EPIDEMIOLOGY OF TUBERCULOSIS AND MOLECULAR CHARACTERIZATION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX SPECIES IN GOATS AND THEIR OWNERS IN ADAMI TULU JIDO KOMBOLCHA DISTRICT, EAST SHEWA ZONE OF OROMIA

Msc Thesis

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Masters Program in Tropical Veterinary Public Health

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College of Veterinary Medicine and Agriculture, Bishoftu

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A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical Veterinary Public Health

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by: Chala Feyera Entitled: Epidemiology of Tuberculosis and Molecular Characterization of *Mycobacterium tuberculosis* Complex Species in Goats and Their Owners in Adami Tulu Jido Kombolcha District, East Shewa Zone of Oromia and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Science in Tropical Veterinary Public Health.

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STATEMENT OF AUTHOR

First, I declare that this thesis is my original 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 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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ACRONYMS

AFB	Acid-Fast Bacilli
ATJK	Adami Tulu Jido Kombolcha
ALIPB	Aklilu Lemma Institute of Pathobiology
BCG	Bacillus Calmette–Guérin
BTB	Bovine Tuberculosis
CDC	Center for Disease Control
CIDT	Comparative Intradermal Tuberculin
DNA	Deoxyribonucleic Acid
DR	Direct Repeat
ECDC	European Union for Disease Control and Prevention
EFSA	European Food Safety Authority
ESGPIP	Ethiopian Sheep and Goat Production Improvement program
EU	European Union
FEDO	Finance and Economic Development Office
FMOH	Federal Ministry of Health
HIV/AIDS	Human Immune Deficiency Virus/Acquired Immune Deficiency Syndrom
ILRI	International Livestock Research Institute
IP	Intraperitonial
IV	Intravenous
LA	Livestock Agency
LJ	Lowenstein-Jensen
MTBC	*Mycobacterium tuberculosis* Complex
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
PPD	Purified Protein Derivative
PZA	Pyrazinamide
SIDT	Single Intradermal Tuberculin
TB	Tuberculosis
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

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ABSTRACT

Cross-sectional and case-control studies were conducted from November, 2013 to July, 2014 to investigate epidemiology of tuberculosis (TB) and perform molecular characterization of *Mycobacterium tuberculosis* complex (MBC) species in goats and their owners in Adami Tulu Jido Kombolcha (ATJK) district by comparative intradermal tuberculin (CIDT) test, bacteriological and postmortem examination and molecular characterization techniques. A cross-sectional study was conducted on a total of 97 flocks comprising 527 goats from rural and urban villages by CIDT test and flock prevalence was 15.5% (95% CI: 5.7-19.0) at ≥2mm cut-off points and 12.4% (95% CI: 8.1-22.8) at ≥4mm cut-off points while animal level prevalence was 4.6 % (95% CI: 2.8-6.3) at ≥2mm cut-off points and 3.8% (95% CI: 2.2-5.4) at ≥4mm cut-off points. None of animal characteristics considered were associated with CIDT positivity of goats (P>0.05). A case-control study was conducted on a total of 70 flocks comprising 283 goats by CIDT test and 31.6% of flocks owned by TB positive cases and 15.6% of flocks owned by TB free controls were positive to bovine tuberculin test at ≥2mm cut-off. The overall flock prevalence was 24.3% (95% CI: 14.0-34.6) at ≥2mm and 20% (95% CI: 10.4-29.6) at ≥4mm cut-off points. At both cut-off points the CIDT positive flock proportion was not statistically significant (P>0.05). However, 14.4% of goats owned by TB positive cases and 4.1 % of goats owned by TB free controls were positive to CIDT test. The overall animal level prevalence was 9.9% (95% CI: 6.4-13.4) at ≥2mm a cut-off while it was 8.8% (95% CI: 5.5-12.2) at ≥4mm cut-off. Animal prevalence was statistically significant P=0.004 at both cut-off points. The risk of acquiring caprine TB infection of goats being owned by TB positive cases was 0.40 times higher than being owned by TB free controls at both cut-off points. 38 specimens of human sputum and 7 tuberculous tissue lesions obtained from CIDT test positive goats were cultured and 31.6% human sputum and 14.3% of tuberculous tissue lesions showed growths on Lowenstein-Jensen (LJ) media. Genus typing of goat isolates using multiplex polymerase chain reaction (m-PCR) revealed a product characteristic to genus Mycobacterium. Furthermore, 91.7% of the 12 heat-killed positive human isolates had intact direct repeat (RD) 9 locus up on deletion typing for RD9, and subsequently classified as *M. tuberculosis.* Both human and goat isolates were spoligotyped and two lineages; Euro-American (E-A) and Indo-Oceanic (I-O) lineages were recognized from human isolates. The most common patterns were Spoligo International Type Number (SIT) 53 consisting of 3 isolates accounting for 30% of the isolates. SIT44, SIT54 SIT523 were among the recognized patterns in the present finding. The patterns of four strains were not recognized by SpolDB3, the international spoligotyping database indicating that they could be new strains. The spoligotyping result of goat isolate was new to Mbovis.org strain typing database. Findings from this study add useful epidemiological data regarding human and caprine TB infection at ATJK district. Therefore, further studies must be designed to get actual profile of the disease. Diagnosis of TB should be done to the extent of strain typing so as to establish lineages particular to the region which provides easy implementation of treatment and control strategy.

Key words: Tuberculosis, Adami Tulu, Human, Goats, Prevalence, Transmission, Strain typing.

1. INTRODUCTION

Despite the existence of anti-TB drugs for the last 60 years, TB continues to be a major threat worldwide. In 2009, WHO estimated the global incidence of TB with 9.4 million cases. Most of the estimated number of TB cases occurred in Asia (55%) and Africa (30%). The 22 high burden TB countries account for 81% of all estimated cases worldwide (WHO, 2010). Ethiopia ranks 3rd in Africa and seventh among the world's 22 high burden TB countries. The country had 314,267 TB cases in 2007, with an estimated incidence rate of 378 cases per 100,000 populations (WHO, 2009). According to the Ministry of Health hospital statistics data, TB is one of the leading causes of morbidity, the fourth most common cause of hospital admission, and the second most common cause of hospital death in Ethiopia (FMOH, 2009).

TB is a term that encompasses various diseases caused by bacteria of the *M. tuberculosis* complex (MTBC) of which, *M. tuberculosis* and *M. bovis* are highly pathogenic and clinically similar to each other, with humans and cattle being their natural hosts, respectively. Zoonotic TB is a recognized public health threat in the developing world occurring by the aerogenous route, ingestion of infected milk or less frequently, by contact with mucous membranes and broken skin (WHO, 1994). Pigs are most susceptible to *M. bovis*, with sheep and horses showing a higher natural resistance (Radostits *et al*., 2000; Thoen *et al*., 2006).

Caprine TB is caused by *M. bovis* and *M. caprae*, member of the MTBC, causing disease in a wide range of mammals, including humans (Aranaz *et al.,* 1999). Caprine TB, therefore, poses a risk to goat health and production and a public health threat and considerable economic losses due to animal production losses and abattoir condemnation of infected carcasses (Aranaz *et al.,* 1999). Recently, reports of caprine TB have increased in several countries; even in those practicing a long standing test and slaughter policy (Sharpe *et al.,* 2010). *M. caprae* has been known to infect humans of older age and livestock (Kubica *et al.,* 2003; Erler *et al.,* 2004).

In Ethiopia, mixed farming of cattle and goats is a common practice which poses a high risk of inter-and intra species transmission and spread of *M. bovis* infection (Ameni *et al.*, 2010). This mixed farming of small and large ruminants is especially a risk to goats in countries like Ethiopia where bovine TB (BTB) is endemic (Hailemariam, 1975).

Different studies have reported is8olation of *M. tuberculosis* from cattle (Ameni *et al.*, 2013), camel (Gumi *et al.*, 2012) and goat (Hiko and Agga, 2011; Mamo *et al.*, 2012; Deresa *et al*., 2013) in Ethiopia. The latter studies highlighted the potential transmission of *M. tuberculosis* from human to goat. The source of *M. tuberculosis* infection in animals is most frequently considered to be active TB patients expelling *M. tuberculosis* through sputum primarily, less often through aerosol, urine or feces (Berg *et al*., 2009). Smaller studies have reported isolation of *M. bovis* from humans (Kidane *et al*., 2002), indicating the transmission of *M. bovis* from cattle to human. The primary sources of infection for humans are consumption of unpasteurized milk and prolonged close association between humans and animals (Cosivi *et al.,* 1998). Isolation of *M. bovis* has also been made from goats, many other livestock and wildlife species (Ayele *et al*., 2004; OIE, 2009). However, there is no study indicating isolation of neither *M. bovis* nor *M. caprae* from both goat and human in Ethiopia.

Statement of the problem/rationale/

Although no estimates have been given for the TB burden at the national level in Ethiopia, evidence of caprine TB generally detected on postmortem findings during slaughter suggests that the disease burden is high. In the 1996, reports from the ILRI showed that prevalence of TB in Ethiopian goats was 4.2% and study conducted on 1990 randomly selected male goats that were slaughtered at Luna Export abattoir of central Ethiopia using post mortem examination, mycobacterial culturing and molecular typing techniques also showed 3.5% prevalence of caprine TB (Deresa *et al.*, 2013).

Despite the increasing report on the occurrence of caprine TB in different regions of Ethiopia (Hiko and Agga, 2011; ILRI, 1996; Nigussie, 2005; Tafese *et al.*, 2011; Mamo *et al.*, 2012; Deresa *et al.*, 2013), there is little information on the epidemiology, transmission pattern and species of MTBC infecting goats and their owners in ATJK district.

ATJK district is found in mid-Rift valley where there is scarcity of wood for sufficient shelter construction and those people who own goats share the same night shelter with flock of goats. Usually they protect their animals from predators and thefts by keeping their animals either in common night shelter or in very close proximity to their houses and they have close physical contact with their animals. Goats have also common watering and grazing points with cattle and other livestock that might favor the transmission of mycobacterial species among these domestic animals. These conditions are potential risk factors for transmission of zoonotic diseases such as TB of animal origin to human or vice versa. This husbandry practice that is trained by inhabitants of the area was observed previously whereby traditional housing and grazing of natural pasture are the predominant husbandry practices (Tafese *et al*., 2011). On the other hand, the community is highly dependent on animals and animal products for livelihood. Rural inhabitants and some urban dwellers in ATJK district still consume unpasteurized milk mixed with coffee potentially contaminated with TB. Goats are mainly utilized for milk and meat production and generate income to the owners through sale of live goats and their milk in the study area. The community's habit of consumption of raw animal product particularly milk of goats is highly common. However, there is little knowledge about the transmission of the disease through close physical contact and consumption of raw food of animal origin especially in the rural community. Thus, it requires addressing epidemiology of TB and molecular characterization of MTBC species isolated from human and goats in the study area.

The previous study conducted by Tafese *et al*., (2011) indicated the existence of caprine TB in the area. However, it did not show the particular Mycobacterium species attributed for the occurrence of the disease in the area. There is a substantial lack of knowledge on the distribution, epidemiological pattern in goats and zoonotic importance of caprine TB in human population in the country in general and in the study area in particular. As such, fundamental question regarding the molecular and strain aspects of mycobacterial species causing TB infection in goats and human in ATJK district has not yet been addressed. Therefore, the present study was designed to investigate epidemiology of TB and molecular characterization of MTBC species isolated from goats and human in ATJK district.

General objective

This study was designed to determine epidemiology of tuberculosis and perform molecular characterization of *Mycobacterium tuberculosis* complex species in goats and their owners in Adami Tulu Jido Kombolcha District, East Shewa Zone of Oromia.

- **Specific objectives**

This study was formulated specifically

- To investigate epidemiology of caprine TB in ATJK district
- To perform molecular characterization of mycobacterial species isolated from human pulmonary TB patients and tuberculin reactor goats in the study area
- To provide baseline data on the status of caprine TB transmission between goats and human in the area that could be used as a reference in the future.

2. LITERATURE REVIEW

2.1. General characteristics of Mycobacterium

2.1.1. Taxonomic classification

Conventional methods for classification and identification of Mycobacteria include observation of rate of growth, colony morphology, pigmentation and biochemical profiles. The conventional methods require 6-8 weeks for identification. Growth rate separates the rapid growers (growth in less than 7 days) from other Mycobacteria (Brooks *et al.* 2007).

The genus Mycobacterium includes obligate parasites, saprophytes and intermediate forms differing in their nutritional requirement (Tauro *et al.*, 1996). The phylogenetic analysis of the genome of pathogenic mycobacteria has shown that all (except *M. avium*) belong to a single genetic species: the MTBC (Haddad *et al.*, 2004). The cause of caprine TB, *M. caprae*, belongs to the MTBC (Aranaz *et al.*, 1996).

Genetically, all the members of this complex are extremely similar; having 99.9% similarity at the nucleotide level and identical 16S rRNA sequences (Brosch *et al.*, 2002). Members of MTBC includes: *M. tuberculosis* (humans), *M. africanum* (humans; Africa), *M. bovis* (bovines), *M. caprae/M. bovis* subsp. *caprae* (goats), *M. microti* (mice), *M. pinnipedii* (seals) (Vasconcellos *et al.*, 2010).

According to novel taxonomy, *M. bovis* was divided to *M. bovis* subsp. *bovis* and *M. bovis* subsp. *caprae* (Niemann *et al.*, 2002); the currently accepted taxonomy for these two members of MTBC is *M. bovis* and *M. caprae*, respectively (Aranaz *et al.*, 2003). The major phenotype characteristic for these two members is the susceptibility to pyrazinamide (PZA) (Aranaz *et al.*, 1999; Niemann *et al.*, 2000). Though there is still some argument on differentiation of these species using PZA sensitivity (Kubica *et al.*, 2003), some studies have shown that *M. bovis* and *M. caprae* can only be differentiated by molecular biology methods like spoligotyping analysis (Kremer *et al.*, 1999; Erler *et al.*, 2004; Prodinger *et al.*, 2005). Study conducted by Aranaz *et al.*, (2003) showed *M. caprae* to be sensitive to PZA and *M. bovis* showing natural resistance to PZA and this study suggested this technique as additional differentiation tool to molecular techniques.

The scientific classification of *M. caprae is*: Kingdom: Bacteria, Phylum: Actinobacteria, Order: Actinomycetals, Suborder: Corynebacterineae, Family: Mycobacteriaceae, Genus: Mycobacterium, Species: *M. caprae (*Aranaz *et al.*, 2003).

2.1.2. *Morphology and staining characteristics*

Most of the members mycobacteria are slightly curved rods, sometimes branching filamentous or mycelium type growth may occur but usually gets fragmented into rods or coccoid cells (Tauro *et al.*, 1996). They are aerobic, acid fast, rod shaped, non motile, non spore forming bacteria (Kaneene, 2004). Historically, they were considered as unencapsuled organisms but it is now known that pathogenic mycobacteria contain a "capsular structure" that protects them from microbiocidal activities of the macrophages and also contributes to the permeability barrier of the mycobacterial cell envelope. The cell envelope (bacterial cytoplasmic membrane, the cell wall and the mycobacterial capsule) is important to enable mycobacteria to survive and grow intracellularly (Goodchild and Clifton-Hadley, 2001).

Though mycobacteria are cytochemically gram positive, they often resist staining with gram stain (Biberstein and Hirsh, 1999). Thus, they cannot be classified as either gram positive or gram negative bacteria (Brooks *et al.*, 2004). For this reason, special staining techniques like Ziehl-Neelsen staining, which uses acid decolorizing agents, are widely used to stain mycobacteria since the bacteria are resistant to acid treatment. Under light microscope, in smear stained with carbon fuchsin; mycobacteria appear red straight or slightly curved rods. Depending on growth condition and age of culture, mycobacteria may vary in shape from coccobacilli to long rods. (Kent and Kubica, 1985; CLSI, 2008).

2.1.3. Growth and cultural characteristics

Mycobacterium species are acid fast organisms and have relatively slow growth rate. They do not grow outside of the host except in cultured media. They cannot tolerate harsh environments such as prolonged exposure to heat, direct sunlight, and or dry conditions but the organisms can survive longer under cold, dark, and moist condition (Goodchild and Clifton-Hadley, 2001). Some of the distinctive characteristics of mycobacteria like acid fastness (resistance to acid decoloratization), resistance to injury (even for many antibiotics) and extreme hydrophobicity are strongly associated to the waxy coat of the bacteria. These, probably, contributes to the low growth of the bacteria by hindering nutrient intake (Barrera, 2007).

Identification of mycobacterial isolates is carried out by determining cultural and biochemical properties. Mycobacteria do not grow on simple laboratory media, but grow slowly on media containing serum, potato and egg. In contrast to *M. tuberculosis*, poor or no growth of *M. bovis* is observed on media containing more than 1% glycerol. Because they are slow growing, isolation of the bacteria can require 3 to 8 weeks of incubation (Murray *et al*., 1998). Under microscopic observation, it is not possible to differentiate Mycobacterium at species level. However, rough colony growth on solid media, unlike smooth colonies of most non-virulent mycobacteria and serpentine/cording feature of colonies under light microscope gives presumptive distinction of *M. tuberculosis* from other mycobacteria (Kent and Kubica, 1985; CLSI, 2008).

2.1.4. Routes of infection

Animal infection routes

Mycobacteria are transmitted by many different routes, depending on the localization of the foci of infection in animal tissues and organs. Routes of entry can be respiratory, alimentary, genital, cutaneous or congenital. By far, the commonest methods of animal infection are the respiratory and digestive tracts. Genital TB is transmitted when sexual organs of male or female contain tubercles. Infection through skin occurs when the lesion develops in superficial lymph nodes. Kids can acquire the infection congenitally particularly in tubercle metritis (Gracey, 1986). Although respiratory transmission is the most important route of infection in groups of animals that remain in close contact, indirect transmission via feed contamination is another important route (Kaneene, 2004).

