0.95(0.44-2.09)	
breed	local	172	10 (5.81)	1	0.001*
	exotic	212	35(16.51)	3.20(1.54-6.68)	
Body condition score	good	178	14 (7.87)	1	0.003*
	poor	75	17 (22.67)	3.43 (0.19-0.89)	
	medium	131	14(10.69)	1.40 (0.62-0.82)	
Management system	Semi-intensive	64	14 (21.88)	1	0.006*
	intensive	320	31 (9.69)	2.61 (1.30-5.25)	
Herd size	medium	107	5(4.67)	1	0.010*
	small	133	23 (17.29)	4.27 (1.56-11.64)	
	large	144	17 (11.8)	2.73 (0.97-7.65)	
Sign of coughing	absent	358	19(5.31)	1	0.000*
	present	26	26 (100)	56.62 (22.63-141.71)	

Logistic regression Model was developed

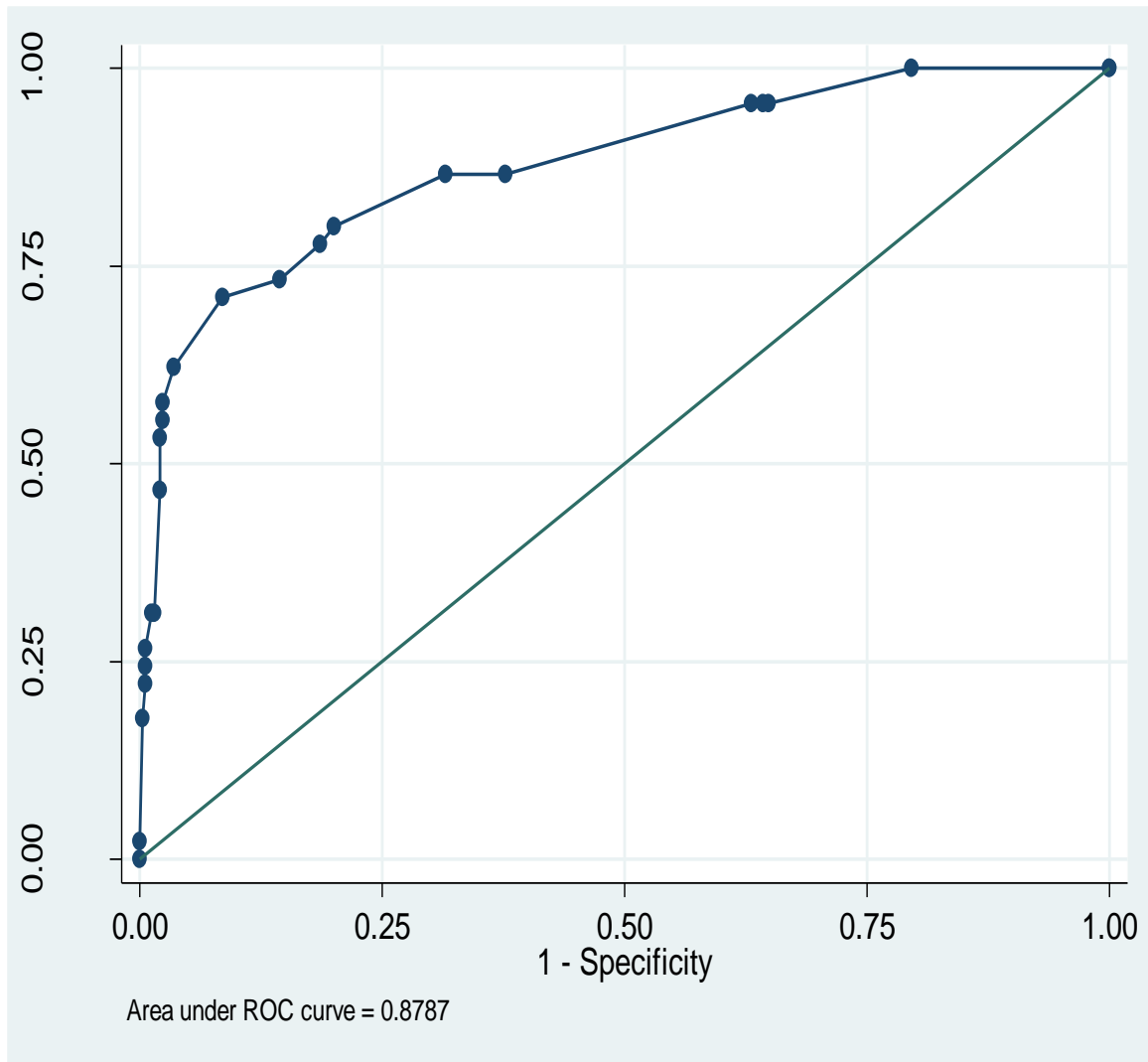
$$\text{Log}(\pi_{ij}/1 - \pi_{ij}) = \beta_0 + \beta_1 X_{1j} + \beta_2 X_{2j} + \dots + \beta_n X_{nj} + u_{0j}$$

Goodness –of-fit test was applied Hosmers –Lemeshow was use .

