

Thesis Ref. No. _____

**PATHOLOGICAL CHARACTERIZATION OF LESIONS AND
BACTERIOLOGICAL ISOLATION OF CAUSATIVE AGENTS OF SWINE
TUBERCULOSIS AT BISHOFTU AND ADDIS ABABA ABATTOIRS, BISHOFTU,
ETHIOPIA**

MVSc THESIS



BY

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ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

JUNE, 2018

BISHOFTU, ETHIOPIA

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JIRATA SHIFERAW ABOSSÉ

**A Thesis Submitted to the College of Veterinary Medicine of Addis Ababa University in
Partial Fulfillment of the Requirements for the Degree of
Master of Veterinary Science in Veterinary Pathology**

**JUNE, 2018
BISHOFTU, ETHIOPIA**

APPROVAL

Addis Ababa University
College of Veterinary Medicine and Agriculture
Department of Pathology and Parasitology

As members of the Examining Board of the final MVSc open defense, we certify that we have read and evaluated the Thesis prepared by: **Jirata Shiferaw Abosse** entitled “**Pathological Characterization of Lesions and Bacteriological Isolation of Causative Agents of Swine Tuberculosis at Bishoftu and Addis Ababa Abattoir, Bishoftu, Ethiopia**” and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Veterinary Science in Veterinary Pathology.

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DEDICATION

This thesis/dissertation manuscript is dedicated to my younger sister, Lalise Shiferaw, whom I lost at her early age with unexpected case of disease. Let God wait her life at heaven!

STATEMENT OF AUTHOR

Firstly, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MVSc) degree at Addis Ababa University, College of Veterinary Medicine and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are permissible without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or imitation of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however permission must be obtained from the author.

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LIST OF ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
AFB	Acid Fast Bacilli
bp	Base pair
bTB	Bovine Tuberculosis
DNA	Deoxyribonucleic Acid
ELISA	Enzyme linked Immunosorbent Assay
HIV	Human Immunodeficiency Virus
HPC	Hexadecylpyridinium Chloride
IFN- γ	Interferon Gamma
IHC	Immunohistochemistry
IS	Insertion Sequence
LJ	Lowenstein Jensen
MIRU-VNTR	Mycobacterium Interspersed Repetitive Unit Variable Number Tandem Repeats
MTBC	<i>Mycobacterium tuberculosis</i> Complex
LSSNPA	Lineage-Specific Single-Nucleotide Polymorphism Analysis
OIE	Office of International des Epizootics
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PPD	Purified Protein Derivatives
RDs	Regions of Differences
REA	Restriction Endonuclease Analysis
RFLP	Restriction Fragment Length Polymorphism
rRNA	ribosomal Ribonucleic Acid
SIT	Shared International Type
TB	Tuberculosis
TNF α	Tumor Necrosis factor Alpha
WHO	World Health Organization

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ABSTRACT

A cross sectional study was conducted at Bishoftu and Addis Ababa Abattoirs from September 2017 to May 2018 to estimate abattoir based prevalence of tuberculosis in swine, to isolate *Mycobacterium* species involved and to characterize the lesions. Five hundred and fifty six (556) swine were examined. Tubercle like granulomatous lesion were detected in 19.6% (109/556) of different organs of which 12% (69/556) was in lymph nodes, 5.7% (32/556) in the lungs, 1% (6/556) in the liver and 0.36% (2/556) in the spleen. Based on microscopic changes 4.7% (26/556) of lesions were tuberculous granulomatous type with central necrosis, calcified foci, epitheloid cells admixed with lymphocyte and connective tissue boundries, 3.6% (20/556) pyogranulomatous and 2.5% (14/556) non necrotic granulomatous lesion without epitheloid cells and connective tissue capsules. In lymph nodes, severe depletion of lymphocytes, calcification, cellular debris and aggregation of macrophages were observed microscopically. The tuberculous lesions found in lymph node were statistically significant ($P < 0.05$) than in other organs. The multivariable logistic regression analysis showed that old aged swine were more likely to have characteristic tuberculous lesion (OR = 3.14, 95% CI, 1.62-6.09) than younger ones. From the tissue cultured, 7.5% (3/40) yield growth on primary culture media. The observed colony morphology was smooth whitish or yellowish color, sticky, off-white and breaks apart easily and two (5% (2/40)) of these growth were acid fast positive by Zeihl-Neelsen staining technique. Based on culture result, and typical histopathologic lesions it could be concluded that pork can be source of tuberculosis to human when consumed under cooked and routine abattoir inspections should be conducted. Therefore, further molecular and biochemical research to isolate the species of microbacteria is highly recommended to elucidate the type of lesion and its magnitude in different body organs by considering large number of swine.

Key words: *Abattoir, Ethiopia, Lesion, Swine, Tuberculosis, Zeihl-Neelsen*

1. INTRODUCTION

Tuberculosis (TB) is considered as a re-emerging, infectious granulomatous disease in animals and people caused by acid-fast bacilli of the genus *Mycobacterium*. Although commonly defined as a chronic, debilitating disease, TB occasionally assumes an acute and rapidly progressive course. The disease affects practically all species of vertebrates (Thoen, 2005). Tuberculosis occurs frequently in man, domestic and wild animals. The tubercle bacilli are *Mycobacterium tuberculosis*, the agent of the disease in primates, *M. bovis* in other mammals and *M. avium* in birds. Swine are susceptible to all the three types of tubercle bacilli (Thoen, 2005; Mohamed *et al.*, 2009). It has been suggested that there is a correlation between the occurrence of TB in swine and a direct or indirect contact of swine with tuberculous humans, cattle or birds. The presence of TB in swine in virtually developed countries, in which swine are farmed, has been reported before ten years ago (Pavlik, 2006).

Swine are natural hosts for mycobacterial infections including those due to *M. bovis* (De Lisle, 1994). The most common cause of swine tuberculosis is *Mycobacterium avium*, but infection with mammalian tubercle bacilli, including *M. tuberculosis*, *M. bovis*, and *M. africanum* occur coincident with infections of cattle, wildlife, and human beings. Lesions occurring in swine naturally infected with *M. bovis* and *M. tuberculosis* are indistinguishable. Granulomatous lesions are most often found in the cervical, submandibular, and mesenteric lymph nodes, but in advanced disease lesions may also be found in the liver and spleen. Typically, enlarged nodes contain small, white or yellow, caseous foci, usually without any evidence of calcification. Swine with disease due to *M. tuberculosis* may have similar regionalized lesions with human beings. Swine are particularly susceptible to *M. bovis*, which is usually acquired from shared grazing or ingestion of contaminated dairy products. This can cause a rapidly progressive, disseminated disease with caseation and liquefaction of lesions (Charles, 2017).

The gross pathology of tuberculous lesions typically appear as yellowish, caseous, and necrotic areas in nodules of firm white to light grey fibrous tissue. Lesions may be purulent (less common) or dry, with caseation or fibrosis, and may be calcified or caseo-calcified. The main sites of lesions vary between species but the lungs and associated lymph nodes are common sites

of lesions in many species other than swine. Moreover, the lymph nodes of the head and neck that are associated with infection by ingestion are common in swine. Other organs including the liver, spleen and the kidneys may also contain lesions. Old tuberculous masses can be large, even involving a whole lung lobe. Skeletal lesions sometimes occur, particularly of the cervical vertebrae in horses and other animals like swine. The degree to which tubercles are surrounded by fibrous tissue varies between species. Thin-walled purulent abscesses may be found in some species such as cervids. Generally lesions are seen in the tonsils and in submaxillary, cervical, bronchial, hepatic and mediastinal lymph nodes; enlargement due to white caseous and sometimes calcified material with a fibrous capsule and fibrous tissue interlaced through the mass. However, these lesions often negative on culture (Cowie *et al.*, 2016). Swine and humans have similar patterns of resistance and susceptibility to virulent mycobacteria and develop lesions with a similar histological characteristic (Theon, 2005).

Generalized infection is uncommon in swine but if it occurs then it results in miliary lesions in multiple organs. Following experimental inoculation on domestic swine with high doses of *M. bovis* intravenously or intratracheally, disseminated granulomatous lesions were found in the lungs, liver and spleen, peritoneum and the associated lymph nodes. Variable from diffuse pulmonary consolidation to multiple discrete granulomas, often also fibrinous and granulomatous pleuritis are often evident in respiratory region of the lungs. Histologically, the typical tubercle consists of a central area of caseous necrosis with variable calcification, surrounded by epithelioid cells, Langerhans' giant cells, lymphocytes and plasma cells, with a fibrous capsule infiltrated by lymphocytes and plasma cells. There may be scattered foci of neutrophils and degenerate leucocytes at the junction between the caseo-necrotic centre and the surrounding mantle of inflammatory cells (Cowie *et al.*, 2016).

Several methods have been employed for the *in vivo* diagnostic of TB regarding both cellular and humoral responses. The Gamma-Interferon assay (IFN) is based on the release of IFN from previous *M. bovis* sensitized blood cells cultured *in vitro*, and detects an early cell-mediated response (Wood and Jones, 2001). It has been evaluated in Brazil with encouraging results (Lilenbaum *et al.*, 1999; Marassi *et al.*, 2010) as well as in many other countries (Wood and Jones, 2001). With regard to humoral responses, it has been stated that B-lymphocytes are

stimulated to induce antibodies production only in advanced stages of bovine tuberculosis (Pollock and Neill, 2002). The single intradermal test conducted on the dorsal surface of the ear, base of the ear or in skin of the vulva is often useful for diagnosis of TB in live swine. Test responses should be observed at 48 hours after injection of tuberculin for development of swelling. Serological tests were found to be less efficient to identify cattle in the early stages of TB infection (Wood and Rothel, 1994), but they have been recommended for diagnosis of anergic animals (Lilenbaum and Fonseca, 2006) and used as a complementary diagnostic herd tool (Lilenbaum *et al.*, 1999; Welsh *et al.*, 2005; De la Rúa Domenech *et al.*, 2006). Nevertheless, due to the limitations of all those methods, the De initiative diagnosis to check whether a certain herd is infected or not requires a clear evidence of the agent based on bacteriological culturing, histopathology and molecular methods (Thoen *et al.*, 2009; Medeiros *et al.*, 2010).