In the case of open pulmonary lesions, mycobacteria are normally discharged and disseminated by the erogenous route but, if swallowed, they may also be excreted in the feces. Disease may spread from the initial focus to regional lymph nodes and then further via the lymphatic and erogenous routes, resulting in generalized tuberculosis. Dissemination can follow, through excretion in feces, urine, semen, uterine discharges or milk, in addition to respiratory spread. If peripheral lymphatics become involved, fistulae may develop and mycobacteria may be discharged (Cosivi *et al*., 1995).

For oral transmission to be accomplished an uninfected animal has to consume feed or water contaminated with mucous or nasal secretions, feces, or urine that contain the infective organisms or receive milk from an infected dam; therefore, *M. bovis* must be able to survive outside an infected host for sufficient time to be ingested by another animal (Kaneene, 2004).

Human infection routes

M. tuberculosis is transmitted predominantly from human to human via droplet respiratory infection when an infected individual coughs or sneezes (Roberts and Buikstra, 2003). The human-to-human transmission of tuberculosis is reliant on the existence of groups of individuals living in close proximity to one another (Taylor *et al*., 2007).

The main routes of *M. bovis* transmission from infected animals to humans are believed to be through ingestion of raw milk and/or inhalation of aerosol from diseased animal, mainly in settings where pasteurization of milk is not widely established. Contact with infected animals is another source of *M. bovis* infection for humans and is a recognized hazard for abattoir workers, veterinarians, and livestock handlers (Grange, 1995). Among such workers who developed the disease, aerosol transmission was considered the most likely route of infection, but there are many occasions on which infection had been spread via cuts and abrasions (e. g, butcher's wart) (Grange, 1994a).

2.1.5. Pathogenesis and lesion development

M. bovis infection first results in the formation of a primary focus, which is usually located in the lungs. In mammals, lymphatic drainage from the primary focus leads to the formation of caseous lesions in an adjacent lymph node; this lymph node lesion, together with the primary focus, is known as the primary complex. This primary complex seldom heals in animals. Results of experimental investigations (Thoen and Garcia-Marin, 2002) involving exposure of animals to *M. bovis* via IV, intratracheal, and IP injection and via the oral route have indicated that the nature and extent of the resultant disease vary with the route of exposure. In cattle and other animals, aerosol spread of tubercle bacilli frequently leads to involvement of lungs and thoracic lymph nodes, whereas exposure by ingestion of contaminated food and water often results in primary foci in lymph tissues associated with the intestinal tract (Thoen and Garcia-Marin, 2002).

At sites of localization of the organisms, granulomas form and develop into tumor-like masses called tubercles in advanced cases (Thoen and Barletta, 2004). Because of the continued growth of the organisms, these tubercles often enlarge to a considerable size. Large masses may develop on the serous membranes of the body cavities. As the granulomas increase in size, necrosis of their central portions may occur. Finally, these central portions are reduced to caseous masses, which have a tendency to undergo mineralization or liquefaction. In mammals, tubercles may become enclosed in dense fibrous tissue and the disease becomes arrested. Advanced lesions associated with clinical disease include caseous nodules or cavities with liquefaction. Bacilli are transferred from the primary foci via lymph and blood vessels; they lodge in other organs and tissues, thereby establishing sites of additional tubercles. When the bloodstream is invaded by numerous tubercle bacilli from a local lesion, many tubercles develop in the major organs (such as the lungs). The acute form of generalized infection (known as miliary tuberculosis) is often rapidly fatal. If small numbers of bacilli enter the circulation from the primary complex, a few isolated lesions develop in other organs; these widely distributed lesions may become encapsulated and remain small for extended periods, usually causing no detectable clinical signs of disease (Thoen and Garcia-Marin, 2002). The progression of the disease from early infection of macrophages to the development of caseous nodules that undergo calcification and liquefaction, as well as the regression, progression, or generalized spread of lesions, depends on the interrelation of the immune response of the host and the proliferation of the bacilli in macrophages (Thoen and Bloom, 1995; Thoen and Barletta, 2004). Results of some experimental studies indicate that the strain of the organism, dose of the organism, route of inoculation, and prevailing conditions for growth of the organism may influence the time required to produce disease (Kaneene, 2004).

Caprine and BTB are closely related in regard to the immune response and pathological characteristics. In natural infections, TB in goats, as in cattle, is primarily a lower respiratory tract disease, with lesions in the lungs and associated lymph nodes. Goats affected with TB initially show dry coughing, progressive emaciation, occasionally diarrhea and death. Post mortem examination of goats infected with *M. bovis* frequently reveal circumscribed pale yellow, white caseous lesions of various sizes, often encapsulated, especially in the lungs and mediastinal lymph nodes or in the mesenteric lymph nodes (Crawshaw *et al.,* 2008). Occasionally, tuberculous lesions may also be found in the upper respiratory tract lymph nodes and other organs like the spleen, or liver (Daniel *et al.*, 2009). Histologically, the lesions are similar to those observed in cattle and humans. Typical tuberculous granulomatous necrotizing lesions are observed characterized by central caseous necrosis, often with some mineralization, surrounded by macrophages, foamy macrophages, numerous giant cells, lymphocytes, and a fibrotic capsule. Acid-fast bacilli are usually present inside the caseous necrosis but in very low numbers (Cvetnic *et al.*, 2007).

Once the bacteria are transported into the deeper tissues by macrophages and perhaps other phagocytic cells, additional macrophages gather at individual infected foci to form granulomas (Adams, 1976). Disease caused by *M. caprae* is not considered to be substantially different from that caused by *M. bovis* and the same tests can be used for its diagnosis (OIE, 2009).

2.2. Risk factors

2.2.1. Animal infection risk factors

There is relatively limited knowledge about the factors that influence susceptibility or resistance to caprine TB and this is an obvious blind spot in the knowledge and evidence base, especially locally. The risk factors which influence susceptibility can be divided broadly into the classical 'nature' (genetics) and 'nurture' (non-genetic or environmental) factors. If these can be identified and quantified, several are likely to be amenable to intervention. Risk factors will vary across regions due to factors such as differing farm structures, farm management practices, BTB control and eradication programmes, regional TB incidences, wildlife densities and the relative importance of specific risk factors within individual areas (Skuce *et al*., 2011).

Environmental factors

The occurrence of TB is linked to both management and ecological factors (population density of various host species, interaction, habitat differences etc) (Morris *et al.,* 1994). Goats may become infected with TB when they share pastures with infected cattle, at watering points, market places and shared night shelters (Crawshaw, 2008; Tschopp *et al.,* 2011). In rural traditional smallholders in Ethiopia, small ruminants are livestock component and are flocked together with cattle during the day, whereas at night they are usually kept inside poorly ventilated farmer's house for protection, thus having daily contact to cattle and human. Such husbandry practices are epidemiologically important for potential disease transmission at human-animal interface (Tschopp *et al.,* 2011).

Wildlife plays an important role in TB epidemiology. Wildlife may contaminate animals through the contamination of the environment by their excretions such as faeces, urine or pus. Cattle share grazing land and water points with infected wildlife, a situation frequently observed in Africa, as suggested in a cross-sectional study including 106 flocks carried out in 2003–2004 in Zambia (Munyeme *et al*., 2008). In the UK, the risk of contracting BTB through inhalation of urine-born aerosols was the highest (Gallagher and Horwill, 1977). No case of MTBC has been reported in the wildlife population in Ethiopia (*Tsochopp et al.,* 2010).

Agent factors

Tubercle bacilli are usually resistant to drying, to moist disinfectants, acids and alkalis. This resistance is due to hydrophobic lipid surface. They also remain viable for long period in the dark putrefying material, feces, sputum and soil (Biberstein and Hirsh, 1999). *M. bovis* is an obligate pathogen and is highly susceptible to sunlight (UV radiation), but it can survive for several months in the environment, in moist soil and in darkness and remains viable for long periods (>6 months) in frozen tissues (Jahans and Worth, 2006). It survives in acid milk for 15 days and milk products such as cheese and butter for weeks but it gets destroyed when milk is heated for 30 minutes at 65^0c, and cream is heated at 850c for 2minutes. The organism can also survive for 2 years in frozen carcasses (Seifert, 1996).

The MTBC is known to infect a wide variety of warm-blooded animals. The sources of these infections have commonly been traced to infected humans who have exposed susceptible animals to infection. In animals, most mycobacterial infections that are reported involve *M. bovis*; therefore, infection with *M. bovis* is of public-health and economic importance (Kaneene, 2004).

The factor that played an important role in the epidemiology of *M. caprae* is its ability to infect human, domesticated animals and wildlife. Twelve cases of *M. caprae* infection have occurred in four humans, three cattle, and five red deer in western Austria since 1994 (Gutie´Rrez *et al.,* 1997; Aranaz *et al.,* 2003). DNA-fingerprinting of the isolates suggested transmission in and between these species over several years (Prodinger *et al.*, 2002). In autumn of 2004, tuberculosis caused by *M. caprae* occurred in Slovenia. Genotyping results revealed the dromedary camel and two bison were infected by the same *M. caprae* (Pate *et al.*, 2006).

Host factors

Zoonotic TB is one of the many sequels of the adaptability of Mycobacterium species in different hosts. The infection is currently posing a major concern in the human population in developing countries, as humans and animals are sharing the same microenvironment and dwelling premises, especially in rural areas (Shitaye *et al.*, 2007).

Age, sex, breed, pregnancy and parity numbers have been found to play important roles in the epidemiology of animal TB (Mamo *et al*., 2012). One of the main individual risk factors identified by numerous studies in both developed and developing countries is the age of animals. The duration of exposure increases with age; older animals are more likely to have been exposed than younger ones, as shown by several cross-sectional studies carried out in Tanzania, Zambia and Chad (Cook *et al*., 1996; Kazwala *et al*., 2001; Cleaveland *et al*., 2007). Animals might get infected at a young age, but only express the disease clinically when they are adults (Griffin *et al*., 1996). Mycobacteria have the ability to remain in a latent state for a long period before reactivation at an older age (Pollock and Neill, 2002). Study conducted by Mamo *et al.*, (2012) confirmed that multivariable logistic regression analysis at ≥2mm cut-off point older small ruminants (five years and above) in Ethiopia had thirteen times the odds of being tuberculin reactors compared with age category less than two years old. Female small ruminants with parity number greater than four had more chance of being bovine tuberculin positivity in relative to those with less than two parity numbers (Mamo *et al.*, 2012).

The body condition score (BCS) relies on palpation of the sharpness, muscle and fat covering the backbones and lumbar processes and is determined on a one (emaciated) to five (obese) scales (Edmonson *et al*., 1989; ESGPIP no. 8). In 1996, Cook *et al*. linked a low BCS to an increased risk of a positive skin test result in their cross-sectional study including 2,226 animals in Zambia (Cook *et al*., 1996). During a matched case-control study including eighty chronic BTB flocks carried out in 1990 in Ireland, Griffin *et al.* demonstrated that an animal's resistance to tuberculosis was reduced by a shortage of feed and/or an unbalanced diet (Griffin *et al*., 1993).

Management

It has been observed that the highest incidence of BTB is generally found in areas where intensive dairy systems are practiced (Cosivi *et al*., 1998). Dairy production in developed countries follows a trend towards increased intensification on a smaller number of larger production units, which implies increased contact between animals and thus an enhanced risk of BTB transmission (Arendonk and Liinamo, 2003). In these intensive systems, aerogenic transmission of BTB seems to dominate (Menzies and Neill, 2000). In Ethiopia, in a 2006-study comparing the effects of zero grazing versus free grazing it was reported that the severity of BTB was significantly higher in cattle kept indoors at a higher population density than in cattle kept on pasture. In addition to close contact, stress caused by overcrowding or nutritional differences between housed and pastured animals was mentioned as contributing to the spread of the disease and reduction of animal's resistance to the disease (Ameni *et al*., 2006).

2.2.2. Human infection risk factors

Close physical contact

Close physical contact between humans and potentially infected animals for prolonged time can be source of infection especially via aerosol. Close contacts of TB patients are at highest risk of becoming infected with TB. Close contacts are more likely to become infected with *M. tuberculis* than contacts that spent less time with a person while the person was infectious (CDC, 2008). Infection can also occur from direct contact with a wound, such as what might occur during slaughter or hunting. At risk groups include animal workers, farmers, meat packers, vets and zoo keepers (Bilal *et al*., 2010). As the lung is the main site of TB in cattle, farmers, veterinary surgeons and other workers in close contact with diseased animals are principally affected by the inhalation route. Workers in abattoirs and butchers are at risk of pulmonary infection by the inhalation of aerosols during the handling of diseased animal meat and carcass ((Bilal *et al*., 2010). 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 TB as zoonosis in Ethiopia (Bogale, 1999).

Feeding habit

The primary sources of infection for humans are consumption of unpasteurized milk, raw or poorly heat-treated meat products from infected animals. Rural inhabitants and some urban dwellers in Ethiopia still consume unpasteurized and soured milk as well as raw meat potentially contaminated with BTB (Ameni *et al*., 2013). Ethiopian milk consumers generally prefer raw milk (as compared to treated milk) because of its taste, availability and lower price (Firdessa *et al*., 2012). In addition, some investigators have pointed out the risk of human infection using raw milk for producing cream, butter or *dahi* (curd) among cattle owners and flocksmen in community (Cotter *et al*., 1996; Bonsu *et al*., 2000). The proportion of patients with extra-pulmonary tuberculosis was significant in those who frequently used to drink raw milk and consume raw meat in particular. In Ethiopia, consuming raw meat is a welcome tradition, thus meat remains to be area of concern or threat to be a source of BTB infection (Regassa, 2005). Though zoonotic risk of BTB is often associated with consumption (ingestion) of dairy products based on unpasteurized milk infected with *M. bovis*, aerosol transmission (inhalation) from cattle-to-human should also be considered as a potential risk factor (Firdessa *et al*., 2012). Furthermore, vegetable contamination represents a potential source of infection to humans, as *M. bovis* survives in soil (Ayele *et al*., 2004).

Human immunodeficiency virus (HIV) infection

HIV has been identified as one of the most important factors contributing to an increase in the incidence of TB, particularly in Africa (WHO, 2008). The chronic nature of BTB in cattle permits spread of the disease long before its presence is even suspected. As a direct consequence, people exposed to either the infected animal or infected products are at risk of contracting zoonotic TB. This risk increases significantly with the presence of progressive immunodeficiency due to HIV infection (Raviglione *et al*. 1995). In addition to the adverse effect of HIV on TB resistance, an adverse effect of TB on HIV resistance is suggested by studies that show that the host immune response to *M. tuberculosis* enhances HIV replication and might accelerate the natural progression of HIV infection (Maher *et al*. 2002). HIV infection compromises the immune system, making even common illnesses to be a serious threat. People with HIV infections are much more susceptible to developing active TB. In many developing countries, TB is the most frequent opportunistic disease associated with HIV infection (Cosivi *et al.*, 1998). HIV sero-prevalence rate greater than 60% has been found in TB patients in various African countries (Raviglione *et al.,* 1995). Person infected with both pathogens have annual risk of progression to active TB 5% to 15%, depending on their level of immune-suppression; approximately 10% non-HIV infected persons newly infected with TB become ill at sometime during their lives. In the remaining 90%, effective host defenses prevent progression TB from infection to disease (Cosivi *et al.*, 1998). The HIV induced immune suppression could lower the host's defense mechanism and occurrence of HIV-TB co-infection in one person makes TB infection very likely to progress to active disease (Grange *et al.*, 1994b).

Miscellaneous

Smoking more than 20 cigarettes a day increases the risk of TB by two to four times (Davies *et al*., 2006). Diabetes mellitus is also an important risk factor that is growing in importance in developing countries (Restrepo, 2007). Other disease states that increase the risk of developing TB are end-stage renal disease, chronic lung disease, malnutrition, and alcoholism (Kumar *et al.*, 2007). A person's genetics also plays a role (Moller and Hoal, 2010).

In general, the existing eating culture (eating of raw meat and drinking of raw milk), the very common close contact of animals with humans (most common in rural areas), inadequate meat inspection and the prevailing low standard of hygienic practices are potential risk factors that favor the spreading of zoonotic TB in developing countries like Ethiopia (Shitaye *et al.,* 2007).

2.3. **Diagnostic methods**

2.3.1. Clinical examination

The diagnosis of TB in live animals mainly depends on clinical manifestations of the disease and tuberculin skin testing. After death, it is diagnosed by post mortem examination and confirmed in the laboratory by histopathological, bacteriological and molecular techniques (Kaneene, 2004).

Depending on which organ or associated lymph node is affected, animals can display a wide range of symptoms e.g. coughing, dyspnoea, gastrointestinal problems, bone deformation, and emaciation (OIE, 2009).

Clinical signs such as chronic debilitation, moist cough, low grade fever and enlargement of local lymph nodes may suggest infection with TB. Animals with pulmonary involvement usually have a moist cough that is worse in the morning, during cold weather or exercise, and may have dyspnea or tachypnea. In the terminal stages, animals may become extremely emaciated and develop acute respiratory distress. In some animals, the retropharyngeal or other lymph nodes enlarge and may rupture and drain. Greatly enlarged lymph nodes can also obstruct blood vessels, airways, or the digestive tract. If the digestive tract is involved, intermittent diarrhea and constipation may be seen (OIE, 2004; Cousins and Florisson, 2005). Goats affected with TB initially show dry coughing, progressive emaciation, occasionally diarrhoea and death (Crawshaw *et al.*, 2008; Quintas *et al*., 2010; Sharpe *et al.*, 2010).

2.3.2. Post mortem inspection

Clinical signs of TB vary depending on the extent and location of the lesions. On gross necropsy examination, TB may be provisionally diagnosed when caseous or calcified foci are observed in various tissues of the body, but this is difficult in the initial stages of the disease (Kaneene, 2004). During necropsy, the lymph nodes especially those associated with the head, thorax and abdomen are closely examined. Careful examination of as few as 6 pairs of lymph nodes, the lungs and the mesenteric lymph nodes can result in 95% of infected animal with microscopic lesions being identified. However, this method is not useful to determine the significance animal that give positive reaction but do not have visible lesions. No visible lesion reactors may be due to early infection, poor necropsy technique or infection with mycobacteria other than MTBC species. Above all, it is not always possible to differentiate TB lesions by gross examinations from those of others infections (Corner, 1994).