4.1. Receiver Operating Characteristic (ROC) Curve

The CIDT test performance was shown graphically by plotting ROC curve , which compares the true-positive rate or sensitivity on the vertical axis with the false-positive rate (1-specificity) on the horizontal axis , the area under the curve is 0.8787 this means 87.87% accuracy of test (figure 4)

Figure 4 .the sensitivity and specificity under ROC curve



4.2. Milk Culture of Mycobacteria

Out of 45 positive animal's milk sample were collected from 23 actively lactating cows, only 13.04% (3/23) were positive for mycobacterial growth (Table3).

Table 4: The result of milk sample culture from positive actively lactating cows

Sample type	Total culture in L-J media containing glycerol	Growth positive	Total culture in L-J media containing pyruvate	Growth positive
Milk sample	23	0 (0)	23	3 (13.04%)

4.3. The Questionnaire Survey

A total of 120 households were interviewed of these 19.17% (23/120) responded that BTB has zoonotic importance, and 17.5% (21/120) of the respondent's knew the means of transmission from cattle to human beings is through the consumption of raw milk according to the response indicated in (Table 4).

Table 5: Knowledge of BTB and its transmission to humans

Statement	Number interviewed	of Respondents who knew(%)
Knew BTB can affect animals	120	19(15.83)
Knew BTB is zoonotic	120	23(19.17)
Knew about pasteurization of milk	120	0(0)
Knew close contact can facilitate BTB transmission	120	3 (2.5)
Knew raw milk consumption obtained from BTB infected cattle transmitted to human	120	21(17.5)

5. DISCUSSION

In the present study, the prevalence of bovine tuberculin positivity at animal and herd levels were 11.72% and 36.76% respectively. This finding was in agreement with the findings of (Ameni and Erkihun, 2007), (Ameni *et al.*, 2001) and (Omer *et al.*, 2001), who recorded animal level prevalence of 11%, 14.2% and 14.5%, in central Ethiopia, southern Ethiopia and Eritrea, respectively. But in contrary to the findings of this (Akililu *et al.*, 2014) and (Fikre *et al.*, 2014) who reported herd level prevalence of 11.4% and 11.3% respectively this finding was higher. At the same time, the current finding compared to the findings of (Gebremedhin *et al.*, 2013) and (Mohammed *et al.*, 2012) who reported animal level prevalence of 6.6% and 7.1% respectively the present finding is higher, this might be due to the sample size difference by Mohammed is lower and due to ever expansion of intensification from time to time. The present finding revealed that there is significant difference on the prevalence of BTB in exotic and local breeds with higher prevalence in exotic breeds having the rate of 16.51% compared to the local breeds having the prevalence of 5.81% (OR=3.09 at 95% CI; 1.22-7.93) which indicated that exotic are two times more likely at risk to the of BTB than local breed. This might be due to less resistant of exotic breeds for to bovine TB compared to indigenous breeds of cattle (Kleeberg, 1984). This finding is not in agreement with (Mohammed *et al.*, 2012) who reported prevalence of 7.1% in exotic breeds and but agreed with of (Sisay *et al.*, 2013) who recorded prevalence of 11.55% in exotic breed compared to local breeds having the rate of 5.3%. In contrary to this finding (Gebremedhin *et al.*, 2013) who reported lower prevalence of BTB in exotic cattle with the rate of 3.5% may be due to the differences of the study sites as he has conducted his study in Western part of Tigray having few number of exotic breed since there are high animal population of local breeds having good performance in his study site.

In the present study the herd prevalence at small, medium and large increase with the herd size, that was herd at large farm were two times more at risk than medium herd size this work agrees with (Asseged *et al.*, 2000) as he reported a similar animal prevalence in and around Addis Ababa, the capital of Ethiopia. In accordance with findings from other studies [Ameni *et al.*, 2002], as Ameni analysis indicates that, as herd size increased, there was a corresponding increase in the prevalence of bovine TB; 4.6%, 6.4% and 10.5% for small, medium and large herd size, respectively which is similar with that of (Fikre *et al.*, 2014, Sisay *et al.*, 2014). All the

above study were agree with the present finding this agreement could be due to the similarity in study subjects, herd size and breed types and production systems in the present as well as in the aforementioned studies.

With regard to the rate of the disease in cattle managed under intensive and semi-intensive, production system the current finding indicated that those raring under intensive were at high risk with the prevalence of 8.07% than under semi-intensive having prevalence of 3.65% cattle kept under high-intensity conditions showed significantly higher skin-test prevalence as compared to cattle kept under extensive conditions. Intensification, stressed animals, and overcrowding are all possible explanations for such relationship. The main routes of BTB transmission are through aerosol as gross lesions usually involve the lungs and thoracic lymph nodes (Radostits *et al.*, 2006), and therefore BTB transmission benefits from overcrowded herds. The current finding is in line with (Ayele *et al.*, 2004) and (Elias *et al.*, 2008). This could be due to the fact that intensive farming system promotes close contact between animals, thereby favoring the spread of the disease from one animal in to another animals.