A major effort to eradicate tuberculosis in swine and in people markedly reduced the incidence of the types of tuberculosis usually seen in swine and people. This is mainly through changes in poultry production system which was the usual source of exposure of swine. A trace-back system identified the source of infected swine for slaughter and reduced infection at points of origin. As a consequence of these measures, TB in swine was reduced largely in incidence. Reduction was achieved without an eradication program directed specifically at swine or poultry. The serovars of avian type TB found in confinement-raised swine have been found in people may translate into increased concern for TB in both swine and poultry (Charles, 2017).

The impact of tuberculosis in swine is complicated. Swine carcasses are condemned due to tuberculous lesions. Losses from test-and-slaughter of swine for the control of bovine TB caused by dissemination of *M. bovis* by infected swine (Machackova *et al.*, 2003; Phillips *et al.*, 2003) and losses from destruction of false positive reactors due to sensitization by mycobacteria species other than tuberculosis (Matlova *et al.*, 2005; Pavlik *et al.*, 2005) may be indirect consequences of swine tuberculosis in other animals. A retrospective meat inspection data analysis report by Shitaye *et al.* (2006) in Ethiopia indicated a 0.009% prevalence of TB in swine slaughtered at Addis Ababa abattoir within ten years (1996–2005).

The epidemiology, economic impact and public health importance of swine tuberculosis is not yet studied in Ethiopia. To study swine tuberculosis, different diagnostic methods employed along with histopathological studies to isolate, identify and characterize the agent of swine TB. Therefore, this study is the first of its kind in providing baseline information with regard to histopathology and epidemiology of swine TB. On the other hand, the current bacteriological study of swine TB is the second of its kind next to Arega *et al.* (2013). To put it in a nutshell, both pathological and bacteriological methods were used for the diagnosis of swine tuberculosis. Correlation of gross, microscopic and bacteriological findings to increase the sensitivity of TB detection provided the true epidemiological status of the disease and useful information in the swine population in Ethiopia that can be utilized for future research and disease control purpose at national and global level TB control efforts and reducing the risk of its zoonotic significance.

The objectives of the current study were to:

- Estimate the abattoir based prevalence of tuberculosis in swine slaughtered at both Addis Ababa and Bishoftu Abattoirs
- Isolate and characterize *Mycobacterium* species from characteristic tubercle lesions
- Characterize lesions of *Mycobacterium* positive swine and
- Assess the distribution and frequency of tuberculous lesions in different tissues or organs of slaughtered swine.

2. LITERATURE REVIEW

2.1. Etiology

2.1.1. Taxonomy of mycobacteria

The genus *Mycobacterium* is classified under the Order Actinomycetales and Family *Mycobacteriaceae* (Quinn *et al.*, 1999). They are grouped in the suprageneric rank of actinomycetes that usually have a high content (61–71%) of guanine plus cytosine (G+C) in the genomic deoxyribonucleic acid (DNA), and a high lipid content in the wall, probably the highest among all bacteria (Palomino *et al.*, 2007). *Mycobacterium tuberculosis* complex (MTBC) has seven approved members and these are *M. tuberculosis*, *M. bovis*, *M. bovis BCG*, *M. africanum*, *M. microti*, *M. tuberculosis sbsp. canetti* and *M. bovis sbsp. caprae* (Corner *et al.*, 1990). Four members of this group cause human tuberculosis i.e. *M. bovis*, *M. tuberculosis*, *M. africanum* and *M. canetti* (Higgins *et al.*, 2011; Bilal *et al.*, 2010).

Mycobacteria comprise more than 80 species, within the complex of related and poorly studied organisms (Rainy *et al.*, 1995). Avian type mycobacteria (*Mycobacterium avium*) have survived for more than four years in poultry lot soil, in cages, and in sawdust used as litter. The three mycobacterial species that cause TB in swine are *Mycobacterium avium*, *Mycobacterium bovis* and *Mycobacterium tuberculosis*. These three species infect poultry, cattle and people, respectively. However, each of these three occasionally infects other animal species. *Mycobacterium avium* usually is the organism that infects swine. There are 19 known serovars. In the USA serovars 1, 2, 4 and 8 are the most common isolates from swine. Serovars 4 and 8 have been found in poultry, large confined swine herds and people (Charles, 2017).

2.1.2. Physical and biochemical characteristics

2.1.2.1. Morphology and staining

Mycobacteria are non-motile, non-spore forming, pleomorphic bacilli or coccobacilli. In tissues they appear as rods, which may be straight, curved or in the form of clubs, measuring 1.0-4.0µm

in length and 0.2-0.3µm in width. They occur singly, in pairs or as small bundles. On laboratory media they may appear as cocci or rods measuring 6-8µm (Quinn *et al.*, 1999). The distinguishing features of pathogenic mycobacteria are the formation of characteristic cords (Grange, 1995). Mycobacteria are similar to other bacteria in many respects except for a unique cell wall that is made up of four parts. The first part is a peptidoglycan layer that is similar to that found on other bacterial species. The second layer contains arabinogalactan, which is made up of branched macromolecules of arabinose and galactose (Jovan *et al.*, 2011). The third layer, which contributes to the thickness of mycobacterial cell walls, is made of mycolic acids that are long branched chains of fatty acids with differing 50 and 30 carbon atom lengths. The mycolic acids are responsible for the acid fast staining reaction of mycobacteria cells. The fourth, outer, layer consists of a mixture of lipids and related compounds such as trehalose-containing glycolipids and peptidoglycolipids called mycosides. Trehalose-containing glycolipids include a “cordfactor” compound that was previously believed to be responsible for the “serpentine cords” of virulent MTBC but this was later proved to be inaccurate (Chukwu *et al.*, 2013). This waxy lipid envelope confers an extreme hydrophobicity, resistance to injury, including that of many antibiotics, and a distinctive immunological property which renders the bacteria acid- and alcohol-fast and also a feature that can be exploited to identify mycobacteria via the Ziehl Neelsen staining technique. Mycobacteria when stained are acid fast as they resist decolorizing with strong acid and alcohol solutions (Quinn *et al.*, 1999).

2.1.2.2. Growth requirement and cultural characteristics

Mycobacterium bovis is a slow growing, facultative intracellular, aerobic and gram positive bacterium with a dysgonic colony shape when cultured on Löwenstein- Jensen (L-J) (Kubica *et al.*, 2006). To date, the most frequently used media for isolation of *M. bovis* are L-J medium and Ogawa medium and the former contains asparagines (Seifert, 1996). On the other hand, *M. bovis* is microaerophilic, i.e. it grows preferentially at a reduced oxygen tension. *M. tuberculosis* is mesophile and neutrophile as its multiplication is restricted to conditions offered by warm-blooded animals: about 37⁰C and a neutral pH. The temperature and hydrogen ion concentration ranges, in which the bacillus is able to multiply, are relatively narrow (Palomino *et al.*, 2007).

Table 1: Clinical significance, growth characteristics and biochemical differentiation of pathogenic mycobacteria

	<i>M. tuberculosis</i>	<i>M. bovis</i>	<i>M. avium complex</i>	<i>M. avium</i>
Significance of infection	Important in humans and occasionally in dogs.	Important in cattle and occasionally in other domestic animals and humans	Important in free-range poultry. Opportunistic infections in humans and domestic animals	Important in cattle and other ruminants
Cultural characteristics and growth requirements				
Growth rate	Slow (3-8 weeks)	Slow (3-8 weeks)	Slow (2-6 weeks)	Very slow (up to 16 weeks)
Optimal incubation temp.	37 ⁰ C	37 ⁰ C	37 ⁰ C to 43 ⁰ C	37 ⁰ C
Atmospheric requirements	Aerobic	Aerobic	Aerobic	Aerobic
Colonial features	Rough, buff, difficult to break apart	Cream-colored, roughness, break apart easily	Sticky, off-white, break apart easily	Small hemispherical, some pigmented
Essential growth supplement	None	None	None	Mycobactin
Effect of added glycerol	Enhanced growth (eugonic)	Growth inhibited (dysgonic)	Enhanced growth (eugonic)	
Effect of added pyruvate	No effect	Enhanced growth	No effect	
Biochemical differentiation				
Niacin accumulation	+	-	-	-
Pyrazinamidase production	+	-	+	-
Nitrate reduction	+	-	-	-
Susceptibility to TCH (10 µg/ml) TM	Resistant	Susceptible	Resistant	-

Source: Kubica *et al.*, 2006

2.2. Epidemiology of *Mycobacterium* Infections

The disease is found in mammals throughout the globe, but some countries have been able to reduce or limit the incidence of the disease through process of 'test and cull' of the animal. Most of Europe and several Caribbean countries, including Cuba, are virtually free of *M. bovis*. Bovine tuberculosis is endemic to many developing countries particularly African countries (Abubakar *et al.*, 2011). *Mycobacterium bovis* combines one of the widest host ranges of all pathogens with a complex epidemiological pattern, which involves interaction of infection among human beings, domestic animals and wild animals (Grange, 1995; Mamo *et al.*, 2012; Gemechu *et al.*, 2013). However, only little is done particularly in developing countries on the epidemiology of this organism and the epidemiological requirements for its control (Ali, 2006).

The marked resistance of mycobacteria to environmental factors makes contaminated premises a long-term threat to swine. The TB organisms usually are spread to swine by tuberculosis-infected poultry, wild birds, cattle or people, or by soil or bedding materials contaminated by them. The route of exposure usually is by ingestion although spread by inhalation occurs occasionally. Infectious feeds that have spread TB bacilli to swine include offal from infected poultry and cattle, uncooked garbage, unpasteurized milk and dairy products. Exposure of swine to lots contaminated by the feces of tuberculosis-infected poultry often has led to their infection by *M. avium*. Other less common but proven routes of exposure include exposure to serovars of avian type contained in sawdust or wood shavings used as bedding, congenital exposure of fetuses of infected, pregnant sows, and animal-to-animal exposure among swine. Some infected swine have TB lesions in their tonsils or intestine and shed the organisms in their feces (Charles, 2017). The usual habitat lipid-rich walls render mycobacteria hydrophobic and resistant to adverse environmental influences. Environmental mycobacteria are found in soil, on vegetation and in water. Obligate pathogens, shed by infected animals, can also survive in the environment for extended periods (table 2).