Postmortem examination of goats infected with *M. bovis* frequently reveals circumscribed pale yellow, white, caseous lesions of various sizes, often encapsulated, especially in the lungs and mediastinal lymph nodes, or in the mesenteric lymph nodes (Crawshaw *et al.*, 2008; Quintas *et al.*, 2010; Sharpe *et al.*, 2010). Similar gross lesions have been described in goats infected with *M. caprae* (Álvarez *et al.*, 2008).

2.3.3. Tuberculin skin test

Several diagnostic assays developed so far have been proved to be effective in diagnosing TB in cattle (Rua-Domenech *et al*., 2006). The most widely used field surveillance test is the tuberculin skin test that measures the cell mediated immunity (CMI) response to *M. bovis* exposure (Monaghan *et al.*, 1994).

The tuberculin skin tests are the international standards for ante mortem diagnosis of tuberculosis in flocks/flocks and individual animals and humans (Pollock and Neill, 2002).

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 reactive antigens are common to the members of the MTBC and tuberculin can be used to measure the CMI response as evidence of exposure to *M. bovis*. However, many of these antigens are also found in non-pathogenic environmental mycobacterial species and this cross reactivity to common antigens can result in a reduced specificity of the test, giving rise to non-specific reactors (false positives). Where this problem occurs, an *M. avium* derived tuberculin is included to perform the CIDT (Francis *et al*., 1978).

Monaghan *et al.,* (1994) reviewed the most common tuberculin tests in use today, namely, the caudal fold test and the single intradermal tuberculin (SIDT) test, which both use only bovine tuberculin purified protein derivatives (PPDs) and the CIDT test, which uses bovine and avian tuberculin PPD in combination. The principle behind tuberculin test is that when PPD is injected intradermally into normal animal, there is no local response but when they are inoculated into exposed animals, there is an allergic reaction, which is typically delayed type hypersensitivity reaction (Monaghan *et al.,* 1994).

The diagnosis of TB in goats is performed using the same tests applied in cattle: the tuberculin test can be performed as SIDT test, using only bovine PPD, or as the CIDT test, using both bovine and avian PPDs. The CIDT test is used to differentiate between animals truly infected with *M. bovis/M. caprae* from those sensitized to bovine PPD as a result of exposure to other mycobacteria (Gutie´Rrez *et al*., 1997).

The CIDT test involves the intradermal injection of tuberculins, PPD from *M. bovis* and *M. avium,* and the subsequent detection of swelling and indurations at the injection site 72 hrs later. The relative change in skin thickness at the two sites is used to differentiate

M. bovis infection from infection with non-tuberculous mycobacteria. According to the *Office des Internationale Epizooties* (OIE) (OIE, 2004) recommendation, the difference between the increase in skin thickness following the intradermal administration of bovine PPD (B) and the increase in skin thickness following the intradermal administration of avian PPD (A), B-A, should be >4 mm for the animal to be considered positive for BTB. Thus, the OIE advocates the use of this cutoff point for the diagnosis of BTB in live animals (Ameni *et al*., 2008). However, >2mm cut-off point of the CIDT test was assessed with the Ethiopian Arsi breed cattle and came up with better sensitivity and specificity with significantly higher prevalence value than at >4mm cut-off value (Ameni *et al*., 2008). Thus, this study suggests the use of both cut-off points for the diagnosis of animal TB by tuberculin skin test in live animal. The use of > 2mm cut-off of was to discriminate resistant animals positive to TB but which show negative or suspect at >4mm cut-off.

2.3.4. Isolation and identification of etiological agent

The definitive diagnosis of TB depends on the isolation and identification of the mycobacteria in clinical specimens. The main factors which influence the success of primary isolation of *M. bovis* from clinical specimens are the culture media, the decontamination procedure and incubation condition (Corner, 1994). Culture is a technique based on the cultivation of live bacilli on appropriate culture media (WHO, 1998). Robert Koch was the first person to invent a new solid media made up of potato and agar to cultivate *M. tuberculosis* (Koch, 1882). Depending on the decontamination method and the type of culture medium used, as few as ten viable tubercle bacilli might be enough to isolate *M. tuberculosis* using culture techniques (WHO, 1998). Before inoculation the samples are digested, decontaminated and concentrated. The commonly used decontaminants for *M. bovis* preparation are hexadeculphyridinium chloride, benzalkonium chloride (Zepharin), oxalic acid, NaOH, and sodium hypochlorate (Corner, 1994; OIE, 2009).

Using media of two different types ensures maximum recovery of most strains of Mycobacterium. Egg based media, LJ and Ogawa, and agar based solid media, Meddilebrook 7H-10 and 7H-11, are widely used solid media worldwide for the isolation of Mycobacterium (CLSI, 2008). Commonly used medium is LJ medium. It is made selective by addition of malachite green. Other components include egg yolk, mineral salt solution & asparagines (Kent and Kubica, 1985). A processed specimen inoculated on LJ media and monitored twice a week for the first 4 weeks then once a week until the end of 8 weeks for the growth of mycobacterial species. Since TB is a slow growing organism, positive result (colonies) usually appear after 3 weeks of incubation. On LJ media *M. tuberculosis* colonies appear as colorless, flat, dry and rough with irregular edges. Acid fastness can be confirmed by smear microscopy (CLSI, 2008).

2.3.5. Biochemical test

Identification of mycobacterial isolates depends on colony morphology, staining characteristics as well as biochemical tests such as niacin production, nitrate reduction, urease test and pyrazinamidase activity (deamination of PZA to pyrazinoic acid in 4-7 days) and drug sensitivity tests, etc (OIE, 2009). A combination of positive activity for niacin, nitrate reduction, and negative activity for catalase at $68^{o}C$ and arylsulphatase were considered as characteristics of *M. tuberculosis* while negative activity for niacin, nitrate reduction, catalase at $68^{o}C$, and arylsulphatase were considered as characteristics of *M. bovis* as described by (Vestal, 1977). PZA activity is a stable feature commonly used to distinguish *M. bovis* from other members of the MTBC. *M. bovis* isolates are naturally resistant to PZA (Aranaz *et al*., 1999).

Table 1: Some properties of tubercle bacilli

TCH= thiophen-2-carboxylic hydrazide; PZA=pyrazinamide; R= resistant; S= sensitive

Species	Host range	Niacin accumulation	Nitrate reduction	TCH	PZA
M. africanum	Humans	+/-	+/-	R/S	S
M. bovis	Badgers, deer, cattle, elephants, goats, humans,	-	-	S	R
M. caprae	Goats, deer, humans cattle	-	+	S	S
M. canettii	Humans	+	+	R	S
M. microti	Voles	+	-	S	S
M. tuberculosis	Humans	+	+	R	S

Adapted from Aranaz *et al. (*1999); Cole, (2002).

2.3.6. Molecular characterization

Polymerase chain reaction (PCR)

Molecular techniques have been developed over recent years and have been used to characterize members of the MTBC and to provide information on transmission of mycobacterial diseases between animals and humans. However, because of limited resources and lack of expertise, these techniques are not commonly used in most developing countries (Warren *et al.*, 2006).

PCR has been widely evaluated for the detection of MTBC in clinical samples (mainly sputum) in human patients and has recently been used for the diagnosis of TB in animals. A number of commercially available kits and various 'in-house' methods have been evaluated for the detection of the MTBC in fresh and fixed tissues (OIE, 2009). Amplification products have been analyzed by hybridization with probes or by gel electrophoresis. Commercial kits and the in-house methods, in fresh, frozen or boric acid-preserved tissues, have shown variable and less than satisfactory results in inter-laboratory comparisons (Noredhoek, 1996; OIE, 2009). False positive and false negative results, particularly in specimens containing low numbers of bacilli, have reduced the reliability of this test. Variability in results has been attributed to the low copy number of the target sequence per bacillus combined with a low number of bacilli. Variability has also been attributed to decontamination methods, DNA extraction procedures, techniques for the elimination of polymerase enzyme inhibitors, internal and external controls and procedures for the prevention of cross-contamination. Improvement in the reliability of PCR as a practical test for the detection of MTBC in fresh clinical specimens will require the development of standardized and robust procedures. Cross contamination is the greatest problem with this type of application and this is why proper controls have to be setup with each amplification (Miller, 2002). Although direct PCR can produce a rapid result, it is recommended that culture be used in parallel to confirm a viable *M. bovis* infection (OIE, 2009).

A typical amplification reaction includes the sample of target DNA, a thermostable DNA polymerase, two oligonucleotide primers, deoxynucleotide triphosphates (dNTPs), reaction buffer, magnesium and optional additives. The components of the reaction are mixed and the reaction is placed in a thermal cycler, which is an automated instrument that takes the reaction through a series of different temperatures for varying amounts of time. This series of temperature and time adjustments is referred to as one cycle of amplification. Each PCR cycle theoretically doubles the amount of targeted template sequence (amplicon) in the reaction. Ten cycles theoretically multiply the amplicon by a factor of about one thousand; 20 cycles, by a factor of more than a million in a matter of hours (Miller, 2002).

Each cycle of PCR amplification consists of a number of steps. Each step denatures the template producing two oligonucleotide-primed single-stranded DNA templates, sets up the polymerization reaction, and synthesizes a copy of each strand of the template being targeted. These steps should be optimized for each template and primer pair combination. The initial step in a cycle denatures the target DNA by heating it to 95°C or higher for 15 seconds to 2 minutes. In the denaturation process, the two intertwined strands of DNA separate from one another, producing the necessary single-stranded DNA template for the thermostable polymerase (Hellyer *et al*., 1996).

m-PCR is used to test acid fast organisms which are neither recognized by spoligotyping nor show growth characteristics typical to *M. bovis* (Jahans and Worth, 2006). A single step m-PCR assay for distinguishing (1) between the MTBC and mycobacteria other than TB targeted the 16S and 23S rDNA and (2) between *M. tuberculosis* and *M. bovis* species targeted the *oxyR* gene have been developed (Kurabachew *et al*., 2004). Besides a single tube m-PCR has been developed and said valuable where bovine and human TB coexist, and the distinction of *M. bovis* from *M. tuberculosis* is required for monitoring the spread of *M. bovis* to humans (Bakshi *et al*., 2005).

Table 2: The presence and absence of genomic regions of difference in MTBC members.

No	RD *locus*	*M. tuberculosis*	*M. canettii*	*M. africanum*	*M. microti*	*M. pinnipedii*	*M. caprae*	*M. bovis*
1	ThD1	present/absent	absent	absent	absent	absent	absent	absent
2	RD9	present	present	absent	absent	absent	absent	absent
3	RD10	present	absent	absent	absent	absent	absent	absent
4	RD1^{Mic}			present	absent	present		
5	RD12	present	absent	present	present	present	absent	absent
6	RD4	present	present	present	present	present	present	absent
7	RD1	present	present	present	present	present	present	present
8	RD2^{Seal}			absent	absent	absent		
9	RD^{Can}	absent		present	absent	absent	absent	absent

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

Spoligotyping

Spoligotyping (spacer oligonucleotide typing) or (reverse line blot hybridization technique) is a novel molecular typing method that is based on DNA polymorphism present at one particular chromosome locus, the direct repeat (DR) region, which is unique to the MTBC bacteria (Gori *et al*., 2005; Jahans and Worth, 2006). 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, thus, useful for differentiation of Mycobacterium strains (Wayne, 1984). Thus, the clinical usefulness of spoligotyping is determined by its rapidity, both in detecting causative bacteria and in providing epidemiologic information on strain identities (Gori *et al*., 2005).

A spoligotyping kit for detection of the MTBC contains one membrane, positive control (1)(*M. tuberculosis* strain H37Rv), positive control (2) (*M. bovis* BCG P3), primer DRa (biotinylated) and primer DRb. Amplification of the spacers is accomplished by using the DRa and DRb, which enable to amplify the whole DR region. Only a very small amount of template DNA is required (Sharma and Gupta, 2002). The use of a standard nomenclature for the spoligotypes according to the database Mbovis.org (http://www.mbovis.org) is encouraged to allow international comparison of profiles (OIE, 2009).

2.4. Prevention, control and treatment

At least three important reasons for controlling BTB should be considered: animal welfare, the financial burden to farmers with diseased animals, loss in productivity due to infected animals, and animal market restriction set by countries with advanced eradication programs and the risk of zoonotic transmission (Cousins, 2001; Firdesa *et al*., 2012). This chronic disease can take years to develop. Animals infected with BTB can therefore slowly develop symptoms and may, if not carefully observed by animal keepers, suffer unnecessarily (Firdesa *et al*., 2012).

The basic strategies required for control and elimination of BTB are well known and well defined. However, because of financial constraints, scarcity of trained professionals, lack of political will, as well as the underestimation of the importance of Zoonotic TB in both the animal and public health sectors by national governments and donor agencies, control measures are not applied or are applied inadequately in most developing countries (Cosivi *et al.*, 1998).

In order to reduce the risk associated with consumption of contaminated milk and meat, it is necessary that specific hygiene rules for food of animal origin be laid down to prevent infected animals from entering the food chain. Meat inspection system should be strengthened and designed to prevent the consumption of contaminated products by people. All animals entering the food chain should be subjected to ante-mortem and postmortem inspection. The tuberculin test is valuable in the control of zoonotic TB because early recognition of preclinical infection in animals intended for food production and early removal of infected animals from the flock eliminates a future source of infection for other animals and for humans. In the case of cattle, a tuberculin test should be performed in the course of the twelve months prior to presentation for slaughter (FSAI, 2008). Milk should be pasteurized or effectively treated with heat prior to human consumption or further processing, as this is the generally agreed critical and effective control measure to prevent transmission of zoonotic TB through milk (FSAI, 2008).

Efforts should continue in the control or elimination of TB in cattle and other animals used for food production as this may be expected to reduce or eliminate the ultimate source of *M. bovis* infection. The test-and-slaughter policy, which is the mainstay of BTB control programme in any given country, can be modified to accommodate a step-wise basis involving segregation and phased slaughter of reactor animals (WHO, 1994). This will make control of TB in cattle more practicable, especially in developing countries. Also, the role of wild fauna in the epidemiology of TB in livestock and humans need not be ignored, as they have been reported to serve as a reservoir of the pathogen (CFSPH, 2007). Animal husbandry practices should be improved upon to reduce contact between domestic livestock and wild ruminants especially during grazing (Nwanta *et al.*, 2010).

Besides public education regarding the risks of consumption of unpasteurized dairy products and precautions that should be taken in dressing or processing animal carcasses or when cooking meat from animals that are particularly susceptible to the disease may be useful in reducing the risks of transmission through ingestion or indirect inoculation (Cosivi *et al.*, 1995; LoBue, 2006). Morris *et al* (1994) considered one area which is undoubtedly crucial to effective TB control but receiving little attention; the influence of farmer's attitude and behavior, livestock trading practices and flock management in determining how well TB is controlled in individual flocks. However, attempts to eradicate TB from cattle flocks in some developed countries have been frustrated by the presence of reservoirs of *M. bovis* infection in wildlife (Barrow and Gallagher, 1981).

M. bovis is resistant to PZA, which is widely used in the treatment of infections caused by MTBC in humans (Krauss *et al*., 2003). Cattle should not be treated at all and as such farm animals with tuberculosis must be slaughtered (culled) (Krauss *et al*., 2003; CFSPH, 2007). This is because the risk of shedding the organisms, hazards to humans and potential for drug resistance make treatment controversial (Nwanta *et al.*, 2010).

3. STUDY MATERIALS AND METHODS

3.1. Description of study area

The study was conducted in ATJK district east Showa zone of Oromia regional state which is found 165 kms to southeast of Addis Ababa from November, 2013 up to July, 2014. East Showa zone covers the largest part of the mid-Rift Valley. The district lies at latitude of 7.58ºN and 38.43ºE longitudes. Its agro-ecological zone is semi-arid and sub-humid in which 90% of the area is lowland while the remaining 10% is intermediate with altitude ranges from 1500 –2000 meter above sea level. The mean annual rainfall ranges from 750-1000mm and the distribution is highly variable between and within years (Debele *et al*., 2010). The area has average maximum and minimum annual temperature of 27.2 and 12.7 ^{0}C, respectively and a relative humidity of 60% (Ebro *et al*., 1998). The total land area of the district is estimated to be about 75,223 ha, of which, 36,661 ha is used for crop production, whereas, 17,113 ha is used for grazing (Tafese *et al.*, 2011). ATJK district has 43 rural kebeles and three towns on the main road from Addis Ababa to Hawassa and a small rural town 20kms from Bulbula town towards southwest called Jido town. The district is endowed with beautiful natural lake; Batu lake and surrounded by Abijata and Langano lakes which are becoming tourist attraction places recently.

According to census data collected by district's Finance and Economic Development Office (FEDO) and Livestock Agency (LA) in 2012/2013, there were 167,169 human populations whereby 84,163 were males and 83,006 were females. Among these 140,542 were rural and 26,627 were urban dwellers (Personal communication. ATJK district FEDO, 2014). The major animal species kept in the area include cattle, goat, sheep, horses, donkeys, mule, poultry and rarely camel. The total livestock population of the district was 221,428 cattle, 133,160 goats, 27,488 sheep, 25,530 donkeys, 1,800 horses, 1,000 mule and 143,900 poultries (Personal communication. ATJK district LA, 2014). The existing farming system is a mixed crop livestock farming system. The agriculture is cereal based mainly maize and sorghum and entirely depends on oxen and draught power to till the land. Next to cattle, goats are the most important animal species kept in the area for milking, for sale of live goat as a source of income and meat purposes. Milk is collected twice a day from lactating goats, and either consumed directly raw, mixed with coffee or mixed with cow milk and processed at home into butter and cottage cheese. Butter can be marketed in the local market and cottage cheese, which is un-cooked, is always consumed at home. Live animals can be slaughtered at the farm for meat consumption and hides sold at the local market. Most of the farmers rear goats for the measure of social status among the community. The great majority of farmers are small holders keeping livestock in traditional husbandry systems. Overall zebus are the main cattle breed and the predominant goat breeds in the study area are Arsi-Bale goats (ILRI, 1996). Traditional housing and grazing of natural pasture are the predominant husbandry practices observed. The predominant goats are managed under extensive production system mixed with cattle and other livestock.