This study revealed that the rate of the disease in poor, medium and good body condition animals were 22.67%, 10.69% and 7.87% respectively which cleart indicated that poor body condition animals were highly affected with the disease compared to the medium and good body condition animals. In agreement with the current finding (Mahmmmed *et al.*, 2012, Fikre *et al.*, 2014 and Akililu *et al.*,2014), also reported that poor body condition animals are highly susceptible compared to medium and good body condition animals. Similarly, tuberculin reactivity was significantly affected by the body condition of the animal cattle. This could be because the tuberculin reaction is dependent on immune competence, which in turn may be associated with the physical condition of the animal such that animals with better physical condition are immune competent and thus give a better reaction to tuberculin. But animal with poor body condition could be immune compromised and hence may not react to tuberculin although they might have been infected by *Mycobacterium*(Cook *et al.*, 1996).Similar to the observation of the present study previous studies also reported higher prevalence in animals with poor body condition as compared to those with good body condition scores (Cook *et al.*, 1996, Kazwala *et al.*, 2001and Asseged *et al.*, 2004).However it is difficult to decide whether BTB has caused poor body condition or animals in poor conditions were susceptible than those in good body condition.

The current finding on the distribution of the disease in different age category of cattle also indicated that the rate was slightly higher in young animals followed by old and adult animals with the prevalence of 13.14%, 12.50% and 10.55% respectively where there is no significance difference among the age groups as only few number of old age animals were included in the current study. But in contrary to the current result analysis for the effect of risk factors revealed that the animal prevalence of BTB increased with age up to the age of 7 years, and was then observed to decrease slightly (Mohammed *et al.*, 2012).

The culture result of this finding on selective media important for Mycobacteria growth indicated that out of the 23 Milk samples subjected to culture on L-J media containing pyruvate only 3 (13.04%) showed growth positive, where as in L-J with glycerol there were no growth. This results indicated that L-J medium could be used for primary isolation, sensitivity testing, identification and sub-culturing of the majority of Mycobacteria as reported by Maureen (1981). This result has similarity with that of reported by Ameni *et al.* (2003) who finds (13.3%) from milk samples collected from Canadian cattle, this similarity might be due to sampling of milk from dairy reactors to CIDT in both studies, but (Saad El-din *et al.*, 2013) reported low growth of 3(6%) and 1 (2%) milk samples from tuberculin positive and negative reactors. But the present study is higher compared to the study conducted by (Akililu *et al.*, 2014 and Gad *et al.*, 2000) who reported milk culture positivity of 9% and 9.3% respectively. At the same time, the present milk sample culture positivity indicated that it is much lower compared to the report of (Hamid *et al.*, 2003) who recorded 28.07% and 25% culture positivity from milk of tuberculin positive buffaloes and cows respectively.

The questionnaire survey had provided information regarding the knowledge and practices of livestock keepers about zoonotic diseases in the study districts of Tigray region of Ethiopia. Like in most African countries, in Ethiopia, illiteracy is yet another unsolved problem in most rural communities particularly in the study districts. High number of respondents had, therefore, no detailed and accurate knowledge about tuberculosis and its zoonotic importance as indicated in this study 84.17% of the households who don't know that BTB affected cattle. At the same time, 80.83% of the households didn't know BTB is zoonotic that can be transmitted by consumption of raw milk obtained from BTB animals. The current assessment of the knowledge of the society on BTB is in agreement with the findings of (Mahmmed *et al.*, 2012 ; Sisay *et*

al.,2013 ; Fikre *et al.*,2014 and Akililu *et al.*,2014) who reported that, 81.8% , 72.2% , 70% and 79.3% , respectively. Close physical contact b/n owner and cattle and the consumption of raw milk products facilitate the transmission of BTB (WHO, 1993; Cosivi *et al.*, 1998).

6. CONCLUSION AND RECOMMENDATIONS

This study using comparative intradermal tuberculin test and culture of milk suggests that BTB in intensive dairy farms among different breeds, age, production systems and body condition of animals in Adigrat district is very common which requires urgent intervention to control the disease. Furthermore, the economic implications in terms of trade restrictions and productivity losses have direct and indirect implications for human health and the food supply. The results of this study revealed that the livestock owning community members from the study districts had low knowledge of the cause, source of infection and the mode of transmission of tuberculosis infection. Therefore the following points are recommended

- ❖ Boiling of milk before consumption should be practice by the society.
- ❖ Animals should be tested and certified before introducing to new farm by the veterinary clinician annual two times To comprehensively control BTB individual animal and herd prevalence should be identified.
- ❖ Public sensitization on the means of transmission, its risk, and handling of tuberculin reactor and risk of consumption raw milk should be created by the public health office and the veterinarian of the district jointly.
- ❖ Disease animal movement restriction should be practiced by the regional bureau of agriculture to prevent the spread of the disease to new farm.
- ❖ Policy maker should be introduce test and slaughter policy for Eradication of the disease.