Table 2: Mycobacteria which are pathogenic for animals and humans

<i>Mycobacterium</i> species	Main host	Species occasionally infected	Disease
MTBC			
<i>M. tuberculosis</i>	Humans, primates	Dogs, Cattle, Psittacine, birds, canaries,	Tuberculosis
<i>M. bovis</i>	Cattle	Deer, badgers, Opossums, humans, cats, mammals	Tuberculosis
<i>M. africanus</i>	Humans		Tuberculosis
<i>M. canettii</i>	Humans		Tuberculosis
<i>M. microti</i>	Voles	other mammalian species	Tuberculosis
<i>M. caprae</i>	Goats	Cattle	Tuberculosis
<i>M. pinnipedii</i>	Seals, Sea-lions	other mammalian species, humans	Tuberculosis
<i>M. avium complex</i>	Most avian species except psittacines	Swine, cattle	Tuberculosis
<i>M. marinum</i>	Fish	Humans, aquatic mammals, amphibians	Tuberculosis
<i>M. ulcerans</i>	Humans	Koalas, possums	Buruli ulcer
<i>M. leprae</i>	Humans	Armadillos, chimpanzees	Leprosy
<i>M. lepraemurium</i>	Rats, mice	Cats	Rat and feline leprosy
<i>M. avium subsp. Paratuberculosis</i>	Cattle, sheep, goats, deer	Other ruminants	Paratuberculosis (Johne's disease)
<i>Unspecified acid-fast bacteria</i>	Cattle		Associated with skin tuberculosis
<i>M. senegalense, M. farasinogenes</i>	Cattle		Implicated in bovine farsy

Source: Charles, 2017

2.2.1. Source of infection and mode of transmission

The main reservoir of *M. bovis* is cattle, which can transmit the infection to many mammalian species including man (Tadayon *et al.*, 2013). The main reservoir of *M. avium*, a causative agent of swine TB, is birds. Organisms leave the host in respiratory discharges, faeces, milk, urine, semen and genital discharges. These body excretions may contaminate grazing pasture, drinking water, feed, water and feed troughs or fomites, which may act as sources of infection to other animals (Russel, 2003). Inhalation of *M. bovis* bacilli is the most common route of infection with only a small number of mycobacteria required to cause an infection and spread of the infection can happen between animals when that are confined together in the same air space, such as during housing over the winter period (Sakamoto, 2012). But, ingestion is the main route of transmission in swine TB. A secondary source of infection is the ingestion of contaminated milk or contaminated pasture and water, though environmental contamination is not believed to be a significant source of infection for bTB. Infection of the reproductive system can lead to genital transmission of the bacilli but this is particularly rare event as is congenital infection (Mathema *et al.*, 2006; Nahar *et al.*, 2011).

2.2.2. Risk factors: Animal population

The most common risk factors of TB disease in animals are environment, agent and host range. It is rarely diagnosed in living swine. Paddocks that have been treated with poultry manure up to one year previously (or in the case of bovine TB, which have been grazed by infected cattle or badgers). Avian TB as the name implies is found in wild birds. The organism is shed in large numbers via droppings and therefore food, grain or bedding contaminated by birds becomes a potent source. Peat often contains *M. intracellulare*. Peat is used both for bedding and gut stimulation in the young piglets. It should only be used if it has been pasteurized. Water contaminated by *M. avium/intracellulare* is often a source. In industrialized countries most tuberculous lesions in swine are caused by bacteria of the *M. avium* complex (*M. avium* *ss. hominisuis*, *M. avium* *ss. avium*) and *M. intracellulare*. Lesions are most often observed in lymph nodes associated with gastrointestinal tract. The probability of infection with *M. bovis* is influenced by factors, which are linked to environment, host and the pathogen itself (Regassa, 2005; Ameni *et al.*, 2011).

2.2.3. Risk factors: Human Population

The risk factors for occurrence of TB in human populations are; close physical contact, increasing demand for milk, feeding habit, HIV infection and decreased immunity against TB disease. Close physical contact between humans and potentially infected animals is present in some communities, especially in developing region (Ameni *et al.*, 2007; Samuel, 2010). For example, in many African countries cattle are an integral part of human social life; they represent wealth and are at the center of many events and therefore gatherings. In addition, with 65% of Africa, 70% of Asian, and 26% of Latin America and Caribbean population working in agriculture, a significant proportion of the population of these regions may be at risk for bovine TB (Cosivi *et al.*, 1998).

2.3. Distribution of *Mycobacterium*

Although bovine tuberculosis was once found worldwide, control programs have eliminated or nearly eliminated this disease from domesticated animals in many countries (Regassa, 2005). Nations currently classified as tuberculosis free include Australia, Iceland, Denmark, Sweden, Norway, Finland, Switzerland, Luxembourg, Latvia, Slovakia, Lithuania, Estonia, the Czech Republic, Canada, Singapore, Jamaica, Barbados and Israel (Ali, 2006; Shimeles, 2008; Shitaye *et al.*, 2009). Eradication programs are in progress in other European countries, Japan, New Zealand, the United States, Mexico, and some countries of Central and South America. Bovine tuberculosis is still widespread in Africa, parts of Asia and some Middle Eastern countries (Ashford *et al.*, 2001).

2.3.1. Status of bovine/swine tuberculosis in Ethiopia

Studies in Ethiopia revealed a higher prevalence of bTB in cattle kept indoors compared to free grazing animals and a higher susceptibility to *M. bovis* infection of exotic Holstein cattle compared to local zebu cattle (Bilal *et al.*, 2010; Nemomsa *et al.*, 2014). Moreover, a study by Ameni *et al.* (2010) revealed a better performance of single intradermal comparative cervical tuberculin (SICCT) in Ethiopia if the cut-off value for positive test interpretation was lowered from > 4 mm (OIE standard cut-off) to > 2 mm. The spoligotyping pattern of 17

strains of *M. bovis* isolated from a herd with a high prevalence of bTB was identical for all animals and recently published (Firdessa *et al.*, 2013). Berg *et al.* (2011) conducted a comprehensive investigation on bTB in Ethiopia and showed a wide spread distribution of the disease at an average prevalence of approximately 5% (Mekibeb *et al.*, 2013).

A higher estimated prevalence was recorded by Arega *et al.* (2013) in swine from Addis Ababa and Special Oromia Zone as compared to those slaughtered at Bishoftu was due to reflection of the husbandry system in that, swine are reared in Addis Ababa and the nearby Special Oromia Zone, where poor husbandry practices such as feeding swill, poultry litter, abattoir offal and garbage; sheltering with other domestic animals and confinement in poor housing system and where they have close contact with other animals and humans. The traditional small scale production, which is the predominant swine production system, in Ethiopia is characterized by absence or minimal health care, lack of supplementary feeding and proper housing. It has been reported that swine production is aggregated in the central part of the country (Abdu and Gashaw, 2010).

The distribution and mean prevalence of bovine tuberculosis in districts of Ethiopia were conducted in Addis Ababa, Amhara, Oromia and Southern Nations and Nationalities Peoples regions while no valid published study was obtained from Benishangul-Gumuz, Harari and Dire Dawa regions (fig 1). On the other hand, few studies were undertaken in Afar, Gambella, Somali and Tigray regions. Variable animal level prevalence of bTB were recorded in the districts of the regions ranging from 0.8% to 54.6%; the highest prevalence being reported in intensive farms in and around cities while the lowest prevalence being recorded in grazing animals in rural areas. The quantitative review showed that a pooled prevalence estimate of bTB in Ethiopia is 5.8%. The pooled prevalence was affected both by breed and management systems. The estimate computed for intensive and or semi-intensive production systems was higher than that of extensive production systems, reflecting the effect of the intensification on the prevalence of bTB. Holstein-Friesians and their local crosses had higher pooled prevalence compared to local zebu breed. Perhaps these could be strong epidemiological evidences on the importance of genetics and management on prevalence of bTB in Ethiopia (Sibhat *et al.*, 2017).

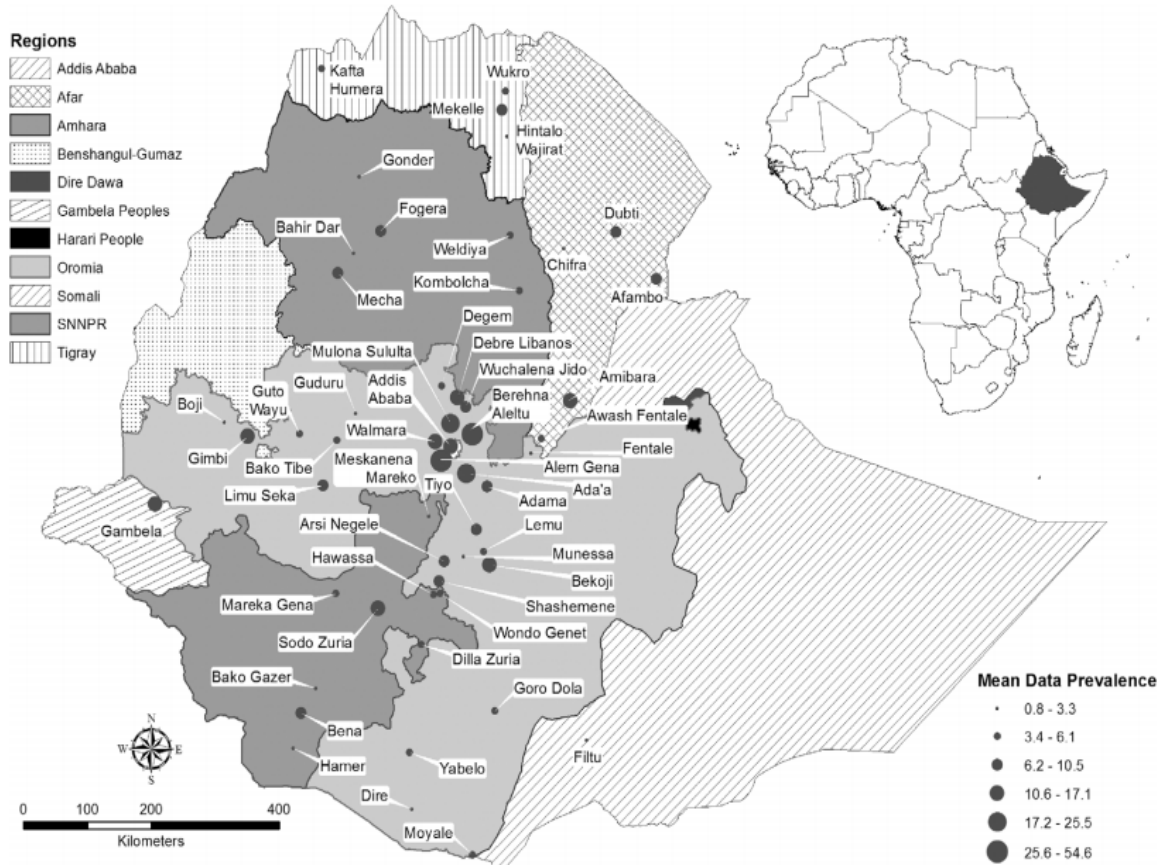


Fig 1. Distribution and mean prevalence of bovine tuberculosis in districts of Ethiopia (Sibhat *et al.*, 2017).