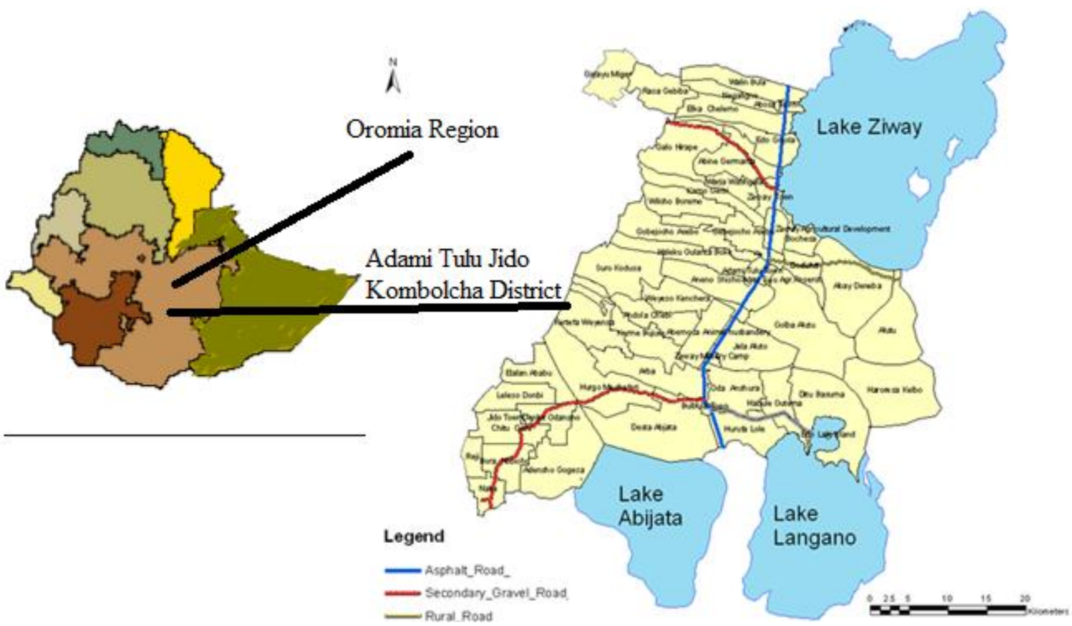

Fig.1: Map of the study area. (Source: personal communication. ATJK district LA, 2014).

3.2. Study design and population

Two types of study designs were used to undertake study; cross-sectional study to determine prevalence of caprine TB and case-control study to investigate transmission of TB between goats and their owners in the study area. In cross-sectional study, the study animals were goats from smallholder farmers of ATJK district from 9 selected Kebeles and two towns. In case-control study, cases of human TB were recruited from health facilities (Batu Share hospital and Batu health centers) who were diagnosed as smear positive pulmonary TB patients and matched control human who were free of TB, living in the same village with the cases with similar life status as well as attitude and with no history of TB for the last decade were selected. Visits to settlements for each study were made early in the morning when goats were kept in their enclosures. Testing was performed after informed verbal consent was given by the individual owners.

3.2.1. Inclusion and exclusion criteria

Acid-fast bacilli (AFB) smear positive individuals with age of above 18 years old were included. However, individuals who do not have goats and youths below 18 years of age were not included. Control individuals residing in the same village as the cases and with similar life status as well as attitude but with no history of TB for the last decade only were included. Potential immuno-suppression that would interfere with the skin test, goats currently treated for an acute disease, goats younger than 6 months of age, peri-parturient goats, and goats showing any clinical symptoms of diseases were not included in the study.

3.3. Sample size and sampling

Determination of prevalence of caprine TB was conducted by cross sectional study. ATJK district comprises three towns and 43 rural Kebeles. The selection of animals was based on a stratified sampling method. First East Shewa administrative zone was selected. Then ATJK district was selected based on the previous information for the presence of caprine TB in the area. Urban and rural environments were considered as strata. Two towns and 9 villages were selected and 97 households were selected among them. Villages and households were selected purposively based on the inclusion criteria (accessibility, willingness of the households to participate in research). In each household, all goats more than 6 months old were recruited. Sample size was determined according to Thrusfield, (2005) using single proportion formula. Taking estimated prevalence of 9.6% caprine TB in individual animal previously reported by Tafese *et al*., (2011) on household farmer's goats of the area and taking a confidence interval of 95% and 5% absolute precision.

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

Where: - n = the required sample size, Pexp = expected prevalence

d= desired absolute precision

Therefore, the minimum sample size required was 133 goats. However, to increase the representativeness of the study sample and reduce the design effect sample size was increased by 4 folds which results in 532 goats at the study area. Study animal-related information on each tested goat (such as sex, age, BCS and parity number) was collected and recorded at the time of the test (Annex 1 and 2).

To assess transmission of TB between goats and their owners, a total 70 households comprising 38 TB positive cases and 32 TB free controls were included in the study based on the inclusion criteria described. Goats of cases and control were traced back and tested by CIDT for determination of infection by MTBC species. Accordingly, a total of 283 goats of both cases and controls were tested.

3.4. Study methodology

3.4.1. Comparative intradermal tuberculin (CIDT) test

Testing of animals was based entirely on owner willingness for both types of the study (case control and epidemiology). After having obtained informed consent from the owners, testing was done, either at the individual household or after gathering animals from all owners in a common place. First, injection site was shaved, 12cm apart and cleansed. Then, a cutaneous fold was formed with thumb and the point finger within each clipped area and the skin fold was measured using caliper in millimeters and recorded as A1 and B1, respectively (Annex 3). CIDT was carried out by injecting both bovine and avian tuberculin PPD intradermally into neck region of goats. Aliquots of 0.1 ml of 2500 IU per ml of avian purified PPD and 0.1 ml of 3000 IU per ml of bovine PPD (Lelystad, The Netherlands; Animal Health and Veterinary Laboratories Agency) were injected to the dermis of the study animals. All goats which were selected and tested were identified by number marked on their inner ear written by permanent marker. The skin thickness at each injection site was measured again after 72 hours and coded as A2 and B2, respectively. The interpretation of the result was made on the basis of the difference in skin thickness at the bovine and avian PPD injection sites. Two cut-off points were used to determine caprine TB status of an animal; the animal was considered as positive for caprine TB if the skin thickness at bovine PPD injection site minus the skin thickness at avian PPD injection site (B2-A2) was ≥4mm (WHO, 2008; Bezos *et al*., 2011) or if the difference ((B2-B1)-(A2-A1)) was ≥2mm (Ameni *et al*., 2003; Bezos *et al*., 2011). A flock was considered as positive if there was at least one tuberculin reactor animal in the flock (Mamo *et al*., 2012). Those animals whose skin fold thickness measured below 2mm were considered as negative for MTBC.

Fig. 2: Measuring of skin folds before PPD injection

Fig. 3: Appearance of skin folds just after PPD injection

Fig. 4: Identification of the animal after after PPD injection

3.4.2. Post mortem examination

Goats which were strong bovine tuberculin reactors and owned by TB positive patient were purchased, slaughtered and investigated for gross tuberculous lesions. Detailed post mortem examination was performed following a procedure described by Corner (1994). All pulmonary lobes and the lymph nodes of the head (retropharyngeal, mandibular), thorax (mediastinal and bronchial), abdomen (mesenteric lymph node) and other organs including liver and intestines were examined thoroughly. The carcass including other internal organs and lymph nodes were examined under a bright-light source. The lung and the lymph nodes were cut into approximately 2cm thick slices using separate sterile scalpel blades. The cut surfaces were examined visually under bright light for the presence of lesions compatible with TB. Tissues with gross nodular lesions of caseous necrosis and/or calcification were obtained and suspected for TB. Accordingly, liver, large intestine wall, omentum, both right and left lung lobes, cranial mediastinal lymph nodes and mesentery tissues were among the tissues with gross nodular lesions of caseous necrosis and/or calcifications suspected for TB. These tissues were collected for bacteriological culture. These tissues were carefully removed from the carcass and placed into sterile universal bottles with 5ml of 0.9% saline solution and also kept in icebox with solid packs to keep the cold chain. The samples were stored in deep freeze for two days and were transported to Aklilu Lema Institute of Pathobiology (ALIPB).

Fig. 5: TB lesion on right diaphragmatic lobe of lung and omentum

3.4.3. *Interviewing goat owners*

Verbal consent was obtained from the respondents and the objective of the study was explained to them before start of the interview. The interviews were conducted by the investigator in local languages (Afan Oromo or Amharic) using questionnaire (Annex 4) consisting of closed and open questions which address the knowledge, attitude and practices for the individuals in relation to TB in humans and goats and assess the public health risk. The questionnaire focused on demographic characteristic of the interviewee like housing practices, knowledge about caprine TB, transmission ways of TB between human and animals and habit of animal product consumption.

3.4.4. *Sputum culture*

Ethical clearance for the study of both animal and human component was approved by Aklilu Lemma Institute of Pathobiology Ethical review committee (Ref. No. IBR/04-c/2014).

Sputum samples were collected from AFB positive pulmonary TB patients (after informed consent was obtained) in sterile universal tubes using sterile leak proof disposable plastic material that was labeled with patient code and were taken to ALIPB TB laboratory, Addis Ababa. The samples were processed using standard operating procedures in the TB laboratory. Briefly, the processed sputum and tissue were inoculated onto a duplicate set of LJ slants (Annex 5); one supplemented with 0.4% sodium pyruvate (labeled as P) and the other with glycerol (labeled as G). Cultures were incubated aerobically at 37°C for 8-12 weeks with weekly observation for growth according to (McIlroy *et al.,* 1986; OIE, 2004). Growth of mycobacteria was monitored every week for up to 8-12 weeks.

Slow growth and colony characteristics were considered as an evidence of growth of mycobacteria. The observation of the isolates eugenic growth on LJ media supplemented with glycerol were tentatively identified as *M. tuberculosis* while those eugenic growth on pyruvate containing LJ media were regarded as suggestive of *M. bovis.* Finally, isolates were harvested for molecular typing analysis by scrapping the growth from a slope into 300µl of sterile distilled water in PCR tube and heat killed in water bath at 80°C for 45minutes and kept at −20°C until the day of molecular characterization.

3.4.5. Tissue culturing and identification of Mycobacteria

Tissue specimen processing and culturing for mycobacteria were carried out at TB laboratory of ALIPB in accordance with the guidelines of the OIE (OIE, 2009). LJ medium was used for bacteriological culture. In the laboratory, individual animal tissue specimen was sectioned using sterile blades in sterile Petri dishes to obtain fine pieces. Then 0.9% saline water was added and homogenized with a mortar and pestle under a biological safety cabinet. About 5ml of the homogenate was transferred to sterile 15ml centrifuge tube, with screw cap. Then, homogenate was decontaminated using equal amounts, about 5ml, of 4% NaOH for 15 min and then centrifuged at 3,000 rpm for 15 min. The supernatant was discarded and about 2ml of 0.9% saline water was again added to re-suspend the sediment. This suspension was neutralized by 1% (0.1 N) HCl using phenol red as indicator. Then after, the same procedures described above for sputum culture were followed. Positive culture was heat killed in water bath at 80°C for 45 min and stored at −20°C and further molecular characterization was performed.

3.4.6. Molecular characterization

Multiplex polymerase chain reaction (m-PCR)

For this molecular technique the procedure described by Wilton and Cousins (1992) was followed. This PCR analysis differentiates MTBC from other mycobacterial species. The set of primers targeted MTBC species. The primers used were MYCGEN-F, 5'-AGA GTT TGA TCC TGG CTC GA 3', (35ng/µl); MYCGEN-R, 5'TGC ACA CAG GCC ACA AGG GA 3', (35ng/µl); MYCAV-R, 5'-ACC AGA AGA CAT GCG TCT TG 3', (35ng/µl); MYCINT 5'-CCT TTA GGC GCA TGA TGT CTT TA 3', (75ng/µl); TB1-F, 5'-GAA CAA TCC GGA GTT GAC AA 3', (20ng/µl); TB1-R, 5'-AGC ACG CTG TCA ATC ATG TA 3', (20ng/µl). Heat killed culture positive samples were used as source of DNA template. DNA amplification was done in thermocycler (Applied Biosystems, GeneAMP 9700) with 20µl reaction volumes. Each PCR tube consisted of 5.2µl H_2O Qiagen, 8µl HotStarTaq MasterMix (MgCL2, dNTP, Taq polymerase and PCR buffer), 0.3 µl forward and reverse primer per each sample (concentration given above) and 5µl of genomic DNA templates of samples or controls making the total volume of 20 µl. The reaction was carried out for 10minutes at 95^0c for enzyme activation 35 cycles of 1minutes at 95^0c for denaturation, 1minutes at 61^0c for annealing, and 2minutes at 72^0c for extension, and final extension at 72^0c for 10minutes.

M. tuberculosis (H37RV), *M. bovis* and *M. avium* were used as positive controls while H_2O Qiagen was used as a negative control. 1.5% agarose gel was prepared using Ethidium bromide at ratio of 1:10. 100bp DNA ladder and orange 6xloading dye were used in gel electrophoresis. The product was electrophoresed in 10xTAE running buffer at 100volt and 400mAm for 30minutes. Finally, bands were visualized using gel scanner (alpha innotech, (Alpha Innotech Corporation) in a multi–image™ light cabinet).

Deletion typing

The RD9 deletion typing was carried out on 12 isolates that showed positive culture on LJ media and on an isolate from goat that showed positive for genus *Mycobacterium* by genus typing. Each sample was tested in a separate PCR tube. Primers directed against the RD9 were used to generate a deletion profile that would allow species identification of the isolate. Deletion typing was performed to further characterize culture positive isolates to identify strains from the MTBC. Primers that were used include RD9int-ACA CGC TGG CGA AGT ATA GC, RD9-F CTC GTC GAA GGC CAC TAA AG and RD9-R AAG GCG AAC AGA TTC AGC AT to check for the presence of RD9 locus. The HotStarTaq Master Mix system from Qiagen was used for PCR, with primers described previously. The reaction mixture was 10µl of HotStarTaq Master Mix, 0.3 µl x 3 of each primer (flank R, F and int), 2 µl DNA template and 7 µl distilled water to a final volume of 20µl. *M. tuberculosis* H37Rv and *M. bovis* were used as positive control while Qiagen water was used as negative control. The mixture was heated in Program Thermal Controller (Applied biosystem; PTC- 100TM) using an initial hot start of 95^0C for 15 minutes, followed by 35 cycles of 95^0C for 1 minute, 55^0C for 1 minute, and 72^0C for 1 minute; a final extension step of 72^0C for 10 minutes to complete the cycle. PCR products were electrophoresed in 1.5% agarose gel in 10xTAE running buffer, Ethidium bromide at ratio of 1: 10, 100bp DNA ladder and orange 6xloading dye were used for electrophoresis. The gel was visualized in Multi–image ™ light cabinet using Alpha innotech (Alpha Innotech Corporation).

Spoligotyping

Spoligotyping was performed following Kamerbeek *et al*., (1997) and according to the spoligotype kit supplier's instructions (Ocimum Biosolutions Company, Iisselstein, The Netherlands). The DR region was amplified by PCR with oligonuclotide primers derived from the DR sequence (DRa: 5'-GGT TTT GGG TCT GAC GAC-3' and DRb: 5'-CCG AGA GGG GAC, GGA AAC-3'). A total of 25µl of the following reaction mixture was used for PCR: HotStarTaq Mater Mix, 2µl of each primer (20pmol each), 5µl of the suspension of heat-killed cells and 3.5µl of distilled water. The mixture was heated for 15minutes at 95^0c and subjected to 30 cycles of 1minutes at 96^0c 1minutes at 55^0c and 30 seconds at 72^0c. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequences within the DR locus. After hybridization, the membrane was washed twice for 10 minutes in 2xSSPE-0.5% sodium dodecycle sulphate at 60^0c and then incubated in 1:4000 diluted streptavidin peroxidase conjugate for 45 minutes at 42^0c. The membrane was washed twice for 10 min in 2 x SSPE - 0.5% sodium dodecyl sulphtes at 42°C and rinsed with SSPE for 5minutes at room temperature. Hybridizing DNA was detected by the enhanced chemiluminescence method (Amersham Plc. Lt, UK) and by exposure to X-ray as specified by the manufacturer.

3.5. Data management and analysis

All the data obtained from the study were entered into MS Excel 2007 data sheet. Then, coded and were analyzed using STATA (11.0) statistical software. The individual animal prevalence level was defined as the number of positive reactors per 100 animals tested. The flock level prevalence was calculated as the number of flocks with at least one-reactor animal per 100 flocks tested. Individual animal characteristics (age, sex, BCS and parity number) were expressed in percentages. The risk factor associated with caprine infection was assessed using Chi-square ($\chi 2$) test. Uni-and multivariate logistic regressions were used to investigate possible associations between the prevalence and the explanatory variables. For all analyses, a p-value of less than 0.05 and 95% confidence level was taken as statistically significant.

4. RESULTS

4.1. Flock and animal level prevalence of tuberculosis in Adami Tulu Jido Kombolcha District

Cross-sectional study was conducted to determine prevalence of caprine TB and a total of 97 flocks that belong to 97 households from urban and rural villages were CIDT tested. Two cut-off points of skin test (≥4 mm and ≥2 mm) were employed for the estimation of the prevalence at household/flock level. Flock prevalence was 13.6% (9/66) in rural and 9.7% (3/31) in urban villages at ≥4mm cut-off while it was 18.2% (12/66) and 9.7% (3/28) at ≥2mm, respectively. The overall flock prevalence in the district was 12.4% (95% CI: 5.7 to 19.0) at ≥4mm and 15.5% (95% CI: 8.1 to 22.8) at ≥2mm cut-off points cut-off points, respectively. Prevalence of individual goats was 4.4% (17/387) and 2.1% (3/140) at ≥4mm and ≥2mm cut-off in rural and urban villages of ATJK district, respectively. The overall goat level prevalence was 3.8% (95% CI: 2.2 to 5.4) at ≥4mm cut-off points and 4.6 % (95% CI: 2.8 to 6.3) at ≥2mm cut-off points.