7. REFERENCES

- Akililu, B., Gobena, A., Teshale, S., Faanta, D., Akafate T. and Ketema, T.(2014): Epidemiology and public health significance of Bovine tuberculosis in and around Suluta District central Ethiopia *African J. of Micro. Res.* Vol. **8**(24), pp. 2352-2358,
- Amanfu,W. (2006): The situation of tuberculosis and tuberculosis control economic interest, *Tuberculosis*, **86**:330–335.
- Ameni, G.,Amenu, K. and Tibbo, M.(2002):Bovine tuberculosis prevalence and risk factor assessment in cattle and cattle owners in Wuchala-Jida District Central Ethiopia *Int.J.appl.Res.vet.Med* **1**(1);17-26
- Ameni,G. and Wudie, A.(2003): preliminary study on bovine tuberculosis in Nazreth municipality abattoir of central Ethiopia *.Bull. of ani. health and prod. for Africa* **51**:125-132.
- Ameni, G., Regassa, A., Kassa, T. and Medhin, G. (2001): Survey on bovine tuberculosis and its public implications to cattle raising families in Wolaita-Soddo, Southern Ethiopia. *Ethiopian J. of Ani. Prod.*, **1**: 55–62.
- Ameni,G. and Erkihun, A. (2007): Bovine tuberculosis on small-scale dairy farms in Adama Town,central Ethiopia and farmer awareness of the disease. *Rev. Sci. Tech. Off. Int. Epiz;* **26**(3): 711-719.
- Anaelom, N., Ikechukwul, O., Soday,E. and Nnaemek, U. (2010): Zoonotic Tuberculosis: A review of Epidemiology, clinical presentation, prevention and control. *J. of Pub. Health and Epid.***2**: 118-124.
- Ashenafi,D., Mamo, G., Ameni, G. and Simenew K (2013): Epidemiology andMolecular characterization of causative agents of bovine tuberculosis in ruminants.*J. of Bact. and Para.* **4** (1): 161-167.

- Ashford D.A., Whitney, E., Raghunathan, P. and Cosivi, (2001): Epidemiology of selected mycobacteria that infect humans and other animals. *Rev.Sci.Tech. – Office Inter.des Epiz.* **20**, 325-337.
- Asseged, B., Liibke-becker, A., Lemma, E., Taddele, K., Britton, S., (2000): Bovine Tuberculosis: A cross-sectional and Epidemiological study in and around Addis Ababa. *Bull.of Ani. Heal. and Prod. in Africa.* **48**: 71-80.
- Asseged, B., Woldesenbet, Z., Yimer, E. and Lemma, E. (2004): Evaluation of Abattoir Inspection for the diagnosis of *Mycobacterium bovis* infection in cattle at Addis Ababa abattoir. *Trop. Ani.Heal. Prod.*, **36**: 537-546.
- Ayele, W., Neill, S., Zinsstag, J., Weiss, M. and Pavlik, I. (2004): Bovine tuberculosis, an old disease but new threat to Africa. *Inter. J. of tuberculosis Lung Dis.*, **8**: 924-937.
- Awah-Ndukum J, Kudi AC, Bradley G, Ane-Anyangwe I, Titanji VPK, Fon-Tebug S and Tchoumboue J (2012): Prevalence of bovine tuberculosis in cattle in the highlands of Cameroon based on the detection of lesions in slaughtered cattle and tuberculin skin tests of live cattle. *Vet. Med.* **57**(2): 59–76.
- Biberstein, E. and Hirsh, D. (1999): *Mycobacterium* species: The agents of animal Tuberculosis. In. Massachusetts, USA. Pp. 158-164.
- Brosch, R., Gordon, S. and Marmiesse, M. (2002): A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proceeding Natural Academy of Science.* **99**: 3684-3689.
- Carter, G. and Chengapa, M. (1991): The *Mycobacterium*. In: Essential of veterinary Bacteriology and Mycology, 4th ed. Philadelphia. Lea and Febiger.Pp: 202-216
- Central Statistical Authority (CSA). (2010): Agricultural Sample Survey, statistical bulletin, CSA, Addis Ababa, Ethiopia; **193** (2):124-127.
- Cole, S. (2002): Comparative and functional genomics of the *Mycobacterium tuberculosis* complex. *Micro.* **148**: 2919-2928.
- Cosivi, O., Grange, J. M., Daborn, C. J., Raviglione, M. C., Fujikura, T., Cousins, D., Robinson, R. A., Huchzermeyer, H. F., De kantor, I. and Meslin, F. X. (1998): Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. *Emer. Infec. Dis.* **4**, 59- 70.