2.4. Pathogenesis

2.4.1. Route of entry and distribution of mycobacterium

The methods by which tubercle bacilli gain entrance to the animal body include: the respiratory, alimentary, genital, cutaneous and congenital routes. After infection the bacteria may localize in tissue related to the route of infection and associated lymph nodes (Menziez and Neill, 2000). In swine, tubercle bacilli appear to infect the tonsils and intestinal mucosa initially and then spread to the regional lymph nodes, especially those of the cervical area, less often to mesenteric nodes. Lesions in the nodes tend to develop slowly and, in most cases, the bacilli are successfully walled off. Only occasionally does the infection generalized usually in older breeding stock infected with *M. bovis* (Charles, 2017).

The entry of mycobacteria through mucous membranes or into alveolar spaces leads to the recognition of bacterial cell wall components and the activation of inflammatory signaling pathways in phagocytes. The mycobacteria are then phagocytised by macrophages, and neutrophils are attracted to and accumulate at the site of initial infection. These cells interact with other cells involved in the innate and acquired immunological responses (Arentz and Hawn, 2007). In immunocompetent human beings, approximately 90% of infections are controlled through this initial immune response, and specific CD4⁺ T-cells and activated macrophages eliminate the mycobacteria or control its multiplication for years or decades (latent tuberculosis). Consequently, only a small proportion of infected individuals develop active tuberculosis (O'Garra *et al.*, 2013). It is unknown whether latent infections or even the elimination of mycobacteria after a primary infection occur in cattle. It has been hypothesized that some cattle with positive skin tests may be latently infected, and this hypothesis is based on the failure to detect tuberculous lesions or to culture *M. bovis* from several organs (Pollock and Neill, 2002).

2.4.2. Role of bronchial obstruction

The obstruction may be due to external compression by a lymph node or by bronchial tuberculosis. Chronic bronchial obstruction by any means causes post obstructive pneumonia. This is endogenous lipid pneumonia similar to that produced by TB (Hunter *et al.*, 2015). Alveolar macrophages trapped behind an obstruction become foamy with lipids derived from continued synthesis of pulmonary surfactant (Betancourt *et al.*, 2010; Tamura *et al.*, 1998). Post obstructive pneumonia today, also known as golden pneumonia, is most commonly caused by cancers that obstruct bronchi. According to Akinosoglou *et al.* (2013) report, 13% of post obstructive pneumonia lesions have been reported to undergo necrosis to produce cavities. While this lesion lacked the lymphocytes and inflammatory cells characteristic of TB, the cavity was limited to a single lobe and had formed by coughing out fragments of necrotic lung as is characteristic of post-primary TB. The remaining necrotic material in the lung was lipid rich resembling caseation necrosis. This demonstrates that post obstructive lipid pneumonia can be a predisposing factor for pulmonary necrosis and cavitations (Hunter, 2016).

2.4.3. Antigen storage in alveolar cells

Alveolar cells preferentially accumulate secreted, but not somatic, antigens of MTB (Mustafa *et al.*, 2014). Typically, there is little or no inflammation around them until initiation of caseation necrosis when massive inflammation develops. In addition, the pathogenicity of MTB has recently been linked to its ability to release selected mycobacterial proteins (Majlessi *et al.*, 2015). The development of post primary TB is characterized by prolonged asymptomatic accumulation of host lipids and secreted mycobacterial antigens in alveolar cells behind an obstructed bronchus. While the process can be much longer or shorter, there is typically a delay of 1-2 years between infection with MTB and onset of clinical tuberculosis (Salgame *et al.*, 2015). This suggests that the delay between infection and onset of clinical pulmonary TB is due to the time required for accumulation of sufficient host lipids and mycobacterial materials for a necrotizing reaction sufficient to produce a cavity from which MTB can escape to infect new hosts (Hunter, 2016).

2.4.4. Gross lesion

The typical gross lesion of tuberculosis is known as a tubercle, which is a circumscribed yellowish granulomatous inflammatory nodule approximately 2–20mm in diameter that is more or less encapsulated by connective tissue and often contains central caseous necrosis and calcification. A primary lesion or focus of infection is established following the interaction of the host and the agent at the site of entry within 8 weeks of bacterial entrance (Radostits *et al.*, 1994). The *Mycobacterium* is then taken by the alveolar macrophages to the circulation and establishes in the lymph nodes. Cellular responses attempting to control the disease results in the accumulation of large number of phagocytes and lead to the formation of a macroscopic lesion referred as tubercle. Tubercle is formed by containment of the bacteria results in the formation of non-vascular nodular granulomas. Lesions show typically a centre of caseous with some degree of calcification surrounded by a cell wall of epithelioid cells, lymphocytes and neutrophils (Doherty *et al.*, 1996).

In localized TB, grossly visible lesions usually are apparent only in cervical, submaxillary or mesenteric lymph nodes. Lesions vary considerably in appearance, depending on the type of

infection. Localized avian and human type infections tend to produce enlarged, firm nodes with no discrete purulent foci. Calcification is seldom apparent and lesions are not easily enucleated. Nodes affected by *M. bovis* tend to be well encapsulated, relatively easy to enucleate, and there often is marked calcification in the lesions. *M. bovis* tends to generalize more readily than do other forms of tuberculosis. Lesions in the organs vary considerably. Initially, the lesions are miliary and widely distributed. Those in the lungs tend to develop along pleural and septal lymphatics and resemble dewdrops. Those in the liver tend to have caseated or liquefied centers. Splenic lesions occur commonly and, when well developed, they often protrude. Serous membranes seldom are affected. It is not possible to accurately identify tuberculosis, or the particular variety of tuberculosis on the basis of gross or microscopic lesions. Lesions, including discrete granulomas containing acid-fast organisms, can be caused by other agents and are easily mistaken as tuberculosis (Charles, 2017).

Swine and horses may be infected by *M. bovis*, *M. tuberculosis*, or *M. avium*, and the lesions caused by these mycobacteria may not be differentiated morphologically (Thoen, 1994). The digestive route of infection is common in these two species, and lesions are frequently found in oropharyngeal lymph node, the gastrointestinal tract, and mesenteric and portal lymph nodes. Lesions in swine and horses are often disseminated, and tubercles or miliary granulomatous inflammation can be found in the liver, lungs, and spleen (fig. 2). Lesions in swine and horses show a more proliferative character than those in cattle are less prone to caseous necrosis and calcification (Domingo *et al.*, 2014).

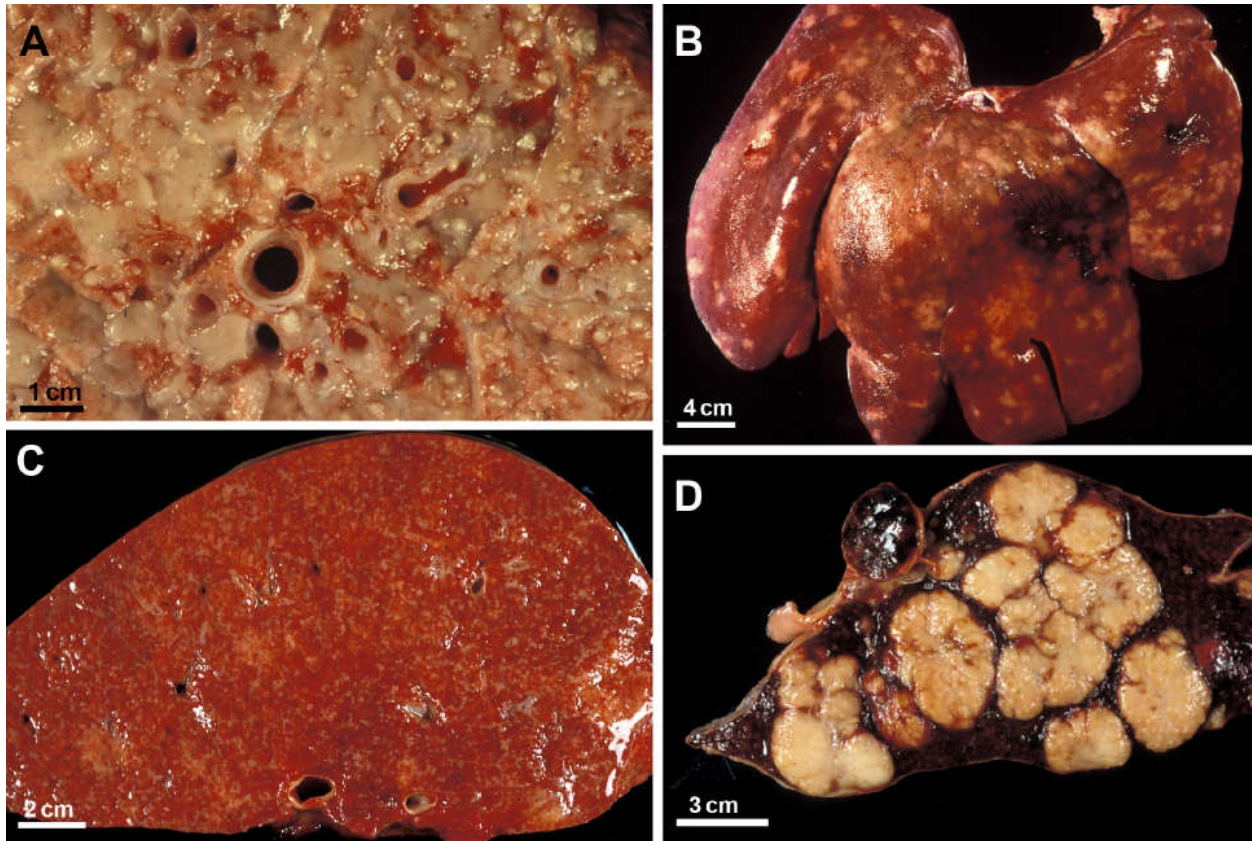


Fig 2. Gross tuberculosis lesions in swine and horse (Domingo *et al.*, 2014). **Note:** A. Miliary tuberculosis in the lung of a swine. Caseous necrosis is usually minimal. (B) Multifocal hepatic tuberculosis in the liver of a swine. (C) Miliary tuberculosis in the lung of a horse. Small granulomatous lesions that resulted from haematogenous dissemination are shown. (D) Multiple tuberculous lesions in the spleen of a horse. The lesions have a productive character and resemble mesenchymal tumours.