Many animal characteristics (goat sex, goat age, BCS and parity number) and Chi square were used to assess the risk factors predisposing animals to caprine TB infection. But none of the considered animal characteristics were found associated with caprine TB (P>0.05). Table 3 summarizes the level of association of animal characteristics with caprine TB infection in the cross-sectional study.

Table 3: The level of association of animal characteristics with caprine TB infection in the cross-sectional study at ≥2mm cut-off point

Animal characteristics		No of animals (%)				
No		Tested	positive	negative	χ2	P-value
1.	Study village				2.55	0.11
	Urban	140(26.6)	3(2.1)	137(97.9)		
	Rural	387(73.4)	21(5.4)	366(94.6)		
2.	Sex				0.13	0.71
	Male	95(18.0)	5(5.3)	90(94.7)		
	Female	432(82.0)	19(4.4)	413(95.6)		
3.	Age				0.05	0.82
	≤ 1.5	51(9.7)	2(3.9)	49(96.1)		
	>1.5	476(90.3)	22(4.6)	454(95.4)		
4.	BCS				0.43	0.81
	Poor	114(21.6)	4(3.5)	110(96.5)		
	Medium	299(56.7)	14(4.7)	285(95.3)		
	Fat	114(21.6)	6(5.3)	108(94.7)		
5.	Parity				0.71	0.87
	No delivery	152(28.8)	6(3.9)	146(96.1)		
	Gave birth 1-2	133(25.2)	5(3.8)	128(96.2)		
	Gave birth 3-4	97(18.4)	5(5.2)	92(94.8)		
	Delivered 5 and above	145(27.5)	8(5.5)	137(94.5)		

BCS: body condition scoring

4.2. Prevalence of CIDT reactivity in flock and goats owned by cases (TB cases) and controls (TB free owners)

Case-control study was conducted on a total of 283 goats; 160 owned by households with TB and 123 owned by households not diagnosed with TB, were CIDT tested in 70 flocks from ATJK districts. Two cut-off points of skin test result (≥4mm and ≥2mm) were employed for the estimation of the prevalence at household/flock and goat levels. Table 4 shows the flock and goat prevalence of caprine TB using the two cut-off points. Prevalence of flocks owned by TB positive cases was 26.3% (10/38) at ≥4mm cut-off while prevalence of flocks owned by TB free controls was 12.5% (4/32) at ≥4mm cut-off. The overall prevalence of flocks was 20% (95% CI: 10.4-29.6) at cut-off ≥4mm and 24.3% (95% CI: 14.0-34.6) at cut-off ≥2mm. At both cut-off points the CIDT-positive flock prevalence was not statistically significant. Prevalence of individual goats owned by TB positive cases was 13.1% (21/160) and that of TB free controls was 3.3% (4/123) at ≥4mm cut-off. Prevalence of goats owned by TB positive cases was 14.4% (23/160) and that of TB free controls was 4.1% (5/123) at ≥2mm cut-off. The overall prevalence of goats in case-control study was 8.8% (95% CI: 5.5-12.2) at a cut-off ≥4mm while it was 9.9% (95 CI: 6.4-13.4) at cut-off ≥2mm. Unlike for the flock prevalence, goat prevalence was statistically significant P=0.004 at both cut-off points in goats owned by households with TB patients in relation to goats owned by TB free households from the same village (Table 4).

Table 4: Caprine TB prevalence in flocks and animals owned by households with TB positive case and TB free control households in the same village at different cut-off points.

	≥4mm cutoff			≥2mm cutoff		
Household Status	TB positive case	TB Free control	Total	TB positive case	TB Free control	Total
Flock Prevalence						
No. of flocks tested	38	32	70	38	32	70
No. of flocks positive	10	4	14	12	5	17
Prevalence (95% CI)	26.3 (11.6-41.0)	12.5 (0.4-24.6)	20 (10.4-29.6)	31.6 (16.1-47.1)	15.6 (2.3-28.9)	24.3 (14.0-34.6)
P-value		0.150				0.121
Goat Prevalence						
Number of goats tested	160	123	283	160	123	283
Number of goats positive	21	4	25	23	5	28
Prevalence (95% CI)	13.1 (7.8-18.4)	3.3 (0.07-6.4)	8.8 (5.5-12.2)	14.4 (8.9-19.9)	4.1 (0.5 7.6)	9.9 (6.4-13.4)
P-value			0.004			0.004

4.3. Knowledge, attitude and practice of goat owners with regards to tuberculosis

A comparative assessment of different risk factors was made between TB positive (case) and TB negative (control) goat owners. 70 households; 38 TB positive cases and 32 TB free controls were traced back. Several demographic characteristics and other factors were considered to investigate their possible association with caprine TB recognition of the respondents, however, there was no statistically significant association ($P>0.05$). Goat owners who were TB positive were more likely to have CIDT test positive animals and more likely to have awareness about TB than the TB free control group living in the same village. Higher proportion of TB positive goat owners did not know TB transmission ways and consume mixed animal products than TB free control group living in the same village. The results of selected characteristics of study participants are summarized in Table 5.

Table 5: Comparison of risk factors for human TB

		Number (%)				
No	variables	TB positive Cases(n=38)	TB free controls(n=32)	Total n=70	χ2	P-value
1.	Residence				0.32	0.57
	Urban	18(47.4)	13(40.6)	31(44.3)		
	Rural	20(52.6)	19(59.4)	39(55.7)		
2.	Owner sex				0.37	0.54
	Male	26(68.4)	24(75.0)	50(71.4)		
	Female	12(32.4)	8(25.0)	20(28.6)		
3.	Owner age				0.41	0.52
	<18 years	0(0.0)	0(0.0)	0(0.0)		
	18-45 years	33(86.8)	26(81.3)	59(84.3)		
	>45 years	5(13.2)	6(18.8)	11(15.7)		
4.	Occupation				1.33	0.52
	Farmer	21(55.3)	19(59.4)	40(57.2)		
	Civil servant	7(18.4)	8(25.0)	15(21.4)		
	Others	10(26.3)	5(15.6)	15(21.4)		
5.	Education				0.14	0.99
	No formal education	15(39.5)	12(37.5)	27(38.6)		
	Primary school	14(36.8)	13(40.6)	27(38.6)		
	Secondary school	8(21.1)	6(18.8)	14(20.0)		
	College/university	1(2.6)	1(3.1)	2(2.8)		
6.	Share night shelter				0.00	0.99
	Yes	13(34.2)	11(34.4)	24(34.3)		
	No	25(65.9)	21(65.6)	46(65.7)		
7.	His goats are TB positive				2.41	0.12
	Yes	12(31.8)	5(15.6)	17(24.3)		
	No	26(68.4)	27(84.4)	53(75.7)		
8.	Knows/heard about caprine TB				0.46	0.50
	Yes	7(18.4)	4(12.5)	11(15.7)		
	No	31(81.6)	28(87.5)	59(84.3)		
9.	Knows TB transmission ways				6.10	0.11
	Do not know	29(76.3)	27(84.4)	56(80.0)		
	Raw milk	3(7.9)	5(15.6)	8(11.4)		
	Raw meat	3(7.9)	0(0.0)	3(4.3)		
	Aerosol, raw milk and meat	3(7.9)	0(0.0)	3(4.3)		
10.	Habit of milk consumption				1.78	0.41
	Boiled milk only	2(5.3)	0(0.0)	2(2.8)		
	Raw milk	11(28.9)	9(28.1)	20(28.6)		
	Mixed (boiled, raw, mixed with coffee)	25(65.8)	23(71.9)	48(68.6)		
11.	Habit of meat consumption				0.02	0.90
	Cooked only	1(2.6)	1(3.1)	2(2.9)		
	Raw only	0(0.0)	0(0.0)	0(0.0)		
	Mixed (raw, cooked)	37(97.4)	31(96.9)	68(97.1)		

The result of logistic regression taking skin test as a binary outcome and TB status of the owner (confirmed positive versus negative as main exposure variable) is summarized in Table 6. The risk of acquiring the disease was tested by unadjusted effect of being owned by confirmed case compared to being owned by control and it was found that goats owned by TB positive patients have odds of acquiring the infection 0.40 times higher at both cut-off points than those owned by TB free controls.

Table 6: Effect of being owned by households with confirmed TB positive patients on skin test result of goat in ATJK district.

Factor	Cut-off ≥4mm	cut-off ≥2mm
	OR (95%CI)	OR (95%CI)
Unadjusted effect of being owned by confirmed case compared to being owned by control	0.40(0.11-1.43)	0.40(0.12-1.30)
Effect of being owned by confirmed case compared to being owned by control adjusted for owner characteristics		
Sex of respondents	1.47(0.35-6.22)	1.46(0.39-5.46)
Age	0.84(0.08-8.35)	1.36(0.22-8.25)
Share house with goats	0.25(0.06-06.96)	0.30(0.09-1.03)
Knowledge about caprine TB	0.11(0.22-0.52)	0.16(0.04-0.69)
Effect of being owned by confirmed case compared to being owned by control adjusted for goat characteristics		
Goat sex	0.52(0.01-20.57)	0.54(0.01-21.32)
Goat age	0.48(0.01-18.87)	0.49(0.01-19.37)
BCS		
Poor	1	
Medium	2.61(0.83-8.21)	1.74(0.65-4.66)
Fat	0.62(0.06-6.01)	0.39(0.04-3.52)
Parity		
Did not deliver	1	
1-2 kids	1.7(0.60-4.91)	2.36(0.87-6.43)
3-4 kids	2.56(0.81-8.07)	2.38(0.76-7.47)
≥ 5 kids	0.15(0.00-6.18)	0.16(0.00-6.54)

4.4. CIDT test result

Goats' skin at their neck region was re-measured by caliper 72hr later post CIDT test. Accordingly, goats which were positive to CIDT test showed swelling reaction on their neck at PPD injection site of different size for both avian and bovine PPD injections.

Fig. 6: TB positive goat skin fold's thickness 72h of injection of PPD

4.5. Post mortem examination

Post mortem examination was performed on goats that showed strong bovine tuberculin reactors. Figures below show visceral organs with tuberculous lesions from slaughtered goats.

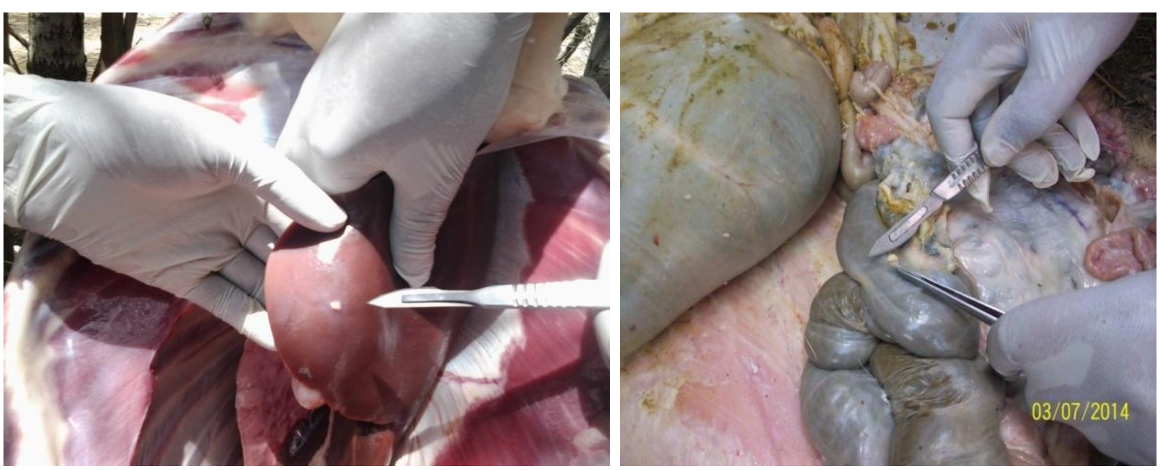

Fig. 7: Tuberculin lesion on liver and intestinal wall

4.6. Bacteriological examination of human and goat samples

Seven different tuberculous tissue lesions were obtained from two CIDT test strong positive goats that belong to AFB smear positive household and cultured on LJ media. Out of 7 tissues cultured, only 14.3% (1/7) was culture positive.

Similarly, human sputum was collected from AFB smear positive patients from Batu Share hospital and two health centers in Batu town. Out of 38 human sputa collected and cultured 31.6% (12/38) of them showed growths on the LJ media. Heat-killed cells of each positive isolate were prepared by mixing, about two loops full of cells (about 20µl cell pellet) in 200µl distilled water followed by incubation at 80^0C for 1 hour and then stored at -20^0C until used for molecular typing. Culture positive isolates were also prepared as 20% glycerol stocks and stored at -80^0C.

4.7. Molecular analysis

4.7.1. Genus typing

The goat isolates was amplified by m-PCR for genus typing and the process revealed a product characteristic to genus *Mycobacterium*. Fig.8 shows electrophoretic separation of PCR products of m-PCR typing of the genomic DNA of mycobacteria isolated from TB lesions of goat. Since goat isolate was single and because of resource limitation this isolate was directly subjected to RD9 deletion together with other human isolates.

4.7.2. Deletion typing

A total of 12 AFB and culture positive human isolates and one goat culture positive isolate were subjected to RD9 deletion typing. Out of the 12 heat-killed positive human isolates tested for RD9, 91.7% (11/12) had intact RD9 locus and were subsequently classified as *M. tuberculosis* while one human isolate and goat isolate were RD9 deleted. Fig.8 shows electrophoretic separation of PCR products of RD9 deletion typing.

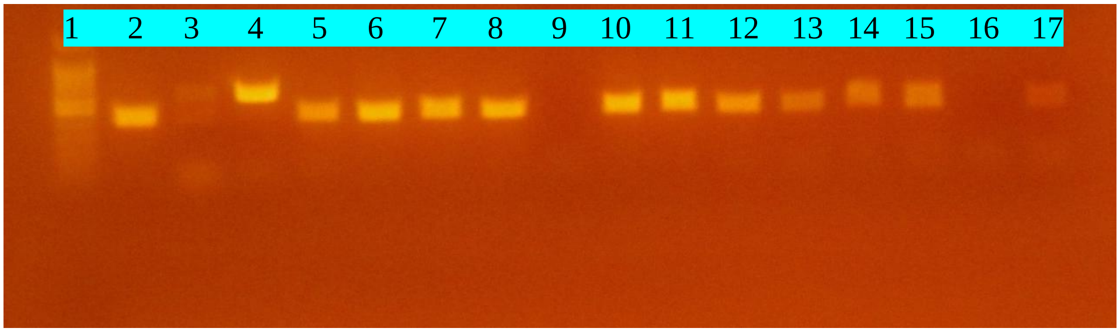

Fig. 8: Electrophoretic separation of PCR products by RD9 deletion typing of the genomic DNA of mycobacteria isolated from human sputum and tuberculous lesion of goat. Lane 1= 100bp ladder, Lane 2= *M.tuberculosis* positive control Lane 3= distilled water negative control, Lane 4=*M. bovis* positive control Lane 5-8; 10-15; 17 = positive samples for *M. tuberculosis*, Lane 9 negative sputum sample and 16= negative goat sample.

Therefore, the PCR analysis of the 13 isolates from human sputa and animal tissue indicated the presence of mycobacteria characteristic to MTBC in 84.6% (11/13) of the samples. The remaining isolates did not give signal characteristic to MTBC. Both human and goat isolates which showed positive results were spoligotyped for further strain identification.

4.7.3. Spoligotyping

A total of 10 strains from sputum samples were spoligotyped. Two lineages were recognized in this finding in the strain collection based on spoligotype features characteristic for each lineage; the Euro-American (E-A) and Indo-Oceanic (I-O) lineages. Half of the lineages were E-A while the rest half were I-O lineages. All of the 10 isolates that were characterized to the strain level by spoligotyping gave good and interpretable patterns characteristic to both the genus Mycobacterium and MTBC. The most common patterns were Spoligo International Type Number (SIT) 53 consisting of 3 isolates accounting for 30% of the isolates. SIT44, SIT54 SIT523 were among the recognized patterns in the present finding. The patterns of four strains were not recognized by SpolDB3, the international spoligotyping database (Brudey *et al*., 2006) indicating that they were new strains. Among the unregistered strain patterns, one belongs to E-A while the rest three belong to I-O lineage. Table 7 shows the spoligotypes obtained from the present study. Goat isolate was also spoligotyped and gave good pattern. But it was not

registered in the spoligotypes the Mbovis.org database. The strain lacked spacers 2-5, 9-10, 12, 14-16, 36 and 39–43.

Table 7: The result of spoligotyping of isolates from sputum samples

ID	Species	Binary form	Octal code	SIT	Family	Lineage
H37RV	Mtb					
Qiagen H_2O						
D01	Mtb	1111111111111111111111111111111100001111111	777777777760771	53	T1	E-A
B05	Mtb	1111111111111111111111111111111100111111111	777777777763771	54	33	I-O
730	Mtb	1110100111111111111111011000110000111011111	723777754303571	N0	33	I-O
763	Mtb	1111111111111111111111011111111100001111111	777777757760771	44	T1	E-A
7416	Mtb	1111111111111111111111111111111111111111111	777777777777771	523	33	I-O
763	Mtb	1111111111111111111111111111111100001111111	777777777760771	53	T1	E-A
781	Mtb	1111111111111111111111111111111100001111111	777777777760771	53	T1	E-A
816	Mtb	1110100111111111111111001000110000111111111	723777744303771	N0	33	I-O
G01	Mtb	1111111110111101111111111111111100111111111	777367777763771	N0	33	I-O
813	Mtb	1111111111111101101111111111111100001110111	777766777760731	N0	T1; X1	E-A
H37Rv	Mtb					

Mtb = *M. tuberculosis*; SIT= Spoligo International Typing; E-A = Euro-American; I-O = Indo-Oceanic

5. DISCUSSION

Reports of caprine TB are increasing in several countries. Recent studies are demonstrating the increasing incidence of caprine TB infection and its effect on goat and public health and economic losses due removal of infected animals and limitations to trade (Aranaz *et al*., 2003; Kubica *et al*., 2003). There is no comprehensive data on the occurrence TB in goats in Ethiopia regardless of the evidences of its existence in certain regions of the country (Nigussie, 2005; Hiko and Agga, 2011; Tafese *et al*., 2011; Mamo *et al*., 2012; Ameni *et al*., 2013; Deresa *et al*., 2013). Most of these studies focused on prevalence of TB in goats using abattoir studies in the central Ethiopia. The present study has shown the occurrence of TB in human and goats at ATJK district.