- Cook, A., Tuchill, L. and Buve, A. (1996): Human and Bovine Tuberculosis in the Monze District of Zambia: A cross sectional study. *Brit. Vet. J.* **152**: 37-46.
- De la Rúa-Domenech, R. (2006): Human Mycobacterium bovis infection in the United Kingdom: Incidence, risks, control measures and review of the zoonotic aspects of bovine tuberculosis. *Tuberculosis (Edinb)* **86**: 77–109.
- Dlugovitzky, D., Bay, M., Rateni, L., Fiorenza, G., Vietti, L., Farroni, M. and Battasso, O. (2000): Influence of disease survey on nitrate and cytokine production by peripheral blood mononuclear cells (PBMC) from patients with pulmonary tuberculosis (TB). *Clin. and Exp. Imm.*, **122**: 343 – 349.
- Doherty, M., Basset, H., Quinn, P., Davis, W., Kelly, A. and Monaghan, M. (1996): A sequential study of the bovine tuberculin reaction. *Immunology*, **87**: 9-14.
- Elias, K., Hussein, D., Asseged, B., Wondwossen, T. and Gebeyehu, M. (2008): Status of bovine tuberculosis in Addis Ababa Dairy farms. *Rev. of sci.and techn., Office Inter. des Epiz.*, **27**: 915-923.
- Fikre , Z., Gebremedhin, R., Gebretsadik, B., Gezahegne. M., Tesfaye, S. and Gobena A.(2014).Prevalence of bovine tuberculosis and assessment of Cattle owners' awareness on its public health implication in and around Mekelle, Northern Ethiopia . *J. of Vet. Med. and Ani. Heal.*Vol. **6**(6), pp. 159-167, June 2014.
- Fitzhugh, H., Delgado, C.(2000). Livestock: The next food revolution. In: Synergies in Science. Intercollaboration to Eradicate Hunger and Poverty.CGIAR,Washington DC. USA Pp. 30- 31.
- Food Safety Authority of Ireland (FSAI) (2008).Zoonotic tuberculosis and food safety , 2nd. Ed.Lower Abbey Street Dublin I, Ireland. Pp. 7-28.
- Gad, EWA., El-Abeey,A.,Mettias,KN.andManal,A.(2000): The present state and public health importance of tuberculosis of bovine udder.*J.Egypt.vet.Med.Ass.***60**(6);189-194

- Garnier, T., Eiglmeier, K., Camus, J., Medina, N., Mansoor, H, Pryor, M. and Duthoy, S. (2003): The complete genome sequence of *Mycobacterium bovis*. *Proc. of Nat.of Acad.Scin.***100**: 7877-7882.
- Gebremedhin, R., Gobena, A., Gebretsadik, B. and Gezahegne M. (2013).Epidemiology of mycobacterial infections in cattle in two districts of Western Tigray Zone, northern Ethiopia..*African Journal of Microbiology Research* Vol. **7**(31), pp. 4031-4038, 2 August, 2013.
- Glickman, M. and Jacobs, W. (2001): Microbial pathogenesis of *Mycobacterium tuberculosis*: dawn of a discipline. *Cell*, **104**: 477-485.
- Grange JM. (2001): *Mycobacterium bovis* infection in human beings. *Tuberculosis (Edinb)* **81**: 71-77.
- Groenen, P., Bunschoten, A., van Soolingen, D. and van Embden, J. (1993): Nature of DNA polymorphism in the direct repeat cluster of *Mycobacterium tuberculosis*: application for strain differentiation by a novel typing method. *Mol. Micr.*, **100**: 1057-1065.
- Gupte, S. (2006): The short text book of Microbiology. 9thed. JapeeBrathersMedical Publishers Ltd. New Dehi, India, Pp.509.
- Hadded, N., Masslot, M. and Durand, B. (2004): Molecular differentiation of *Mycobacterium* isolates. Review of main techniques and applications. *Res. in Vet. Sci.*, **76**:1-18.
- Hamid, J.,Puran, D.and Asif, S.(2003):Bovine tuberculosis in dairy animals at Lahora , threat to public health. <http://priory.com./vet/bovine.tb.htm/>.
- Indrigo, J., Hunter, R. and Actor, J. (2002): Influence of trehalose 6,6'-dimycolate (TDM) during *Mycobacterial* infection of bone marrow macrophages. *Micr.*,**148**: 1991- 1998.
- Kazwala, R., Kambarage, D., Daborn, C., Nyange, J., Jiwa, S. and Sharp, J. (2001): Risk factors associated with the occurrence of bovine tuberculosis in cattle in the Southern Highlands of Tanzania. *Vet. Res. Comm.*,**25**: 609-614.