2.4.5 Histopathological lesions

Histologically, small tuberculous granulomas are formed by neutrophils, epithelioid macrophages that sometimes have foamy cytoplasm, and a few Langerhans-type multinucleated giant cells (fig 3). This lesion grows over time, and caseous necrosis develops in the centre of the tubercle and appears as amorphous eosinophilic material with necrotic cell debris and central mineralization (Neill *et al.*, 2001). Epithelioid macrophages, Langerhans-type multinucleated giant cells, and lymphocytes surround this central zone of necrosis. Progressively, a more or less complete connective tissue capsule form through the cytokine-induced proliferation of

fibroblasts (Marshall *et al.*, 1996) and the apposition of the pre-existing fibrous tissue of the interlobular septa. A low or very low number of acid-fast bacteria may be found in the caseous eosinophilic material or within the epithelioid cells or the multinucleated giant cells in most bTB lesions in cattle and other domestic animals like swine. However, the apparent absence of acid-fast bacteria in histological slides with typical tuberculous lesions is not sufficient to rule out the morphological diagnosis of tuberculosis (Domingo *et al.*, 2014).

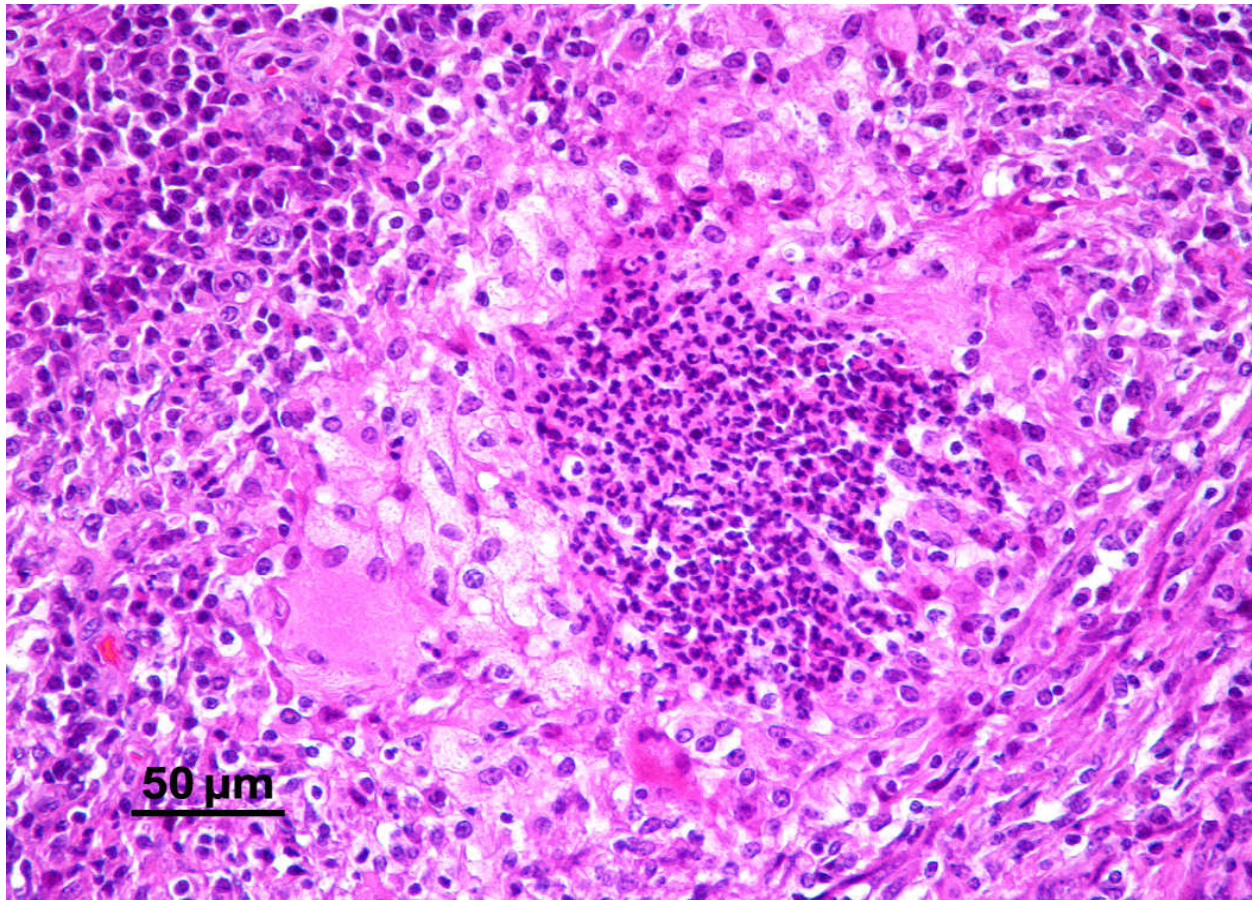


Fig 3. Histological section of tuberculous lesion from lymph node of cow (Domingo *et al.*, 2014). **Note:** A histological section of a mediastinic lymph node of a cow showing a tuberculous granuloma in its initial stage. Neutrophils are abundant in the centre of the granuloma and are surrounded by macrophages, Langerhans-type multinucleated cells, and lymphocytes. Many macrophages exhibit a foamy cytoplasm. H&E staining was used.

2.4.6. Immunity against mycobacterial infection

The macrophages have a central role in processing and subsequent presenting of mycobacterial antigens to antigen specific T-lymphocytes (Ali, 2006). Both humoral and cell mediated immune responses can be induced to mycobacterial infection, but the cell mediated immunity is generally accepted to have the most significant role in protections (Fentahun and Luke, 2012). Mycobacterial infection triggers a Th1-induced cell mediated immune response (CMI) which leads to release of cytokines of such as tumor necrosis factor- α , Interleukin-12 (IL-12) and interferon gamma (IFN- γ). This pathway is essential to activate macrophages (Orme and Cooper, 1999). Depending on the balance of cytokines involved, three outcomes are possible: 1) macrophages kill and eliminate the bacteria, 2) the bacteria lies dormant (latency), 3) the bacteria cannot be contained by the immune system and the disease develops to active TB (Welsh *et al.*, 2005).

2.5. Tuberculosis like Lesion and Causes of Carcass Condemnation in Swine

Tuberculosis-like lesions (TBL) can be an important cause of condemnation in swine at abattoir inspection representing significant important economic losses (Lara *et al.*, 2011). In swine, these lesions are described as necrotic-calcified, proliferative or purulent gross lesions compatible with tuberculosis (TB) (Santos *et al.*, 2010; Di Marco *et al.*, 2012). Although TBL in swine are frequently limited to head lymph nodes, different body locations such as other lymph nodes and thoracic or abdominal organs can be also affected (Martín-Hernando *et al.*, 2007; Di Marco *et al.*, 2012). Granulomatous and pyogranulomatous lesions can be identified in TBL according to the cellular components (Gómez-Laguna *et al.*, 2010). Granulomas, as the main lesions associated with TB, have been widely classified within different stages of development that may help in the interpretation of disease progression (Martín-Hernando *et al.*, 2007). More advanced stages of granulomas have been associated with primary sites of infection (Di Marco *et al.*, 2012; Martín-Hernando *et al.*, 2007), but also with a lower bacterial load (Di Marco *et al.*, 2012).

Mycobacterium avium complex (MAC), *Mycobacterium tuberculosis complex* (MTBC) and *Rhodococcus equi* have been reported as the species most frequently associated with TBL, and these infections typically result in indistinguishable gross lesions in swine (Gómez-Laguna *et al.*,

2010; Miranda *et al.*, 2012; Bailey *et al.*, 2013). Other genera such as *Corynebacterium* spp., *Streptococcus* spp. or *Staphylococcus* spp. have also been isolated in caseous lymphadenitis in swine, highlighting the potential diversity of pathogens that might be associated with TBL in this species (Contzen *et al.*, 2011; Lara *et al.*, 2011; Oliveira *et al.*, 2014). This diversity of microorganisms together with the zoonotic nature of several of them, are factors that should be considered by public health authorities. Detailed studies evaluating the relative importance of microorganisms other than *Mycobacterium* spp. identified from TBL in swine are scarce (Lara *et al.*, 2011).

2.6. Pulmonary Necrosis in *Mycobacterium Tuberculosis* Life-cycle

A long history of human co-evolution with *Mycobacterium tuberculosis* complex (MTBC) suggests that unique immune mechanisms have evolved explaining substantial resistance of modern humans to the disease (Fabri *et al.*, 2011). However, other species also proved to be an ideal host for MTBC, such that the bacteria lost the need for any other environmental niche and relied entirely on modifying human body for every stage of its life cycle. It is generally accepted that to establish new infection, MTBC reaches terminal airways in small aerosol particles generated during cough. Those aerosols are generated from lung cavities where MTBC accumulates in large quantities, perhaps in biofilms, at the air interface, effectively sequestered from host immunity. Thus, from the evolutionary standpoint, MTBC is an obligate lung pathogen, such that substantial MTBC induced destruction of lung tissue is absolutely required for direct transmission to other humans. Although the bacteria are capable of achieving this goal only in a relatively small fraction of the infected hosts, this is sufficient for stable colonization of its unique natural habitat. Therefore, understanding the mechanisms of host susceptibility enabling MTBC, transmission is necessary to counter its evolutionary refined virulence strategy most effectively (Montoya *et al.*, 2014).

2.6.1. Necrosis occurs at various stages of tuberculosis infection

There are at least two distinct stages of MTBC infection at which necrosis can occur: (1) at the initial stage of lung colonization, which leads to necrosis of individual or small clusters of macrophages in a primary granuloma, and (2) during advanced disease where large areas of the

lung are effaced by coalescing granulomas and tuberculous pneumonia. Thus, necrosis delimits the life cycle of MTBC within an individual and plays an important role in both establishing persistent infection initially as well as in the final exit and transmission to a new host. There are many important mechanistic differences between the two stages (Fabri *et al.*, 2011).