The present cross-sectional study result shows that the prevalence of individual goats was 4.6% at ≥2mm cut-off points and 3.8% at ≥4mm cut-off points. The presently recorded prevalence was in agreement with previous study which was 3.8% at ≥2mm cut-off point recorded in small ruminants in four districts of Afar Pastoral Region of northeastern Ethiopia (Mamo *et al*., 2012). Comparative result with the present study was reported in Ethiopian goats (ILRI, 1996) which was 4.2%, by Hiko and Agga, (2011) who reported 4.2% in goats slaughtered at Mdjo abattoir and with a report by Tafese *et al*., (2011) who recorded a prevalence of 3.1% in goats of Adami Tulu research center with SIDT skin test in the same study area and report by Deresa *et al* (2013) who reported 3.5% in goats brought from different regions of Ethiopia and slaughtered at Luna Export abattoir. This comparative result could be attributable to extensive management system employed at each study sites and similar environmental conditions of the present study area with some of them. Tafese *et al*. (2011) conducted SIDT test and reported higher animal prevalence of 9.6% for goats of smallholder farmers of the same study area than the present study. This variation could be due to study methodology, number of animals included in the study and other conditions at the time of the study that might affected the result adversely. However, this result was different from the result of a previous study done in Hamer pastoral district of southern Ethiopia, which indicated the absence of the disease in 186 goats using CIDT test (Tschopp *et al*., 2010) and only 0.2% individual animal prevalence of goats obtained during cross-sectional study of BTB in Somali pastoral livestock, southeast Ethiopia (Gumi *et al*., 2012). This difference might be related to the difference in geographical location of the two studies in which the epidemiology of the disease might vary between these areas. The present study result was far greater than the report of European Union (EU) member states where four member states reported prevalence of 1.2% positive findings in goats in 2011 (EFSA and ECDC, 2013) and report of Pignata *et al*.(2009) who reported CIDT test result of 0.47% prevalence of caprine TB in semi-arid of Paraiba, Northeast Brazil. The present finding shows higher prevalence of caprine TB compared to the findings of Rahmana *et al*. (2013) who reported 1.29% and 1.46 in goats and sheep, respectively in Bangladesh. However, the present study presents lower result compared to study conducted in four slaughterhouses in the northern region of Algeria which found 60 suspicious lesions of TB among 995 inspected goat carcasses giving an infection rate of 6.03% (Naima *et al*., 2011). These differences might be attributable to the differences in the geographical location, number of animals included in the study, study methodology and management systems employed at respective study areas.

Several animal characteristics have been considered as risk factors predisposing goats to caprine TB infection in cross-sectional study. However, none of the considered factors were associated with CIDT positivity of goats at ≥2mm cut-off pints. An important proportion of the goats seem to be free of TB, despite the absence of control measures. In addition, the infected animals do not seem to suffer much from the infection since their body condition seems not affected. Therefore, infected goats are unlikely to excrete much pathogen, compared to clinical TB cases, bringing the transmission by the aerial, digestive and milk routes to a low level subsequently low level of prevalence for this study. In ATJK district, animals are usually kept in free range, in a mostly dry or warm environment, unfavorable to the survival of the mycobacteria (Cleaveland *et al*., 2007). Close contact only occurs at night, when animals are brought together but, even then, animals stay outside, in well ventilated conditions. Finally, most of the flocks are small, which results in relatively little contacts between animals (Cleaveland *et al*., 2007). Therefore, this relatively low prevalence in the present study may be attributable to inefficient close contact between diseased and healthy animals in extensive systems and possibly a decrease in virulence and transmission capacity of the causal strains due to adverse weather (Oloya *et al*. 2006).

In the case-control study the of the present investigation, prevalence of TB in both flock and individual goats was higher (31.6% and 14.4%), respectively in goats owned by TB positive households compared to TB free control farmers at ≥2mm cut-off points. This suggests the existence of transmission of mycobacterial species between human and their goats. This transmission could be possible in both directions. The disease transmission may be cyclical by nature: animal-to-man-to-animal (reviewed in Cosivi *et al*., 1998), underlying the existence of higher risk of dissemination of mycobacteria among the domestic animal and human populations. Similar findings were reported by Cook *et al.* (1996) and Ameni *et al*. (2001; 2013) where animal TB was higher in animals owned by confirmed TB positive patients. In addition, Andersen (1997) indicated that humans acquire the infection primarily by ingesting the agent in raw milk and milk products, and secondly by inhaling it when there is close physical contact between the owner and his/her animal, especially at night since in some cases they share shelters with their animals. Cosivi *et al*. (1998) indicated that pulmonary as well as extra pulmonary cases of human TB of animal origin will continue to be of public health importance especially in areas where the prevalence of infection is high in cattle and where raw milk or its products are commonly consumed.

From the interview, it was investigated that only 18.4% of the goat owning TB positive family knew or have heard about caprine TB. This finding was in agreement with report of Ameni *et al* (2013) who reported 19.9% of TB positive cases of cattle owners knew BTB. However, 81.6% of the TB free goat owners in the present study did not know about caprine TB which disagrees with the same report of Ameni *et al* (2013) who reported that 42.1% of TB free controls did not know about BTB and with that of Bonsu *et al*., (2000) who reported that 30% of the flocksmen did not know BTB in Dagma-West district of Ghana. The present finding on the awareness/knowledge of the study participants concerning caprine TB does not agree with the report of Zeru *et al*.

(2014) who reported that 30.8% of the respondents had recognized or had heard about BTB in and around Mekelle, Northern Ethiopia. From the interview, it was also observed that the majority of study participants in both TB cases and TB free farmers consumed mixed milk (raw milk mixed with coffee), but there was no association between consumption of the mixed milk and occurrence of human TB case. This was different from earlier reports, which associated raw milk consumption with extra pulmonary TB (O'Reilly and Daborn, 1995; Shitaye *et al*., 2007).

The present study demonstrated statistically strong association between bovine tuberculin skin positivity of individual goats being owned by TB positive owners versus being owned by TB free controls. Goat prevalence was significantly higher (P=0.004) at both cut-off points in goats owned by households with TB patients than in goats owned by TB free individual households from the same village. This finding was in agreement with the finding of Ameni *et al.* who found similar result on the study conducted on the transmission of *M. tuberculosis* between farmers and cattle in central Ethiopia (Ameni *et al*., 2013). The risk of acquiring caprine TB infection of goats being owned by TB positive households was 0.40 times higher than being owned by TB free persons at both cut-off points. Moreover, female goats and older goats owned by TB infected owners showed higher proportion of bovine tuberculin test positivity by 0.54 and 0.49 times, respectively than those owned by TB free owners at ≥2mm cut-off points. This might be related to the fact that older animals have longer duration and repeated chance of exposure to mycobacterial infection with their age (Radostits *et al*., 2000). The result was inconsistent with previous studies where cattle TB have been reported (Ameni *et al*., 2007; Cadmus *et al.,* 2010).

Out of samples collected and cultured, only 14.3% tissues with tuberculous lesion and 31.6% of sputa collected from human were culture positive on LJ media. The tissue culture finding is in agreement with that of Deresa *et al*. (2013) who reported 14% of bacterial growth from TB like tissue lesions in abattoir based study in Luna Export abattoir, central Ethiopia. However, the present sputa culture result is inconsistent with that of Gumi *et al*. (2012) who reported 67% AFB positive culture isolates from sputa collected from South-East Ethiopian pastoralists and Ameni *et al*. (2013) who reported 97% culture positivity of sputum from farmers with active pulmonary TB in central Ethiopia. The variation might be due to small number of samples used in the present study compared to those which showed higher prevalence growth rate. But this finding is not comparable with reports of Yalelet, (2010) and Hussein, (2009) in which no growths of *M. bovis* from milk of cows positive to CIDT test was reported. The low culture yield from gross lesions specimens in goat in the present study is consistent with existing data from Ethiopia (Berg *et al*. 2009). This may be due to variable factors like the presence of other granulomatous diseases in the animal or few number viable bacteria in the lesion taken for culture. Moreover, a low isolation rate of mycobacteria in the present study might be resulted from reduced sensitivity of culture, the freeze-thaw cycles that occurred during transportation and contamination of samples (Cleaveland *et al*., 2007).

The molecular typing of goat isolate using m-PCR revealed that the isolate belong to the genus Mycobacterium, and up on spoligotyping the isolate gave good result with interpretable pattern indicating the strain to be *M. bovis*. However, the strain was new and was not registered in Mbovis.org database. Other authors reported isolation of *M. bovis* and *M. tuberculosis* from goats slaughtered at the Mojo export abattoir in Ethiopia; however, their diagnosis was based only on colony morphology and discrimination by culture on growth media with pyruvate or glycerol (Hiko and Agga, 2011).

Out of the 12 human isolates that were subjected to RD9 based deletion typing, 91.7% (11/12) indicated all of them belong to a single species of *M. tuberculosis* from human sputum samples and found to be identical strains. PCR-results showed that none of the patients' samples was infected with *M. bovis*. All the 10 human isolates that were spoligotyped gave good and interpretable patterns in the present study. 100% of the human isolates were identified as *M. tuberculosis* species in the present study. The most common spoligotype identified from TB patients was the T family and the predominant lineage was the Euro-American. Similar to the present study, previous studies in Ethiopia showed that E-A and CAS genotypes were the dominant families (Bruchfeld *et al.*, 2002; Agonafir *et al*., 2010). The present finding is in agreement with report of Yimer *et al*.

(2013) who identified the most predominant families to be T and CAS families which accounted to 30.8% and 38.8% of the total strain, respectively from pulmonary TB patients in Amhara Region, Ethiopia. Dominance of the families resembles the distribution pattern in eastern African countries suggesting a similar transmission trend (Brudey *et al*., 2006). Nonetheless, no *M. bovis* was isolated from the TB positive farmers in this study in agreement with result of the finding of the study conducted by Ameni *et al*. (2013). Unlike the previous findings (Yimer *et al.*, 2013), less diversity of strains in this study might be due to fewer number specimens which were limited due to time bound and inclusion and exclusion criteria. The absence clinical isolation of *M. tuberculosis* neither from goats nor *M. bovis* from human sputum in the present study might suggest that there was no transmission, regardless of the chance being there, of the clinical isolates between the respective hosts in the particular study area which is different from the findings of other investigators who isolated *M. tuberculosis* from animals (Mamo *et al*., 2012; Ameni *et al*., 2013; Deresa *et al*., 2013)

40% of the study subjects were infected with new TB strains that have not previously been described in SpolDB3. These new strains are a valuable contribution to the global database and are important for an understanding of the evolutionary development and diversity of *M. tuberculosis* strains circulating in Ethiopia.

The present finding offer no evidence for transmission of the etiological agent of pulmonary TB from goat to human but the transmission might be from human to human. However, despite close contact between humans and livestock, including consumption of raw milk and meat, it was not possible to indicate the incidence rate and subsequent prevalence of *M. bovis* in human TB patients. The same result was reported from southern Ethiopia where there was no identification of *M. bovis* as an etiological agent of TB lymphadenitis in patients from Butajira and other parts of Ethiopia (Beyene *et al*., 2009).

6. CONCLUSION AND RECOMMENDATIONS

Different authors at different times described the burden of TB infection in Ethiopia in general and caprine infection with mycobacterial species belonging to MTBC in particular in few corners of the country. Evidence of transmission through different routes and impact of the disease on the economy, social, public health and political aspects of the country has also been indicated by different investigators. TB in goats appears to be widespread and serious especially in goat rearing regions of the Ethiopia. A number of studies that are under way in order to elucidate the existing economic and public health impact of the disease and others so far conducted did not indicate the overall prevalence and predictable, if not conventional, transmission of tuberculosis infection in goats at country level. This study revealed a moderately low prevalence of TB in goats residing in ATJK district. Findings from this study add useful epidemiological data/information regarding caprine and human TB infection at ATJK district.

Data on animal TB from different counties show animal TB is one of the most devastating diseases in developing countries in terms of its importance in public and animal health. Moreover, it is known to induce significant decrease in productivity and results in strong restrictions on trade (live animal and animal product) with substantial economic losses. The present study has shown the occurrence of TB in human and goats at ATJK district. Although the extent is higher at rural villages, both rural and urban communities in the study area were found to have low knowledge on caprine TB and ways of TB transmission between animals and humans. Moreover, majority of the community are engaged in husbandry practices such as free movement of goat, sharing of night shelter with goats, consumption of raw animal products. This increases the chance of disease transmission between human and animal as well as between livestock. The study further showed that old and higher parity class animals raised in the areas were more likely to be test positive for caprine TB. Furthermore, animals owned by TB positive patients were found to be at higher risk of being positive CIDT test. This information is useful and could be used by respective authorities in designing appropriate control measures against zoonotic TB. *M. tuberculosis* and *M. bovis* were isolated and characterized as causative agents of TB in human and goats in the study area. The isolation of these species in respective hosts indicates the need for further studies to understand their transmission dynamics and the role of goats in the epidemiology of human and animal TB in the area where potential epidemiological risk factors for infection and transmission between livestock and human exist. In light with the aforementioned findings, the following recommendations are forwarded.

- To find the actual profile of the disease in the study area, further study on the epidemiology TB using increased sample of goats and human pulmonary TB patients is important.
- Isolation of the etiologic agent must be done and prevention and control strategy should be set with.
- Disease surveillance, screening test and culling of tuberculosis infected goats need to be developed.
- Public awareness should be raised on zoonotic TB and preventive measures should be implemented primarily focusing on minimizing contact between animals and humans in both urban and rural communities.
- Diagnosis of TB should not be limited to AFB smear or clinical symptoms and must be done to the extent of strain typing so as to establish lineages particular to the region which provides easy implementation of treatment and control strategy.

7. REFERENCES

Adams, D.O. (1976): The granulomatous inflammatory response. A review. *Am. J. Pathol.*, **84**:164–91.

Agonafir, M., Lemma, E., Wolde, Meskel, D., Goshu, S., Santhanam, A., *et al*. (2010):

Álvarez, J., de Juan, L., Bezos, J., Romero, B., Saez, J.L., Reviriego, F.J., *et al*. (2008): Interference of paratuberculosis with the diagnosis of tuberculosis in a goat flock with a natural mixed infection. *Vet. Microbio.*, **128:** 72–80.

Ameni, G., Amenu, K. and Tibbo, M. (2003): Prevalence and risk factor assessment in cattle and cattle owners in Wuchale-Jida District, Central Ethiopia. *Int. J. Appl. Res. Vet. Med.*, **1**: 17–25.

Ameni, G., Aseffa, A., Engers, H., Young, D., Gordon, S., Hewinson, G. and Vordemieier, M. (2007): High prevalence and increased severity of pathology of bovine tuberculosis in Holsteins compared to zebu breeds under field cattle husbandry in central Ethiopia. *Clin.Vacc. Immunol.*, **14**: 1356–1361.

Ameni, G., Aseffa, A., Engers, H., Young, D., Hewinson, G. and Vordermeier, M. (2006): Cattle husbandry in Ethiopia is a predominant factor affecting the pathology of bovine tuberculosis and gamma interferon responses to mycobacterial antigens. *Clin. Vacc. Immunol*., **13:** 1030–1036.

Ameni, G., Desta, F. and Firdessa, R. (2010): Molecular typing of *Mycobacterium bovis* isolated from tuberculosis lesions of cattle in northeastern Ethiopia. *Vet. Rec.*, **167**: 138–141.

Ameni, G., Hewinson, G., Aseffa, A., Young, D. and Vordermeier, M. (2008): Appraisal of interpretation criteria for the comparative intradermal tuberculin test for diagnosis of tuberculosis in cattle in central Ethiopia. *Clin. Vacc. Immunol.*, **15**: 1272–1276.

Ameni, G., Ragassa, A., Kassa, T. and Medhin, G. (2001): Survey on bovine tuberculosis and its public health implications to cattle raising families in Wolaita Soddo, southern Ethiopia. *Ethio. J. Anim. Prod.*, **1**: 55–62.

Ameni, G., Tadesse, K., Hailu, E., Deresse, Y., Medhin, G., *et al*. (2013): Transmission of *Mycobacterium tuberculosis* between Farmers and Cattle in Central Ethiopia. *Tuberc. Cattle and Farmers.*, **8**:10.

Andersen, P. (1997): A review: host responses and antigens involved in protective immunity to *Mycobacterium tuberculosis*. *Scandinavian J. Immun.*, **45**: 115–131.

Angus, R. D. **(**1978): Production of reference PPD tuberculins for veterinary use in the United States. *J. Biol. Stand*., **6:** 221–227.

Aranaz, A., Cousins, D., Mateos, A. and Dominguez, L. (2003): Elevation of *Mycobacterium tuberculosis* subsp. *caprae* to species rank as *Mycobacterium caprae* comb. nov., sp. nov. *Int. J. Syst. and Evol. Mcrobiol.,* **53**: 1785–1789.

Aranaz, A., Liebana, E., Mampaso, G. E., Galan, J., Cousins, D., Ortega, A. *et al*. (1999): *Mycobacterium tuberculosis* subsp. *caprae* subsp. nov.: A taxonomic study of a new member of the *Mycobacterium tuberculosis* complex isolated from goats in Spain. *Int. J. Syst. Bacteriol*., **49**: 1263–1273.

Aranaz, A., Liebana, E., Mateos, A., Domingue,z L., Vidal, D., Domingo, M., *et al*. (1996): Spacer oligonucleotide typing of *Mycobacterium bovis* strains from cattle and other animals: A tool for studying epidemiology of tuberculosis. *J. Clin. Microbiol*., **34**: 2734–2740.

Arendonk, J.A.M. and Liinamo, A.E. (2003): Dairy cattle production in Europe, *Theriogenol.,* **59**: 563–569.