- Kidane, D., Olobo, JO., Habte, A., Negesse, Y., Aseffs, A., Abate, G., Yassin, MA., Betreda, K., Harboe, M.(2002): Identification of the causative organism of tuberculosis lymphadenitis in Ethiopia by PCR. *J.of Clin. Micr.*, **40** (6), 4230-4234.
- Kleeberg.H.H.(1984):Human tuberculosis of bovine origin in relation to public health *Rev.scient.et.Tech.office intern.des Epiz.***3**:11-32.
- Kubica, T., Agzamova, R., Wright, A., Rakishev, G., Rusch-Gerdes, S. and Niemann, S. (2006): *Mycobacterium bovis* isolates with *M. tuberculosis* specific characteristics. *Eme. Infec. Dis.*, **12**: 763-765.
- Menzies, F. and Neill, S. (2000): Cattle to cattle transmission of bovine tuberculosis. *Vet. J.*, **160**: 92-106.
- Meisinger, G. (1970): Economic effects of the elimination of bovine tuberculosis on the productivity of cattle herds. 2. Effect on meat production. *Mon.Vet.Med* **25**:7–13.
- Mohammed, N., Hailu, M.andGebreyesus, M. (2012): Prevalence and zoonotic implications of bovine tuberculosis in Northwest EthiopiaAndassa Livestock Research Center, P. O. Box 27 Bahir Dar, Ethiopia. *Inter. J. of Med. and Med. Sci. ISSN: 2167-0447 Vol. 2 (9), pp.188-192, September, 2012.*
- Mostowy, S., Inwald, J., Gordon, S., Martin, C., Warren, R., Kremer, K., Cousins, D. and Behr, M. (2005): Revisiting the Evolution of *Mycobacterium bovis*. *J. of Bact.*, **187**:6386-6395.
- Neill, S., Pollock, J., Bryson, D. and Hanna, J. (1994): Pathogenesis of *Mycobacterium bovis* infection in cattle. *Vet. Micro.*, **40**: 41-52.
- Niemann, S., Harmsen, D., Ruschgerdes, S. and Richter, E. (2000): Differentiation of *Mycobacterium tuberculosis* complex isolates by gyrB DNA sequence polymorphism analysis. *J. of clini. Micro.*, **38**: 3231-3234
- OIE, (2009): Bovine tuberculosis.Terrestrial manual: Office International des-Epizootie.
- OIE,(2010): Bovine tuberculosis In Manual of Diagnostic Tests and Vaccines for Terrestrial

- Animals. Part 2, Section 2.3. Chapter 2.3.3. World Organization for Animal Health.
- Omer, M.K., Skjerve, E., Woldehiwet, Z., Holstad, G. (2001): A cross-sectional study of bovine tuberculosis in dairy farms in Asmara, Eritrea, *Trop. Anim. Health Prod* **33**:295–303.
- Orme, I. and Cooper, A. (1999): Cytokine chemokine cascades in immunity to tuberculosis. *Immunology Today*, **20**: 307–312.
- Palomino, J., le'ao, S. and Ritacco, V. (2007): Tuberculosis in 2007 from basic science to patients care. 1sted. www. Tuberculosis Textbook.com. Pp. 687.
- Pollock, J. and Neill, S. (2002): *Mycobacterium bovis* infection and tuberculosis in cattle. *The Vet. J.*, **163**: 115-127.
- Quinen, P.; cartel, M., Malkey, B. and cartel, G. (2004): *Mycobacterium* species. In: Clinical Veterinary Microbiology. 5thed. Mosby International Limited, London, Great Britain. Pp. 156-169.
- Radostits, DM., Blood, DC., Gay, CC.(2006): Veterinary Medicine. A text book of Disease of Cattle, sheep, Pigs, goats and horses, 8th ed. Bailliere Tindal, London,. p. 748-785.
- Rainy, F., Klattle, S., Krappenstedf, R. and Stackebrandet, E. (1995): Ditzia, a new genus including Ditziamalisco. nov. Formerly *Rhod.* *Intern. J. of system. Bact.*, **45**:32-36.
- Saad El-din, N., ,Nagah, M. , Saad, N. and Nahed , M.(2013): Detection of bovine tuberculosis in milk and serum oftuberculin reactors dairy farm animals in AssiutCity,Egypt. Food Hygiene Department, Assuit University Egypt. *Bas. Res. J. of Ani. Scie. Vol. 1(1) pp. 01-06 October 2013.*
- Seifert, H. (1996): Tropical Animal Health. 2nded. Kluwer Academic Publishers, Dordrecht, the Netherlands. Pp. 343-352.
- Sharma, S. and Adlakam, S. (1996): Text book of Veterinary Microbiology. Vikas publishing house Pvt.Ltd, Newdelhi, India. Pp. 150-156.
- Shitaye JE, Getahun B, Alemayehu T, Skoric M, Treml F, Fictum P, Vrbas V, Pavlik I (2007):