The initial contact of MTBC with the host occurs in highly aerated environment, presumably with alveolar macrophages in a context of normal lung tissue. The alveolar macrophages are permissive to MTBC, which establishes its first replicative niche in this cell. Subsequent interactions with lung epithelium and possibly innate T cells lead to recruitment of inflammatory cells from circulation and establishing clusters of myeloid cells that contain the bacteria. These early dynamics cannot be addressed directly in natural lung environment in humans, but the zebrafish model of infection with *Mycobacterium marinum* provides a detailed view of cellular recruitment and interactions that establish nascent microgranulomas (Volkman *et al.*, 2010; Ramakrishnan, 2013; Cambier *et al.*, 2013) before antigen-specific immunity develops. Although zebrafish do not have lungs, this model allows detailed cell trafficking studies *in vivo* and elegantly shows that macrophage death spreads bacilli to adjacent recruited macrophages within the same granuloma. Furthermore, when the recruitment of myeloid cells fails to contain MTBC, the bacilli replicate extracellularly with conspicuous formation of cords (Cronan and Tobin, 2014).

Multiple host responses contribute to macrophage necrosis, including alterations in lipid mediators and increased TNF α production or deficient recruitment of new myeloid cells to the site of infection. Similarly, in the lungs of MTBC infected C3HeB/FeJ mice (abbreviated HeB), early micronecrotic lesions form 2-3 weeks post-infection, when spread of MTBC to adjacent inflammatory cells, as well as robust extracellular replication are observed (Yan *et al.*, 2007). In both the zebrafish model and HeB mice, the microgranulomas undergoing necrosis are composed primarily of myeloid cells (macrophages and some granulocytes). In contrast, different host-pathogen dynamics are observed in the relatively resistant mouse strain C57BL/6 (B6), where lesions are non-necrotic, contain few neutrophils, and bacilli remain intracellular. The early granulomas are composed primarily of myeloid cells that initially act autonomously to restrict the bacterial growth and spread. However, adaptive T cell mediated immunity is necessary to

contain further progression and necrotization of granulomas. The bacterial spread is unstoppable in T-cell deficient mice, where mycobacteria replicate in unrestricted manner and destroy the infected tissue. Large areas of necrotic inflammation, massive bacterial loads, and extensive neutrophil infiltration are typical for this type of progression, which however, lacks the characteristics of organized granulomas (Yan *et al.*, 2007).

The early TB granulomas can follow necrotic and non-necrotic trajectories depending on the myeloid cell intrinsic capacity and help of MTBC-induced T lymphocytes producing IFN γ (Th1-type response) (fig 4). In both cases, however, they serve to constrain MTBC and prevent dissemination. In the case of more efficient immune response in resistant hosts, primary granulomas may be sterilized over time and undergo calcification. In permissive but immune-competent hosts, however, small necrotic granulomas establish a nidus of persistent infection, which can later reactivate and cause post-primary TB (Igor and Gillian, 2016). TB in small ruminants is primarily a chronic infection that causes exudative granulomatous caseous inflammatory lesions in the lungs and associated lymph node (fig. 4).

According to Wangoo *et al.* (2005) small lesions illustrating the four different granuloma stages that are used to classify granulomas in vaccine efficacy studies are shown. All of the sections were stained through the H&E method and are shown at the same magnification: A) Stage I- The granuloma is in its initial stage and exhibits poor organization, few epithelioid macrophages and lymphocytes, and the absence of central caseous necrosis and mineralization. B) Stage II- Greater numbers of epithelioid macrophages are present, necrosis is minimal or absent, and encapsulation is incomplete or absent. C) Stage III- Caseous necrosis in the centre of the granuloma with incipient mineralization. Encapsulation is absent. D) Stage IV- granuloma with evident necrosis and mineralization. Confluent lesions are occasionally present at this stage, and encapsulation is complete (all of the stages are shown by fig 4 below).



Fig 4. Histological sections from the lung of goats with an experimental tuberculosis infection (Domingo *et al.*, 2014).

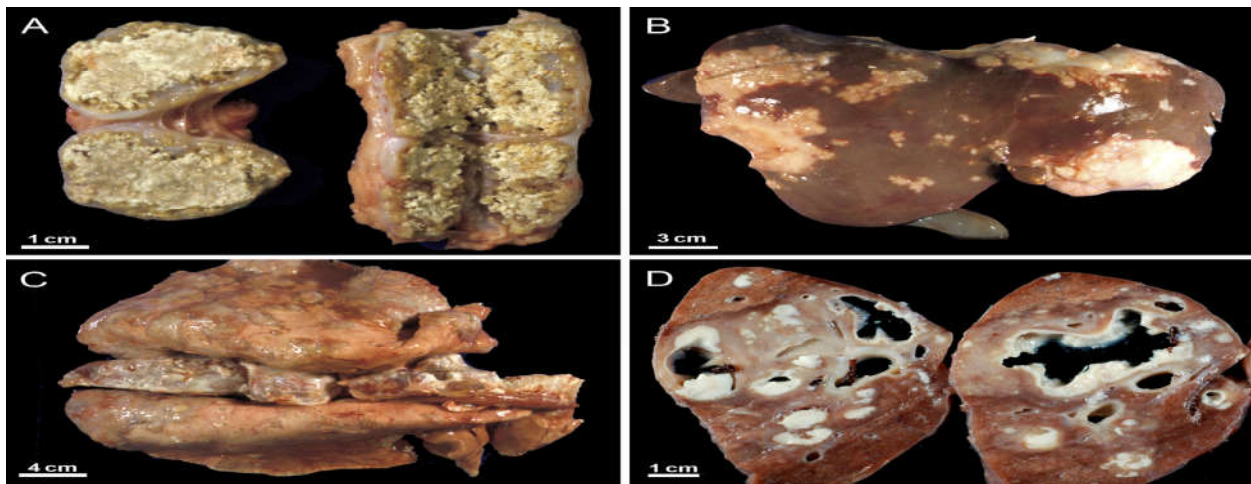


Fig 5. Tuberculosis in small ruminants (Domingo *et al.*, 2014).

A) Tracheobronchial and mediastinal lymph nodes from sheep. Confluent granulomas with caseous necrosis and mineralization. B) and C) Multifocal granulomatous lesions in (B) the liver of a sheep and (C) the lung of a goat. The enlargement of the thoracic lymph nodes and multiple granulomatous lesions involving all pulmonary lobes are evident. D) Extensive cavitory lesions in the lung of a goat.

The exit and transmission strategy of MTBC at the end of its life cycle is entirely dependent on granuloma spread and necrosis leading to formation of lung cavities. Those necrotic lesions become MTBC sanctuaries sequestering the pathogen from the host immune system and allowing its replication and transmission via aerosols. This transition occurs in immune-competent hosts that successfully controlled the primary infection. Thus, the local mechanisms and the dynamics of necrosis at the advanced disease stage are not the same as in primary lesions and the organ and organism scale factors may play bigger or different roles. Two different models of necrosis in advanced TB have been proposed (Igor and Gillian, 2016):

Model 1: This is the gradual necrotization and local expansion of organized granulomas, including formation and coalescence of satellite granulomas. Accumulation of dead macrophages that fail to survive intracellular MTBC infection is a primary source of the caseous necrotic masses (Igor and Gillian, 2016).

Model 2: This is the rapid dissemination of MTBC from chronic lesion causing tuberculous pneumonia, where necrosis formation may be associated with thrombosed blood vessels and infarcted regions of the lungs (Hunter *et al.*, 2015). Subsequently, MTBC bacilli, dead inflammatory cells, and dead lung tissue may be sequestered in a fibrous capsule to re-contain the pathogen. Clearly mechanisms and consequences of lung necrosis need to be considered within the genetic and immunological context of the host and the stage of a disease. We also would like to emphasize the distinction between systemic, lung, and cellular levels that contribute to necrosis and will discuss hypotheses related to necrosis in that order (Igor and Gillian, 2016).

2.6.2. Systemic host factors that contribute to necrosis

The dual role of inflammation was further investigated by Dannenberg (1994) who distinguished cytotoxic delayed type hypersensitivity and macrophage-activating cell-mediated immunity and pointed to a therapeutic potential of manipulating their balance. He used the rabbit model to experimentally reproduce those types and demonstrated that both types of responses were driven by systemic immunity. However, at that stage, it was impossible to identify molecular

determinants of protective versus pathogenic inflammation as specific therapeutic targets. However, specific mechanisms of adaptive immunity causing necrosis in TB granulomas still remain hypothetical (Comas *et al.*, 2010).

2.6.3. Local granuloma factors that contribute to necrosis

Mechanistically, the formation of necrotic TB lesions and liquefaction of cellular debris were attributed to release of hydrolytic enzymes by macrophages and neutrophils. Among them are MMP1 (Matrix Metalloproteinase-1) and MMP8 (Matrix Metalloproteinase-8) (Elkington *et al.*, 2011; Kubler *et al.*, 2015; Ong *et al.*, 2015) and serine proteases (Reece *et al.*, 2010), which degrade extracellular matrix proteins and basement membranes, participating in lung tissue destruction and stimulating fibrosis. Although those enzymes may be attractive therapeutic targets, they are likely to be executors but not the root causes of lung tissue necrosis (Ong *et al.*, 2015).

Recent studies demonstrate that disbalance of inflammatory pathways may lead to necrotizing inflammation. For example, activation of type I interferon (IFN-I) pathway by instilling tlr3 ligand poly (I:C) in the lungs promoted the development of acute necrotic TB lesions via excessive recruitment of myeloid cells (Antonelli *et al.*, 2010). In another model, over expression of IL-13 using T-cell specific promoter generated conditions for the development of well-organized necrotic lung granulomas (Heitmann *et al.*, 2014). Interestingly, the IL-13 over expression did not reduce Th1 responses which would explain the necrotization by suppression of the essential host resistance pathway. Another model of necrotic TB lesions has been generated using temporal inactivation of essential mechanisms of resistance, such as administration of NO (Nitrogen monoxide) inhibitor and neutralization of IFN γ using injections of monoclonal antibodies in mice infected with MTBC intradermally (Reece *et al.*, 2010).