Ayele, W.Y., Neill, S.D., Zinsstag, J., Weiss, M.G. and Pavlik, I. (2004): *Bovine tuberculosis*: An old disease but a new threat to Africa. *Int. J. Tuberc. Lung. Dis.,* **8:** 924-937.

Bakshi, C.S., Shah, D.H., Verma, R., Singh, R.K. and Malik, M. (2005): Rapid differentiation of *Mycobacterium bovis* and *Mycobacterium tuberculosis* based on a 12.7-kb fragment by single tube multiplex-PCR. *Vet.Microbiolo*., **109**: 211-216.

Barrera, L. (2007): The Basics of Clinical Bacteriology. Tuberculosis Book: From Basic Science to Patient Care. Edt Palomino, J.C., Leao, S.C., and Ritacco, V., www.TuberculosisTextbook.com.

Barrow, P. A. and Gallagher, J. (1981): Aspects of the epidemiology of bovine tuberculosis in badgers and cattle. The prevalence of infection in two wild animal populations in South-west England. J. Hyg., **86**: 237-245.

Berg, S., Firdessa, R., Habtamu, M., Gadisa, E., Mengistu, A., Yamuah, L., *et al.* (2009): The burden of mycobacterial disease in Ethiopian cattle: Implication for public health. *Rev. Infect. Dis.*, **4**: 1270-4.

Beyene, D., Bergval, I., Hailu, E., Ashenafi, S., Yamuah, L., Aseffa, A., *et al*. (2009): Identification and genotyping of the etiological agent of tuberculous lymphadenitis in Ethiopia. *J. Infec. Develop. Count*., **3**: 412–419

Bezos, J., Álvarez, J., Mínguez, O., Marqués, S., Martín, O., Vigo, V. *et al.* (2011): Evaluation of specificity of tuberculosis diagnostic assays in caprine flocks under different epidemiological situations. *Res. Vet. Sci.* (in press).

Biberstein, E.L. and Hirsh, D.C. (1999): *Mycobacterium* species: The agents of animal tuberculosis. In: Hirsh, D.C. and Zee, Y.C. (eds). Veterinary Microbiology. Blackwell Science, Inc., Massachusetts, USA, 158-164.

Bilal, Sh., Iqbal, M., Murphy, Ph. and Power, J. (2010): Human bovine tuberculosis – remains in the differential. *J. Med. Microbiol.*, **59**: 1379–1382.

Bogale, A. (1999): Bovine tuberculosis a cross-sectional and epidemiological study in and around Addis Ababa. MSc thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.

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

Brooks, G., Butel, J.S. and Morse, S.A. (2004): Jewetz, Menlick, and Adelberg's Medical Microbiology. 23rd ed. New York, USA. The MacGraw-Hill Medical Publishing, 319-330.

Brooks, G., Carroll, K.C., Butel, J.S. and Morse, S. A. (2007): Jawetz, Melnick and Adelberg's Medical Microbiology 24th Edition. McGraw-Hill Companies, Inc., 320-326.

Brosch, R., Gordon, S.V., Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K. *et al*. (2002): A new evolutionary scenario for the M*ycobacterium tuberculosis* complex. *Proc. Natl. Acad. Sci*., USA, **99**: 3684–3689.

Bruchfeld, J., Aderaye, G., Palme, I.B., Bjorvatn, B., Ghebremichael, S., *et al*. (2002): Molecular Epidemiology and Drug Resistance of *Mycobacterium tuberculosis* isolates from Ethiopian Pulmonary Tuberculosis Patients with and without Human Immunodeficiency Virus Infection. *J Clin Microbiol.* **40**: 1636–1643.

Brudey, K., Driscoll, J.R., Rigouts, L., Prodinger, W.M., Gori, A., *et al*. (2006): *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol.*, **6**:23.

Cadmus, S. I. B., Agada, C. A., Onoja, I. I. and Salisu, I. (2010): Risk factors associated with bovine tuberculosis in some selected flocks in Nigeria. *Trop. Ani.Hlth. Prod.*, **42**: 547–549.

CDC (2008): Centers for Disease Control and Prevention (CDC)-Transmission and Pathogenesis of Tuberculosis. In: Self-Study Modules on Tuberculosis. National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention Division of Tuberculosis Elimination, Atlanta, Georgia.

CFSPH (2007): Centre for Food Security and Public Health (CFSPH)-Bovine Tuberculosis. College of Veterinary Medicine, Iowa State University, www.cfsph.iastate.edu.

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

CLSI (2008): Clinical and Laboratory Standard Institute (CLSI)-Laboratory Detection and Identification of mycobacteria; Approved Guideline- CLSI Document M48-A Wayne, PA.

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

Cook, A.J.C., Tuchili, L.M., Buve, A., Foster, S.D., Godfrey-Faussett, P., Pandey, G.S. and McAdam, K.P.W.J. (1996): Human and bovine tuberculosis in the Monze district of Zambia – a cross-sectional study. *Br. Vet. J.*, **152:** 37–46.

Corner, L.A. (1994): Post-mortem diagnosis of *Mycobacterium bovis* infection in cattle. *Vet Microbiol*: **40**:53–63.

Cosivi, O., Grange, J.M., Daborn, C.J., Raviglione, M.C., Fujikura, T., Cousins, D., *et al*. (1998): Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. *Em. Infec. Dis.,* **4**: 1-17.

Cosivi, O., Meslin, F.X., Daborn, C.J. and Grange, J.M. (1995): Epidemiology of *Mycobacterium bovis* infection in animals and humans, with particular reference to Africa. *Rev. Sci. Tech. Off. Int. Epiz.,* **14**: 733-746.

Cotter, T.P., Sheehan, S., Cryan, B., O'Shaughnessy, E., Cummins, H. and Bredin, C.P. (1996): Tuberculosis due to *Mycobacterium bovis* in humans in the south-west region of Ireland: is there a relationship with infection prevalence in cattle? *Tuberc. Lung Dis.*, **77**: 545-8.

Cousins, D.V. (2001): *Mycobacterium bovis* infection and control in domestic livestock, *Rev. Sci. Tech. Off. Int. Epiz.,* **20:** 71-85.

Cousins, D.V. and. Florisson, N. (2005): A review of tests available for use in the diagnosis of tuberculosis in non-bovine species. Rev. Sci. Tech. *Off. Int. Epiz*., **24**: 1039-1059.

Crawshaw, T., Daniel, R., Clifton-Hadley, R., Clark, J., Evans, H., Rolfe, S. and Rua-Domenech, R. (2008): TB in goats caused by *Mycobacterium bovis*. *Vet. Rec.,* **163**: 127.

Cvetnic, Z., *et al*. (2007): *Mycobacterium caprae* in cattle and humans in Croatia. *Int. J. Tuberc. Lung Dis*., **11**: 652–658.

Daniel, R., Evans, H., Rolfe, S., Rua-Domenech, R., Crawshaw, T., Higgins, R.J., Schock, A. and Clifton-Hadley, R. (2009): Outbreak of tuberculosis caused by *Mycobacterium bovis* in golden Guernsey goats in Great Britain. *Vet. Rec*., **165:** 335-342.

Davies, P.D., Yew, W.W., Ganguly, D., Davidow, A.L., Reichman, L.B., Dheda, K. and Rook, G.A. (April 2006): "Smoking and tuberculosis: the epidemiological association and immunopathogenesis." *Trans. Royal Soci. Trop. Med. Hyg.,* **100**: 291–298.

Debele, G., Misgana, D., Feyisa, H., Firhowet, M., Tesfaye, K. and Mohammed, N. (2010): Study on major causes of kid mortality in Adami Tulu Jido Kombolcha District of Oromia, Ethiopia. *Agri. Biol. J. N. Am.,* **4**: 110-115.

Deresa, B., Conraths, F.J. and Ameni, G. (2013): Abattoir-based study on the epidemiology of caprine tuberculosis in Ethiopia using conventional and molecular tools. *Act. Vet. Scand.,* **55**: 15.

Ebro, A., Eticha, G., Hussen, A. (1998): Thirty years of research experience of Adami Tulu Agricultural Research Centre, Oromia Agricultural Development Bureau. Bulletin, **1**: 1-16.

Edmonson, A.J., Lean I.J., Weaver, L.D., Farver, T. and Webster, G. (1989): A body condition scoring chart for Holstein dairy cows. *J. Dairy Sci.*, **72**: 68–78.

EFSA and ECDC (2013): EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2011. *EFSA J.*, **11**: 3129.

Erler, W., Martin, G., Sachse, K., Naumann, L., Kahlau, D., Beer, J., *et al*. (2004): Molecular fingerprinting of *Mycobacterium bovis* subsp. *caprae* isolates from central Europe. *J. Clin. Microbiol*., **42**: 2234–2238.

ESGPIP and Techinical Bulletin No. 8, “Body condition scoring of sheep and goat, ESGPIP,” Available at: http://www.esgpip.org/PDF/Technical%20bulletin%20No8.html.

ESGPIP and Techinical Bulletin No. 23, “Estimation of weight and age of sheep and goats, ESGPIP, Ethiopia,”Available at
http://www.esgpip.org/PDF/Technical%20bulletin%20No.23.pdf.

Firdessa, R., Tschopp, R., Wubete, A., Sombo, M., Hailu, E., *et al*. (2012): High Prevalence of Bovine Tuberculosis in Dairy Cattle in Central Ethiopia: Implications for the Dairy Industry and Public Health. PLoS ONE **7**: 12.

FMOH (2009): Federal Ministry of Health (FMOH)-Guideline for program and clinical management of drug resistant tuberculosis. 1st ed., Addis Ababa, Ethiopia.

Francis, J., Seiler, R.J., Wilkie, I.W., O’Boyle, D., Lumsden, M.J. and Frost, A.J. (1978): The sensitivity and specificity of various tuberculin tests using bovine PPD and other tuberculins. *Vet. Rec.,* **103**: 420–425.

FSAI (2008): Food Safety Authority of Ireland (FSAI)-Zoonotic Tuberculosis and Food Safety, 2nd ed. Food Safety Authority of Ireland, Abbey Court, Dublin 1, Ireland.

Gallagher, J. and Horwill, D.M., A. (1977): Selective oleic acid albumin agar medium for the cultivation of *Mycobacterium bovis*, *J. Hyg*., **79:** 155–160.

Goodchild, A.V. and Clifton-Hadley, R.S. (2001): Cattle-to-cattle transmission of *Mycobacterium bovis*. *Tuberc.* (Edinb.), **81**: 23-41.

Gori, A., Bandera, A., Marchetti, G., Degli, A.E., Catozzi, L, Piero, G. N.,*et al*.(2005): Spoligotyping and *Mycobacterium tuberculosis. Emerg. Infec. Dis*., **11**:8

Gracey, J.F. (1986): Bacterial, viral and fungal diseases. Meat Hygiene, Bailliere Tindall. 8th ed., 305-371.

Grange, J.M. (1995): Human aspects of *Mycobacterium bovis* infection. In: Thoen CO, Steele JH, eds. *Mycobacterium bovis infection in animals and humans*. Ames, Iowa: Iowa State University Press, 29–46.

Grange, J.M. and Yates, M.D. (1994a): Zoonotic aspects of *Mycobacterium bovis* infection. *Vet. Microbiol.*, **40**: 137–151.

Grange, J.M., Dabron, C. and Cosivi, O. (1994b): HIV related tuberculosis due to *Mycobacterium bovis. Eur. Resp. J.,* **7**: 64-66.

Griffin, J.M., Hahesy, T., Lynch, K., Salman, M.D., McCarthy, J. and Hurley, T. (1993): The association of cattle husbandry characteristics, environmental factors and farmer characteristics with the occurrence of chronic bovine tuberculosis in dairy flocks in the Republic of Ireland, *Prev. Vet. Med*., **17:** 145–160.

Griffin, J.M., Martin, S.W., Thorburn, M.A., Eves, J.A. and Hammond, R.F. (1996): A case-control study on the association of selected risk factors with the occurrence of bovine tuberculosis in the Republic of Ireland, *Prev. Vet. Med*., **27:** 75–87.

Gumi, B., Schelling, E., Berg, S., Firdessa, R., Erenso, G., Mekonnen, W., *et al*. (2012): Zoonotic Transmission of Tuberculosis between Pastoralists and Their Livestock in South-East Ethiopia. *Trop. Anim. Hlth. Prod.,* **44**: 1445-1450.

Gutie´Rrez, M., Samper, S., Jimenez, M.S., van Embden, J.D.A., Martin, J.F. and Martín, C. (1997): Identification by spoligotyping of a caprine genotype in *Mycobacterium bovis* strains causing human tuberculosis. *J. clin. Microbiol*., **35**: 3328-3330.

Haddad, N., Masselot, M. and Durand, B. (2004): Molecular differentiation of *Mycobacterium bovis* isolates. Review of main techniques and applications. *Res. Vet. Sci*., **76**: 1-18

Hailemariam, S. (1975): A brief analysis of activities of meat inspection and quarantine division. Department of Veterinary Service, Ministry of Agriculture, Addis Ababa, Ethiopia.

Hellyer, T.J., Desjardin, L.E., Assaf, M.K., Bates, J.H., Cave, M.D. and Eisenach, K.D. (1996): Specifity of IS-6110 based amplification assays for *Mycobaterium tuberculosis* complex. *J. Clin. Microbiol.,* **34**: 2843-2846.

Hiko, A. and Agga, G. (2011): First-time detection of *Mycobacterium* species from goats in Ethiopia. *Trop. Anim. Hlth. Prod.*, **43**: 133-139.

Hussein, N. (2009): Study on *Mycobacterium tuberculosis* complex infection in livestock and humans in Amibara District of Afar Region. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre-zeit, Ethiopia.

ILRI (1996): International Livestock Research Institute (ILR)-Goat types of Ethiopia and Eritrea: physical description and management system. Nairobi, Kenya, FARM Africa.

Jahans, K. and Worth, D. (2006): The laboratory diagnosis of Tuberculosis. *Government Vet. J.*, **16**: 53-57.

Kamerbeek, J., Schouls, L., Kolk, A., van Agderveld, M., van Soolingen, D., Kuijper, S., *et al*. (1997): Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. *J. Clin. Microbiol.*, **35**: 907-914.

Kaneene, J. B. (2004): Veterinary Medicine Today: Zoonosis Update. *J. Am. Vet. Med. Assoc*., **224**: 5.

Kazwala, R.R., Kambarage, D.M., Daborn, C.J., Nyange, J., Jiwa, S.F.H., and Sharp, J.M. (2001): Risk factors associated with the occurrence of bovine tuberculosis in cattle in the Southern Highlands of Tanzania, *Vet. Res. Commun*., **25:** 609–614.

Kent, P. T. and Kubica, G. P. (1985): Public Health Mycobacteriology: A Guide for the Level III Laboratory. Center for Disease control.

Kidane, D., Olobo, J.O., Habte, A., Negesse, Y., Aseffa, A., Abate, G., *et al*. (2002): Identification of the causative organism of tuberculous lymphadenitis in Ethiopia by PCR. *J. Clin. Microbiol.*, **40**: 4230–4234.

Koch, R. (1882): Classics in infectious diseases. The etiology of tuberculosis: Robert Koch.

Krauss, H., Weber, A., Appel, M., Enders, B., Isenberg, D.H., Schiefer, G.H. *et al*. (2003): Zoonoses: Infectious Diseases Transmissible From Animals to Humans 3rd ed., 213.

Kremer, K., Van Soolingen, D., Frothingham, R., Haas, W.H., Hermans, P.W., Martin, C., *et al.* (1999): Comparison of methods based on different molecular epidemiological markers for typing of *Mycobacterium tuberculosis* complex strains: interlaboratory study of discriminatory power and reproducibility. *J. Clin. Microbiol.*, **37**: 2607–2618.

Kubica, T., Rusch-Gerdes, S. and Niemann, S. (2003): *Mycobacterium bovis* subsp. *caprae* caused one-third of human *M. bovis*-associated tuberculosis cases reported in Germany between 1999 and 2001. *J. Clin. Microbiol*., **41**: 3070–3077.

Kumar, V., Abbas, A.K., Fausto, N. and Mitchell, R.N. (2007): *Robbins Basic Pathology* (8^{th} ed.). Saunders Elsevier, 516–522.

Kurabachew, M., Engerd, O., Sandaae, R., Skuce, R. and Bjorvatna, B. (2004): A multiplex-polymerase chain reaction assay for genus-, group- and species-specific detection of mycobacteria. *Diag. Microbiolo. Infec. Dis.,* **49**: 99-104.

LoBue, P. (2006): Public Health Significance of *M. bovis.* In: Theon, CO., Steel, J.H. and Gildsdorf, M.J (eds). *Mycobacterium bovis*. Infection in Animals and Humans. 2^{nd} ed. Blackwell Publishing, Iowa, USA, 6-12.

Maher, D., Floyd, K. and Raviglione, M. (2002): A strategic framework to decrease the burden of TB/HIV. WHO [cited 7 Jun 2004]. Available at http://www.who.int/gtb/ publications/tb_hiv/2002_296/index.htm.

Mamo, G.K., Abebe, F., Worku, Y., Legesse, M., Medhin,G., Bjune, G. and Ameni, G. (2012): Tuberculosis in Goats and Sheep in Afar Pastoral Region of Ethiopia and Isolation of *Mycobacterium tuberculosis* from Goat. *Vet. Med. Int.,* **2012**: 2012

McIlroy, S.G., Neill, S.D. and McCracken, R.M. (1986): Pulmonary lesions and *Mycobacterium bovis* excretion from the respiratory tract of tuberculin reacting cattle. *Vet. Rec.*, **118**: 718–721.

Menzies, F.D. and Neill, S.D. (2000): Cattle-to-cattle transmission of bovine tuberculosis, *Vet. J.,* **160:** 92–106.

Miller, J., Jenny, A. and Payeur, J. (2002): Polymerase chain reaction detection of *M. bovis* and *M. avium* organisms in formalin-fixed tissues from culture-negative organisms. *Vet. Microbiol.,* **2328**: 1–9.

Moller, M. and Hoal, E.G. (2010): Current findings, challenges and novel approaches in human genetic susceptibility to tuberculosis. *Tuberc.,* **90**: 71–83.