- A prevalence study of bovine tuberculosis by using abattoir meat inspection and tuberculin skin testing data, histopathological and IS6110 PCR examination of tissues with tuberculosis lesions in cattle in Ethiopia. *J.Vet. Med.***51**: 512-522.
- Sisay, W., Daniel, H., Atsedo, G., Yibrah, B. (2014): Detection of Human and Bovine Tuberculosis Using an Existing Diagnostic Practice in Residential Districts of Tigray Region, Northern Ethiopia a *J. Environ OccupSci***2(2)**:77-88.
- Tauro, P., Kapoor, k. and Yadav, K. (1996): An Introduction to Microbiology Viley Eastern limited, Newdelhi, India. p. 138.
- Thoen, C.O. and Chiodin, R. (1993): *Mycobacterium*. In: Edited by Gyles, C.L. and Thoiien, C.O. Pathogenesis of bacterial infection in animals. 2nd ed. Ames: Iowa State University press. Pp. 44-46.
- Thoen, C. and Barletta, R. (2006): Pathogenesis of *Mycobacterium bovis* in animals. Editors: Thoen, C.O., Steel, J.H. and Glisdorf, M.J. 2nd ed. Blackwell Publishing. Pp. 18-33.
- Thom, M., Hope, J., McAulay, M., Villarreal- Ramos, B., Coffey, T., Stephens, S., Vordermeier, H. and Howard, C. (2006): The effect of tuberculin testing on the development of cell-mediated immune responses during *Mycobacterium bovis* infection. *Vet. Imm. and Immunopath.*, **114**: 25-36.
- Thrusfield M. (2007): *VeterinaryEpidemiology*. 2nd ed. London: Blackwell Science Ltd.;p. 178-198.
- Tschopp R, Aseffa A, Schelling E, Zinsstag J (2010): Farmers' perceptions of livestock, agriculture, and natural resourses in the rural Ethiopian highlands. *Moun. Res. and Dev.(MRD)* **30**: 381–390.
- Tyhra C, Kwadwo A. (2011): Responding to Land Degradation in the Highlands of Tigray. Van Arendonk J.A.M., Liinamo A.E., (2003) Dairy cattle production in Europe, *Therio.* **59** 563–569.
- Vestal AL. (1977): In: Procedures for isolation and identification of *Mycobacteria*, U.S. Dep. of Health, Educ. and Welf. CDC Atlanta, Georgia, Publication (77-8230): 15-19.

- Vincent, V., Frebault, L. and Portaels, F. (1992): Proposed minimal standards for the genus *Mycobacterium* and for the description of new slowly growing *Mycobacterium* Species. *Intern.J. of System. Bact.*, **42**: 315-323.
- Welsh, M., Cunningham, R., Corbett, D., Girvin, R., McNair, J., Skuce, R., Bryson, D. and Pollock, J. (2005): Influence of pathological progression on the balance between cellular and humoral immune responses in bovine tuberculosis. *Immu.*, **114**: 101–111.
- WHO, (1993): Report of the WHO meeting on zoonotic tuberculosis (*Mycobacterium bovis*, Geneva, 15 November 1993) with the participation of the Food and Agriculture Organization (FAO). WHO/CDS/VPH/93.130. WHO/FAO, Geneva.
- WHO, (1998): Laboratory services in tuberculosis control. Part II and III. In: Global Tuberculosis Programme, Global Tuberculosis Control. WHO Report 1998. Geneva, Switzerland, WHO/TB/98:237:1-95.
- WHO, (2011): Global tuberculosis control: World Health Organisation report 2011. Pp. 1–258.
- Wolinsky, E. (1979): Non tuberculous *Mycobacteria* and associated diseases. *Ame. Rev. on Resp. Dis.* **119**: 107-59.

8. ANNEXES

Annex 1: Questionnaire Survey

Questionnaire survey was used in the study of “detection of Bovine Tuberculosis in Dairy cattle in Northern Ethiopia: Implications for the small holder Dairy farmers”. This questionnaire has three parts dealing with (A) general information about the farms and their owners, (B) possible risk factors related to bovine tuberculosis, and (C) public health risks and awareness.

Survey performed by Mekelle University

Questionnaire number: _____

Date of interview: ____/____/_____

Interview performed by: _____

Answering the questions is only dependent on your good will and can be withdrawn at any time during the interview. Please answer the questions in absolute number/text or mark the number of the correct option(s)

A. Questions about the farm and its owner

1. General information

a. Dairy farm name: _____

b. Name of the dairy farm owners (or animal attendant working in the farm not less than one year): _____

c. Address: Region/city _____ District/Subcity _____ Kebele _____

d. Age (years): |__|__|

B. Questions related to possible risk factors for bovine tuberculosis

2. Type of house/barn: |__|

1. Indoor 2. Outdoor

3. None, but fenced 4. Cattle share house with the owners

3. Sanitary condition of the barn/house based on odors, waste drainage, cleanness of floor and animals, light source, and animal stocking: |__|