An important concept emerged recently based on studies of heterogeneous TB granulomas in non-human primates (NHP) using combination of live imaging, analysis of RNA expression patterns, MTBC loads, histopathology, and computational modeling (Flynn *et al.*, 2015; Gideon *et al.*, 2015). The iNOS and arginase-1 protein expression in granulomas in situ were used as

surrogate markers for the M1 and M2 macrophage phenotypes, respectively. The M1/M2 balance emerged as a best correlate of the granuloma outcome. Double positive cells were found in granuloma walls, and phenotypically M1 cells increased towards the necrotic center (Flynn *et al.*, 2015).

2.6.4. Factors that contribute to macrophage necrosis

Mechanisms of macrophage death at a cellular level, directly caused by MTBC are under intense investigation. Roughly, they can be divided into two categories: (1) active mechanisms, whereby virulent mycobacteria or host produce toxic molecular mediators that cause macrophage death, and (2) passive mechanisms, where bacillary replication in macrophages results in lysis, essentially a blood-driven death. The former category focuses on specific pathways that determine macrophage death modality. The necrotic macrophage death is perceived as more detrimental for the host that might be associated with necrotic granuloma formation *in vivo*, while apoptotic death has been associated with host resistance and bacterial control (Divangahi *et al.*, 2013).

2.6.5. Macrophage roles in necrotizing responses

A scrupulous work by the Kornfeld laboratory provided convincing experimental evidence that macrophage necrosis may trigger neutrophil recruitment to sites of MTBC infection (Repasy *et al.*, 2015). Using relatively resistant C₅₇BL/6 mice infected with MTBC strains of varying virulence, they showed that higher neutrophil recruitment paralleled higher MTBC virulence and the rates of intracellular bacterial replication. Macrophage death in that model was driven by bacterial load (burst size), achieving which was followed by increase in uninfected and MTBC-infected neutrophils in the lungs (Repasy *et al.*, 2015). The authors also hypothesized that the neutrophil recruitment to sites of MTBC infection fundamentally reflects necrotaxis and that accumulations of dead cells and bacterial products may establish a self-sustaining positive feedback loop of neutrophil recruitment and death, even in the absence of bacterial replication. Thus, initially triggered by bacterial replication and macrophage death, neutrophil recruitment

could accelerate lesion progression in autonomous manner and create the environment favorable for further MTBC replication (Igor and Gillian, 2016).

2.6. 6. Genetic studies of necrotizing granulomas

The mechanism(s) of necrotizing granulomas using an unbiased forward genetic approach from phenotype to gene. To map genetic loci controlling the formation of necrotizing granulomas in the lungs of C3HeB/FeJ (HeB) mice, we performed classical linkage analysis using crosses with the C₅₇BL/6 (B6) mice (Kramnik *et al.*, 2000). The B6 inbred strain is the most widely used inbred mouse strain in TB research, as a wild-type (wt) control for many genetically engineered (mostly knockout) mice of the same background. The wt B6 mice are permissive to infection with virulent MTB but efficiently control MTB replication due to the development of T cell mediated immunity (Cooper, 2009). Following infection, they typically survive greater than 12 months, and TB disease progression may reflect age-related changes in immune responses or immunological exhaustion with up to 80% of their lung occupied predominantly by macrophages and lymphocytes with interstitial fibrosis but lacking necrotizing granulomas (Beamer and Turner, 2009).

Large necrotic foci could be found within the pneumonic areas at that stage surrounded by neutrophils that formed demarcation zone around necrotic foci. The appearance of organized necrotic lesions in the HeB mice followed the pneumonia and coincided with stabilized bacterial growth. In this model, the bacteria resided both extracellularly within the necrotic core, as well as inside macrophages within the granuloma wall, including the most outer layers and outside of the organized lesions (Igor and Gillian, 2016).

2.7. Diagnosis of *Mycobacterium*

A presumptive diagnosis of TB in swine and other susceptible species is often made on history, clinical findings, tuberculin skin tests, study of gross and microscopic lesions and the presence of acid-fast bacteria within discrete granulomas or granulomatous tissue (OIE, 2009; Tessema *et al.*, 2011). Firm, unequivocal diagnosis can be made only after isolation, identification and

typing of the bacteria. Polymerase chain reaction (PCR) and immunohistochemistry (IHC) are increasingly useful for confirmation (Sharifipour *et al.*, 2014).

2.7.1. Clinical examination

Clinical diagnosis of TB infection is difficult due to the chronic nature of the disease and the wide variety of symptoms depending on the location of the infection (Radostits *et al.*, 2007; Tsegaye *et al.*, 2010). Infection with *M. bovis* in cattle is a progressive, two stages. The initial phase involves TB granuloma formation, which occurs at the site of infection and the local lymph node. Enlarged superficial lymph nodes provide a useful diagnostic sign when lungs are extensively involved; there is commonly an intermittent cough. The principal sign of TB is commonly chronic wasting or emaciation that occurs despite good nutrition and care (Smith *et al.*, 2006)

2.7.2. Tuberculin skin test

The standard method for detection of bovine tuberculosis is the tuberculin test, which involves the intradermal injection of bovine tuberculin purified protein derivative (PPD) and the subsequent detection of swelling (delayed hypersensitivity) at the site of injection 72 hours later in cattle and 48 hours later in swine (Ameni and Erkihun, 2007).

2.7.3. Postmortem and histopathological examinations

Postmortem examinations should be supported by a histological examination of samples stained with haematoxylin and eosin (OIE, 2009). Typically, lesions caused by *M. bovis* in cattle are described as having a center of caseous necrosis with some calcification and a boundary of lymphocytes, neutrophils and epithelioid cells are seems to be found in swine (Bekele and Belay, 2011; Ewnetu *et al.*, 2012; Ayana *et al.*, 2013). Some of the epithelioid cells may fused together and form multinucleated giant cells (Quinn *et al.*, 1999). An outer border fibrous of connective tissue is usually present, giving the lesion a focal appearance and providing encapsulation to some extent, which may limit the spread of infection. Since the lesions are not conclusive, it is necessary to demonstrate the etiological agent using Ziehl Nielsen stain (Ayana *et al.*, 2013).

2.7.4. Bacteriology and differential staining

Mycobacterium bovis can be demonstrated microscopically on direct smears from clinical samples and on prepared tissue materials (Quinn *et al.*, 1999). The acid fastness of *M. bovis* is normally demonstrated with the classic typical Nielsen stain, but a fluorescent acid fast stain may also be used. Immunoperoxidase techniques may also give satisfactory results (Regassa, 2005). Final confirmatory diagnosis of bTB depends on isolation and identification of the bacteria, but preliminary examination of stained smears from lesions, sputum, milk, urine, pleural and peritoneal fluids, uterine discharges and feces is very important (Radostits *et al.*, 2007). In the smear, the organism appear red rods against a blue background in Ziehl Nielsen staining, while in the fluorochrome procedures, the acid fast organisms appear as fluorescent rods, yellow to orange (OIE, 2009).

2.7.5. Immunological/serological diagnostic methods

A number of blood tests have been used besides the classical intradermal tuberculin test. Due to the cost and the more complex nature of laboratory based assays, they are usually used as ancillary tests to maximize the detection of infected animals (parallel testing), or to confirm or negate the results of an intradermal skin test (serial testing). There is also evidence that when an infected animal is skin tested, an enhanced blood test can occur during the following week. The gamma interferon assay and the lymphocyte proliferation assay measure cellular immunity, while the ELISA measures humoral immunity (Jovan *et al.*, 2011).

2.7.6. Molecular techniques

2.7.6.1. Multiplex Polymerase Chain Reaction

Multiplex PCR as molecular technique differentiates MTBC from *M. avium*, *M. intracellularae*, and other mycobacterial species (Araújo *et al.*, 2014). Heat killed AFB positive sample DNA is used as source of DNA template. The PCR targets the sequence of the genus *Mycobacterium*, within the 16S rRNA gene (G1,G2) sequences, within the hyper variable region of 16S rRNA, that is known to be specific to *M. intracellularae* (MYCINT-F) and *M. avium* (MYCAV-R), and the MTB70 gene specific for MTBC (TB-A, TB-1B) (Mamo *et al.*, 2013; Rekha *et al.*, 2015).

2.7.6.2. Region of Differentiation Deletion typing

Primers directed against the RD4, RD9, and RD10 loci are used to generate a deletion profile that would allow species identification of the isolates. Mycobacterial genomic DNA will be obtained by heat killing, the isolates at 80°C for 60 min and stored at -20°C until it is subjected to PCR. Isolates will be confirmed as *M. bovis* by deletion typing of the RD4 region according to a PCR protocol (Brosch *et al.*, 2002).

2.7.6.3. Spoligotyping

Another molecular typing method for *M. tuberculosis* complex is the PCR based spacer oligonucleotide typing (spoligotyping). This method was proposed as an alternative to hybridization based fingerprinting methods for diagnosis and epidemiology of tuberculosis (Kamerbeek *et al.*, 1997). Spoligotyping is based on the variability of spacer sequences interspersed with repeat sequences in the polymorphic chromosomal direct repeat (DR) locus. This locus contains multiple, well conserved 36-bp long direct repeats (DR). 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. Spoligotyping is thus not only useful for differentiation of *M. bovis* strains but can also be used to distinguish these from *M. tuberculosis*, a distinction which is often difficult to make by using conventional bacteriological techniques (Kamerbeek, *et al.*, 1997).

2.7.6.4. Multiple independent loci containing variable numbers of tandem repeats

This is a PCR based method that analyses multiple independent loci containing variable numbers of tandem repeats (VNTR) of different families of interspersed genetic elements collectively called mycobacterial interspersed repetitive units (MIRU). In its original format, the PCR primers are each run in separate reactions and the sizes of the products are analyzed by gel electrophoresis. Currently, the most widely used version of MIRU-VNTR analysis includes 12 tandem repeat loci. A set of 24 MIRU-VNTR loci is standardized to increase the discrimination power. The advantages of MIRU-VNTR analyses are that the results are intrinsically digital and analysis can be applied directly to culture without the need for DNA purification (Supply, 2001).

The discriminatory power of MIRU-VNTR analysis is typically proportional to the number of loci evaluated; in general, when only the 12 loci are used, it is less discriminating relative to IS6110 RFLP genotyping for isolates with high-copy number IS6110 insertions but more discriminating than IS6110 RFLP genotyping for isolates with low copy number IS6110. When more than 12 loci are used, or MIRU analysis is combined with spoligotyping, the discriminatory power approximates that of IS6110 RFLP analysis. MIRU-VNTR genotyping has been used in a number of molecular epidemiologic studies, as well as to elucidate the phylogenetic relationships of clinical isolates (Kremer, 2005). VNTR analysis has also been used to evaluate *M. bovis* transmission. In addition to their use for tracing TB transmission at the strain level, MIRU-VNTR markers can also provide useful predictions for classifying strains into genetic lineages (Huang *et al.*, 2013).