Monaghan, M. L., Doherty, M. L., Collins, J. D., Kazda, J. F., and Quinn, P. J. (1994): “The tuberculin test,” *Vet. Microbiol.,* **40**: 111–124.

Morris, R., Pfeiffer, D. and Jackson, R. (1994): The epidemiology of *Mycobacterium bovis infections*. *Vet. Microbiol.*, **40**: 153-177.

Munyeme, M., Muma, J.B., Sjkerve, E., Nambota, A.M., Phiri, I.G.K., Samui, K.L., *et al*., (2008). Risk factors associated with bovine tuberculosis in traditional cattle of the livestock/wildlife interface areas in the Kafue basin of Zambia, *Prev. Vet. Med*., **85:** 317–328.

Murray, P.R., Rosenthal, K.S., Kobayashi, G.S, *et al*., (1998): Mycobacterium. In: *Medical microbiology*. 3rd ed. St Louis: Mosby Year Book Inc., 319–330.

Naima, S., Borna, M., Bakir, M., Djamel, Y., Fadila, B., Jacob, Z. and Djamel, G. (2011): Tuberculosis in cattle and goats in the North of Algeria. *Vet. Res*., **4**: 100–103.

Niemann, S., Richter, E. and Rusch-Gerdes, S. (2000): Differentiation among members of the *Mycobacterium tuberculosis* complex by molecular and biochemicalfeatures: Evidence for two pyrazinamide-susceptible subtypes of *M. bovis*. *J. Clin. Microbiol.*, **38**: 152–157.

Niemann, S., Richter, E. and Rusch-Gerdes, S. (2002): Biochemical and genetic evidence for the transfer of *Mycobacterium tuberculosis* subsp. *caprae* Aranaz *et al.*, 1999 to the species *Mycobacterium bovis* Karlson and Lessel 1970 (approved lists 1980) as *Mycobacterium bovis* subsp. *caprae* comb. nov. *Int. J. Syst. Evol. Microbiol.*, **52**: 433–436.

Nigussie, T. (2005): Preliminary study of tuberculosis in small ruminants slaughtered at HELIMEX export abattoir. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre-Zeit, Ethiopia.

Noredhoek, G.T., Van Embden, J.D.A. and Kolk, A.H.J. (1996): Reliability of nucleic acid amplification for detection of *Mycobacterium tuberculosis*: an international collaborative quality control study among 30 laboratories. *J. Clin. Microbiol*., **34:** 2522–2525.

Nwanta J. A., Ikechukwu,O. J, Sunday, E. W. and Nnaemeka, U.C. (2010): Zoonotic tuberculosis: A review of epidemiology, clinical presentation, prevention and control *J. Pblc Hlth Epi.* **2**: 118-124.

O'Reilly, L.M. and Daborn, C.J. (1995): The epidemiology of *Mycobacterium bovis* infections in animals and man: A review. *Tuberc. Lung. Dis.*, **76**: 1-46.

OIE (2004): Bovine Tuberculosis. *Office International des Epizooties* (OIE)-Manual of Standards for Diagnostic Tests and Vaccines. 5th ed., 451–463.

OIE (2009): World Organization for Animal Health (OIE)-“Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2009. Chapter 2.4.7: Bovine tuberculosis adopted,”May 2009, http: //www.oie.int/fileadmin/Home/eng/Health standards/tahm/ 2.04.07 BOVINE TB.pdf.

Oloya, J., Opuda-Asibo, J., Djonne, B., Muma, J., Matope, G., Kazwala, R., Skjerve, E. (2006): Responses to tuberculin among zebu cattle in the transhumance regions of Karamoja and Nakasongola district of Uganda. *Trop. Ani. Hlth Prod*., **38**: 275–283.

Pate, M., Svara, T., Gombac, M., Paller, T., Zolnir-Dovc, M., Emersic, I., *et al*. (2006): Outbreak of tuberculosis caused by *Mycobacterium caprae* in zoological garden. .*J. Vet. Med. B. Infec. Dis. Vet. Pub. Hlth.,* **53**: 387-392.

Pignata, W. A., Alves, C. J., Azevedo, S. S. de, Dantas, A. F. M., Gomes, A. A. de B., Remígio, F. R.*et al.(*2009): Prevalence for caprine tuberculosis in semi-arid of Paraíba. *J. P. Vete. Brasi.*, **29**: 526-532

Pollock, J.M. and Neill, S.D. (2002): *Mycobacterium bovis* infection and tuberculosis in cattle, *Vet. J.*, **163:** 115–127.

Prodinger, W.M., Brandstätter, A., Naumann, L., Pacciarini, M., Kubica, T., Boschiroli, M.L., *et al*. (2005): Characterization of *Mycobacterium caprae* isolates from Europe by mycobacterium interspersed repetitive unit genotyping. *J. Clin. Microbiol*., **43**: 4984–4992.

Prodinger, W.M., Eigentler, A., Allerberger, F., Schonbauer, M. and Glawischnig, W. (2002): Infection of red deer, cattle, and humans with *Mycobacterium bovis* subsp. *caprae* in Western *Aust. J. Clin. Microbiol.,* **40**: 2270–2272.

Quintas, H., Reis, J., Pires, I. and Alegria, N. (2010): Tuberculosis in goats. *Vet. Rec*., **166**: 437–438.

Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchelift, K.W. (2000): Disease caused by bacteria – *Mycobacterium*. In: Veterinary Medicine: A Text Book of Disease of Cattle, Sheep, Pig, Goat and Horses. 9th ed. Harcourt Publisher Ltd., London, 909–918.

Rahmana, M. M, Mohammad Khana, F., R., Nazira, N. H. and Rahmana, M. B. (2013): Prevalence of bovine and avian tuberculosis in sheep and goat population of Bangladesh. *Sci.J. Microbiol.,* **2**: 1-8.

Raviglione, M.C., Snider, D.E. and Kochi, A. (1995): Global epidemiology of tuberculosis. Morbidity and mortality of a worldwide epidemic. *JAMA*. **273**:220–226.

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

Restrepo, B.I. (2007): Convergence of the tuberculosis and diabetes epidemics: renewal of old acquaintances. *Clin. Infec. Dis.,* **45**: 436–38.

Roberts, C. A. and Buikstra, J. E. (2003): The Bio-archaeology of Tuberculosis: a Global View on a Re-emerging Disease. Tampa: University Press of Florida.

Rua-Domenech, R., Goodchild, A.T., Vordermeier, H.M., Hewinson, R.G., Christiansen, K.H. and Clifton-Hadley, R.S. (2006): Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. *Res. Vet. Sci.*, **81**:190–210.

Seifert, H.S.H. (1996): Tropical Animal Health. Kluwer Academic Publishers, Dordrecht, the Netzerlands, 343-352.

Sharma, R. and Gupta, V. (2002): Molecular typing of micro-organisms: at the centre of diagnostics, genomics and pathogenesis of infectious diseases. *J. Med. Microbiol*., **51**: 7-10.

Sharpe, A. E., Brady, C. P., Johnson, A. J., Byrne, W., Kenny, K. and Costello, E. (2010): "Concurrent outbreak of tuberculosis and caseous lymphadenitis in a goat flock," *Vet. Rec.,* **166**: 591–592.

Shitaye, J.E., Tsegaye, W. and Pavlik, I. (2007): Bovine tuberculosis infection in animal and human populations in Ethiopia: A review. *Vet. Med*., **52**: 317-332.

Skuce, R.A., Allen, A.R. and McDowell, S.W.J. (2011): Bovine tuberculosis: A Review of Cattle-To-Cattle Transmission, Risk Factors and Susceptibility.

Tafese, K., Dawo, F., Sori, T. and Ameni, G. (2011): Prevalence of caprine tuberculosis in mid-rift valley area of Oromia, Ethiopia. *Afric. J. Microbiol. Res.,* **55**: 1473-1478.

Tauro, P., Kapoor, K.K. and Yadav, K.S. (1996): An introduction to Microbiology. Wiley Eastern Ltd, New Delhi, India, 138.

Taylor, M.G., Murphy, E., Hopkins, R., Rutland, P. and Chistov, Y. (2007): First report of *Mycobacterium bovis* DNA in human remains from the Iron Age: *Microbiol.,* **153**: 1243–1249.

Thoen, C.O. and Barletta, R.G. (2004): *Mycobacterium*. In: Prescott JF, Songer G, Thoen CO, eds. *Pathogenesis of bacterial infections in animals*. 3rd ed. Ames, Iowa: Blackwell Publishing, (in press).

Thoen, C.O. and Bloom, B.R. (1995): Pathogenesis of *Mycobacterium bovis*. In: Thoen CO, Steele JH, eds. *Mycobacterium bovis infection in animals and humans*. Ames, Iowa: Iowa State University Press, 3–14.

Thoen, C.O. and Garcia-Marin, J.F. (2002): *Mycobacterium*. In: *Compendium of animal production*. Wallingford, Oxon, UK: CAB International Publishing Inc.

Thoen, C.O., Steele, J.H. and Gilsdorf, M.J. (2006): *Mycobacterium bovis* Infection in Animals and Humans. 2nd ed. Blackwell Publishing, Boston, Mass, USA, 329.

Thrusfield, M. (2005): Veterlinary epidemiology. 3ed. UK London: Blackwell Science, 178-187.

Tschopp, R., Assefa, A. and Schelling, E., *et al.* (2010): Bovine tuberculosis at the wildlife-livestock-human interface in Hamer woreda, south Omo, southern Ethiopia. *PLoS ONE*, **5**: 12-20.

Tschopp, R., Bobosha, K. and Assefa, A. (2011): Bovine tuberculosis at a cattle-small ruminant-human interface in Meskan, Gurage region, Central Ethiopia, BMC. *Infec. Dis.*, **11**: 18-21.

Vasconcellos, S.E., Huard, R. C., Niemann, S., Kremer, K., Santos, A. R., Philip, N., *et al*. (2010): Distinct genotypic profiles of the two major clades of Myc*obacterium africanum*. *BMC. Infec. Dis.,* **10**: 80.

Vestal, A.L. (1977): In: *Procedures for isolation and identification of Mycobacteria*, U.S. Department of Health, Education and Welfare, CDC Atlanta, Georgia, **8230:** 15-9.

Warren, R. M., Gey van Pittius, N. C., Barnard, M., Hesseling, A., Engelke, E., de Kock, M., *et al*. (2006): Differentiation of *Mycobacterium tuberculosis* complex by PCR amplification of genomic regions of difference. *Int. J. Tuberc. Lung Dis.,* **10**: 818–22.

Wayne, L. G. (1984): Mycobacterial speciation. The mycobacteria: a sourcebook. Part A. G. P. Kubica and L. G. Wayne (ed.), Marcel Dekker, Inc., New York, 25–65.

WHO (1994): World Health Organization (WHO)-Zoonotic Tuberculosis (*M. bovis*): A Memoranda from WHO Meeting (with participation of FAO). Bulletin of the WHO., **72**: 851-857.

WHO (1998): World Health Organization (WHO)-Laboratory services in tuberculosis control. Global tuberculosis program. Geneva, Switzerland, 95.

WHO (2008): World Health Organization (WHO)-Global tuberculosis control: surveillance, planning and financing. WHO Report. WHO/HTM/TB/2008.393. Geneva, Switzerland.

WHO (2009): World Health Organization (WHO)-Global tuberculosis control: epidemiology, strategy, financing. WHO Report. Geneva.

WHO (2010): World Health Organization (WHO)-Global Tuberculosis Control 2010, Geneva.

Wilton, S. and Cousins, D. (1992): Detection and identification of multiple mycobacterial pathogens by DNA application in a single tube PCR. *Method. Appl*., **1**: 269-273.

Yalelet, W. (2010): Epidemiology and species characterization of *Mycobacterium tuberculosis* complex in pastoralist and their livestock in two districts of the Afar region, North Eastern Ethiopia. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre-zeit, Ethiopia.

Yimer, S.A., Hailu, E., Derese, Y., Bjune, G.A., Holm-Hansen, C. (2013): Spoligotyping of *Mycobacterium tuberculosis* isolates among pulmonary tuberculosis patients in Amhara Region, Ethiopia. *APMIS*., 121: 878–85.

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

8. ANNEXES

Annex 1: Estimated age for sheep and goats with different numbers of erupted permanent incisors

No. of permanent incisors	Estimated age range	
	Sheep	Goat
0 pair	Less than 1 year	Under 1 year
1 pair	1-1½ years	1-2 years
2 pairs	1½-2years	2-3 years
3 pairs	2½-3years	3-4 years
4 pairs	More than three years.	More than four years
Broken mouth	Aged	Aged

Source: ESGPIP-Techinical Bulletin No.23, "Estimation of weight and age of sheep and goats, ESGPIP, Ethiopia," Adopted from http/www.esgpip.com/PDF/Technical%20Bulletin%20No.%208.pdf.

Annex-2**:** Scales for Body Condition Scoring

Condition	**Score**	**Lumbar region**	**Rib cage**	**Sternum**
Starving	0	Extremely emaciated and on the point of death. It is not possible to detect any muscle or fatty tissue between the skin and the bone.	Skin is sunken between visible ribs.	There is no sternal fat
Very thin	1	The spinous processes are Prominent and sharp. The transverse process are also sharp, the fingers pass easily under the ends, and it is possible to feel between each process. The eye muscle areas are shallow with no fat cover.	Ribs are clearly visible	Sternal fat is easily grasped and moved to from side to Side.
Thin	2	The spinous process feels prominent but Smooth, and individual processes can Be felt only as fine corrugations. The transverse processes are smooth and rounded, and it is possible to pass the fingers under the ends with a little pressure. The eye muscle areas are of moderate depth, but have little fat cover.	Some ribs can be seen. There is a small amount of fat cover. Ribs are still felt.	Sternal fat is wider and thicker but can still be grasped and moved slightly from side to side
Moderate	3	The spinous processes are detected only as small elevations; they are smooth and	Ribs are barely seen; an even	Sternal fat is wide and

		rounded and individual bones can be felt only with pressure. The transverse processes are smooth and well covered, and firm pressure is required to feel over the ends. The eye muscle areas are full, and have a moderate degree of fat cover.	layer of fat cover them. Spaces between ribs are felt using pressure.	thick.. It can grasped but has very little movement.
Fat	4	The spinous processes can just be detected with pressure as a hard line between the fat covered eye muscle areas. The ends of the transverse processes cannot be felt. The eye muscle areas are full, and have a thick covering of fat.	Ribs are not seen.	Sternal fat is difficult to grasp and cannot be moved from side to side.
Very fat	5	The spinous processes can't be detected Even with firm pressure, and there is a Depression between the layers of fat in the position where the spinous processes would normally be felt. The transverse processes cannot be detected. The eye muscle areas are very full with thick fat over. There may be large deposits of fat over the rump and tail.	Ribs are not visible and are covered with extensive fat.	Sternal fat extends and covers the sternum. It Cannot be grasped.

Source: ESGPIP-Techinical Bulletin No.8, "Body condition scoring of sheep and goats, Ethiopia," http://www.esgpip.com/PDF/Technical%20Bulletin%20No.%208.pdf.

Annex-3: Comparative Intradermal Tuberculin Test (CIDT) Data Recording Format for Epidemiological Study of Caprine Tuberculosis in Adami Tulu J/K District

Before   After

Skin fold thickness: A1____________mm   A2__________mm

B1____________ mm   B2__________mm

ID No.	Date	Owner		Sex	Age	BCS
001		Name	Sex			
002						
003						
004						

Annex 4: Questionnaire Format for Case Control Study to Assess Risk Factors of Caprine Tuberculosis in Adami Tulu J/K District

Quest. No. ----- Date -----------------

1. Name of the owner ---

   Age (<18, 18-45, >45), Sex (M, F)

   Address: a) Urban (Town----------------------- kebele, ---------------

   b) Rural (Kebele-----------------------village ----------------

   Education status

   a) No formal education c) Secondary school

   c) Primary school d) college/university graduate

2. Occupation: (farmer, civil servants, others)
3. Which species of domestic animals do you own?

   (Cattle, sheep, goats, horses/mules/donkeys, poultry camel)

4. Do you share housing with your animals? (Yes, No)
5. Do you know caprine tuberculosis? (Yes, No)
6. Which transmission ways do you know through which tuberculosis can be transmitted from goat to man and vice versa?

   a) do not know c) raw meat,

   b) raw milk, d) aerosol

7. Habit of drinking milk a) Raw b) boiled c) mixed
8. Habit of eating meat a) Cooked b) raw d) mixed

9. Animal information

A .Sex (M F) B. BCS (0 1 2 3 4 5)

C. Age (≤1.5 year, ≥1.5 year) C. Parity number (0 1 2 3 4 ≥5)

	Before	After
Skin fold thickness:	A1___________mm	A2_________mm
	B1___________mm	B2________mm

Annex 5: Preparation of Lowenstein-Jensen Egg-based Medium

1. Mineral salts

1.1. Ingredients

Potassium dihydrogen phosphate anhydrous (KH_2PO_4)	2.4g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.24g
Magnesium citrate	0.6g
Asparagines	3.6g
Glycerol (reagent grade)	12ml
Distilled water	600ml

1.2. Preparation

Dissolve the ingredients in order in the distilled water by heating. Sterilize by autoclaving at 121^0c for 30 minutes. Cool to room temperature. This solution keeps indefinitely and may be stored in suitable amounts in the refrigerator.

2. Malachite green solution

2.1.Ingredient

Malachite green dye	2.0g
Sterile distilled water	100ml

2.2. Preparation

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

3. Homogenized whole eggs

Homogenized whole eggs (20-25 eggs, depending on size	1000ml

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

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

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

The complete egg medium is distributed in 6-8ml volumes in sterile 14ml or 28ml McCartney bottles or in 20ml volumes in 20x150ml screw-caped test tubes and tops are securely fastened.

Before loading, heat the inspissator to 80^0c to quicken the build-up of the temperature. Place the bottles in a slanted position in the inspissator and coagulate the medium for 45 minutes at 800c-850c. inspissate the medium within in 15minutes of distribution to prevent sedimentation of heavier ingredients.

Source: WHO (1998)