1. Poor 2. Medium (satisfactory condition) 3. Excellent

4. Ventilation status of the barn/house: |__|

1. Poor 2. Medium (satisfactory ventilation) 3. Excellent

5. The purpose of the Dairy farm: |__|

1. To produce dairy products for home consumption only

2. To produce dairy products for market only

3. To produce dairy products for market and home consumption

6. What is the total milk production on your farm per year? |__|__|__|__|__|__| Liters/year

7. What is the average milk production per cattle per year?

|__|__|__|__|__| Liters/year

8. To whom do you sell the milk (multiple options possible)?

1. To individual consumers 1. Yes |__| 2. No |__|

2. To processing plant 1. Yes |__| 2. No |__|

3. To intermediate cater 1. Yes |__| 2. No |__|

4. To restaurants/cafeteria 1. Yes 2. No
5. Other, 1. Yes 2. No specify: _____
9. How do you get replacement stock (multiple options possible)?
1. My own farm by Artificial Insemination
 2. Insemination by own bull
 3. Purchasing from different cattle sources
 4. Other, specify: _____
10. From what area have you purchased cattle during 2014-15 (specify farm and woreda)?
-
11. To what area have you sold cattle during 2014-15 (specify farm and woreda)?
-
12. What type of cattle do you sell from your farm (multiple options possible)?
1. Weak / poor body condition 1. Yes 2. No
 2. Diseased 1. Yes 2. No
 3. Low productive 1. Yes 2. No
 4. High productive 1. Yes 2. No
 5. Other, Specify: _____
- 3
13. Are animals in your farm mixed with animals from other farms?
1. Yes 2. No
14. Have you in the last six months, had any animal in your herd with chronic cough/chronic body wastage? 1. Yes 2. No
15. Have cattle been tuberculin/PPD tested before on your farm?
1. Yes 2. No
- If yes, what happened with the cattle tested as positive (multiple options possible)?
1. It remained at the farm 1. Yes 2. No
 2. It was slaughtered 1. Yes 2. No
 3. It was sold 1. Yes 2. No
- C. Questions related to public health risk and awareness
16. Do members of your family/farm drink raw milk regularly (once per month or more)?
1. Yes 2. No
17. Do you know that bovine tuberculosis is a cattle disease?
1. Yes 2. No
18. Do you know that bovine tuberculosis can be transmitted to man through raw milk/milk products consumption obtained from bovine tuberculosis infected cattle?
1. Yes 2. No
19. Do you know that bovine tuberculosis can be transmitted to man through raw meat consumption obtained from bovine tuberculosis infected cattle?
1. Yes 2. No
20. Have any of the people living/working on your farm had tuberculosis in the last two years?
1. Yes 2. No
- If anyone has had tuberculosis on your farm, did he/she drink raw milk/milk products?
1. Yes 2. No

Annex-2: Body condition scoring and age determination

Body condition score1: The individual spinous processes are sharp to touch and easily distinguishable.

Bodyconditionscore2: spinous processes can be identified individually when touched but feel round rather than sharp.

Bodyconditionscore3: spinous processes can only felt with very firm pressure and area of either side of tail head have come fat cover.

Body condition score 4: Fat cover around tail head is easily seen as mounds, soft to touch, the spinous process cannot be felt.

Body condition score 5. The bone structure of animal is no longer noticeable and the tail head is almost completely buried in fatty tissue.

The body condition of animals was classified as poor medium and good.

Poor=body condition score 1and 2

Medium =body condition score 3

Good =body condition score 4and 5

Source: - Body condition scoring in cattle: Nicholson and Butterworth, (1986)

Annex-3: Age determination based on dental level

Year characteristics changes

1.5 -2 Incisor I erupts

2 – 2.5 Incisor II erupts

3 Incisor III erupts

3.5 Incisor IV erupts

5 All incisors are wearing

6 Incisor I is level and has emerged from the gum

7 Incisor II is level and the neck is visible

8 Incisor III is level and the neck is visible and incisor IV may be level

9 Incisor IV is level and the neck is visible

10 The dental star is square in incisor I

15 The teeth that have not fallen are reduced to small round pegs.

Source: De- Lahunta and Hable, (1986)

Annex 4: CIDT test FORMAT.

Name of Kebele:

I. D	Owner's name	Age	Breed	BC S	Mgt System	Herd size	Sign Of coughing	A 1	B 1	A2	B 2	Δ A	Δ B	Δ B- Δ A	Result

Notice: BSC=body condition score, A1=avian before injection, A2=avian after injection, B1=bovine before injection, B2=bovine after injection, Δ A=A2-A1, Δ B=B2-B1

Annex 5: Result of PPD after 72hr negative (A) and positive (B)

A. Negative for BTB.



B. Positive for BTB



STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material 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 Mekelle University, College of Veterinary Medicine 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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