2.8. Molecular Epidemiology of Tuberculosis in Ethiopia

The knowledge of molecular epidemiology in the field of tuberculosis have been used to provide novel information about the spread of tubercle bacilli in outbreaks, to track the transmission dynamics of tuberculosis in the population and to distinguish exogenous re-infection from endogenous reactivation. Ethiopia is among the 22 high TB-burden countries that accounts for 81% of estimated cases (WHO, 2014). The prevalence of bTB in Ethiopia is high and molecular typing of *M. bovis* has also indicated the existence of unique strains of *Mycobacterium* (Zeru *et al.*, 2013).

Isolation and molecular characterization of the causative agent of bTB has been carried out in the last decade mainly in cattle by making use of molecular epidemiology as a tool to elucidate bTB in livestock of Ethiopia. A number of isolates have been reported from different regions of the country and the study carried out by Ameni *et al.* (2007) in this regard was the first of its kind and marked major breakthrough in Ethiopia. Human infection due to *M. bovis* is thought to be mainly through drinking of contaminated or unpasteurized raw milk. The high prevalence of TB in cattle, close contact of cattle and humans, the habit of raw milk and meat consumption, and the increasing prevalence of HIV/AIDS may all increase the potential for transmission of *M. bovis* and other *Mycobacteria* between cattle and humans (Ameni *et al.*, 2013; Zeweld, 2014).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted from September 2017 to May 2018 in Addis Ababa and Bishoftu abattoirs. These two abattoirs were selected purposively because they were the only abattoirs slaughtered swine.

Addis Ababa is the capital and largest city of Ethiopia. It has an average annual rainfall of 1151.6mm of which 84% is during the long rainy season that extends from June to September and the remaining during the short rainy season that extends from March to May. Bishoftu is rapidly growing and industrializing town located 47.9 km South-East of Addis Ababa in Oromia Regional State. The geographical location of Bishoftu is at a latitude and longitude of 8°45' N and 38°59' E, respectively, with an elevation of 1920 meters above sea level (CSA, 2017). Apart from Addis Ababa and Bishoftu, swine were brought for slaughter to these abattoirs from surrounding Oromia Zone, Sabata and Burayyu which are at most within 60km radii from Addis Ababa (NMSA, 2015). Swine brought from each area were kept under intensive management system, feed poultry manure and left over (disposed carcass of other animals) from abattoirs.

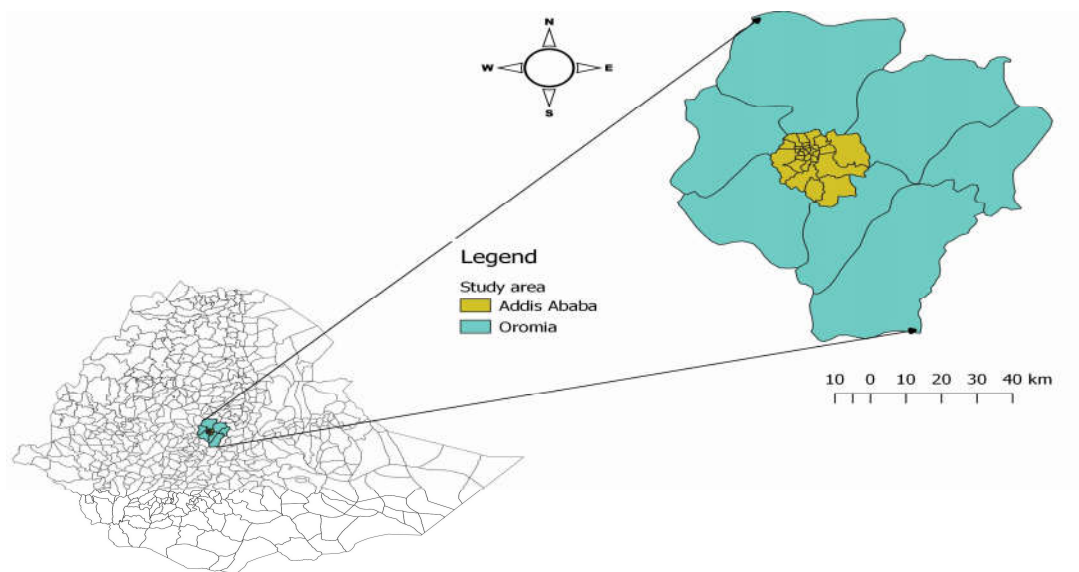


Fig 6. Map of study area- Addis Ababa and its surrounding Oromia Zone

3.2. Study Animals

Swine brought to Addis Ababa and Bishoftu abattoirs during the study periods for slaughter were study animals without discrimination of their age, sex, breed and body condition scores. Accordingly, 335 swine, from Addis Ababa and its surrounding and Bishoftu town, brought to Addis Ababa and 221 to Bishoftu abattoirs in total 556 swine were included in the current study. The average number of swine presented to slaughter per day was about 15 to 25 swine in Addis Ababa abattoir and about 5 to 10 swine in Bishoftu abattoir. Slaughtering of swine was done once in a week, commonly, on Monday.

3.3. Study Design and Sample Size

A cross-sectional study was employed for the current study. Previous studies on the abattoir gross lesions prevalence of swine TB were reported to be 3.6% (Shitaye *et al.*, 2006) and 5.8% (Arega *et al.*, 2013) in central Ethiopia. However, there was no a study report based on histopathological and molecular diagnostic techniques. Therefore, for the current study, the required sample size were calculated by considering 50% expected prevalence (p), 95% confidence interval ($z = 1.96$) and 5% desired absolute precision (d). The sample size was determined by making use of the formula after Thrusfield and Christley, (2018).

$$\text{Hence, } n = \frac{(z)^2 p(1-p)}{d^2}$$

Where $n =$ is the required sample size, $p =$ is the expected prevalence, $z =$ multiplier of the 95% confidence interval and $d =$ desired absolute precision. By making use of the above formula the calculated sample size (n) was 384; however, to increase precision 556 swine were selected from the two abattoirs.

3. 4. Sample Collection and Sample Processing

Non probability sampling method was employed to all slaughtered swine at Addis Ababa and Bishoftu abattoir. Hence, antemortem examination was carried out on all live swine. These included any abnormality in movement (gait), reaction to touch and sound, any visible

discharges from natural orifices, coughing, lesions on body and body conditions. Data such as age, sex, origin and body condition scores were collected during antemortem inspection by discussion with the owners and abattoir swine care providers.

Postmortem inspections were done according to Santos *et al.* (2010) and Di Marco *et al.* (2012) by observation of any visible gross lesions and by palpation of organs and lymph nodes during and/or after the animals were slaughtered. The lymph nodes inspected include submandibular, mesenteric, retropharyngeal, parotid, mediastinal, tracheobronchial lymph nodes as well as lungs, liver and spleen were inspected. Pathology scoring was conducted on tissues with tuberculous lesions to determine the severity of the lesions based on semi-quantitative procedure developed previously by Ameni *et al.* (2006). Briefly, lesions in the lobes of the lungs were scored separately as follows: 0 = no visible lesions; 1 = no gross lesions but lesions apparent on slicing of the lobe; 2 = fewer than five gross lesions; 3 = more than five gross lesions; 4 = gross coalescing lesions. The scores for the individual lobe were added to generate lung score. Similarly, the severity of gross lesions in individual lymph node was scored as follows: 0 = no gross lesions; 1 = small lesion at one focus; 2 = small lesions at more than one focus; 3 = extensive necrosis. Scores of individual lymph node were added and generated the lymph node score. The total pathology score per swine was obtained from the sum of lung and lymph node scores.

Gross lesions indicative of TB were observed on lung and/or lymph nodes to collect tissue samples. Briefly, approximately 2mm thick tissue specimens from active lesions were collected into sterile universal bottles filled with 5ml of 0.9% saline solution and transported on ice box packed with ice packs to keep the cold chain until they reached Aklilu Lama Institute of Pathobiology (ALIPB) TB laboratory for *Mycobacterium* culture. If immediate culturing was not done then samples were kept at 4^oC for two days and refrigerated at -20^oC when to stay for longer time (OIE, 2009). Mycobacterial culturing was conducted under biocontainment safety cabinet level two (BSC-II) facilitated with HEPA filter (negative pressure).

Tissue samples of 3-5mm were also pararely collected in 10% buffered formalin from lung, liver, spleen and lymph nodes including active part of the lesion and some surrounding

apparently normal tissue for histopathology (Talukder, 2007) and taken to pathology laboratory of the National Animal Health Diagnosis and Investigation Center (NAHDIC).

3.4.1. Histopathology

Briefly, the formalin fixed tissues were trimmed to 2-3mm, dehydrated in increasing alcohol concentration, cleared in three passes of xylene and impregnated with molten paraffin. Tissue block were made and then, the tissues were sectioned at 5µm thickness and the sectioned tissue (ribbon) was straighten on water bath. Then, the ribbon was adhered on frost ended and clear slide which is labeled and put in an incubator (annex I). The slides were deparaffinized in 3 changes of xylene, hydrated in decreasing alcohol concentration, placed in hematoxylin, rinsed in tap water, decolorized in acid and checked for differentiation of nucleus and cytoplasm. Then, rinsed again in tap water and stained in Eosin. Dehydrate again in increased alcohol concentration, clear in three changes of xylene and mount cover slide with Canada Balsum. Finally, the slides were read under a microscope (details in annex II) (Talukder, 2007).

Samples from lymph nodes, liver and spleen were categorized according to Varello *et al.* (2008), as follows: Positive: tubercular granuloma displaying central necrosis with or without mineralization surrounded by macrophages, lymphocytes, plasma cells, neutrophils, epithelioid cells, and Langerhan's giant cells, and enclosed partly or completely by a thin capsule. Inconclusive: lesion characterized by irregular with no capsulated clusters of epithelioid macrophages; associated with a not Langerhan's-type multinucleated giant cells and necrosis. Negative: features not consistent with tubercular granuloma, including significant eosinophilic infiltrates and lymphoid hyperplasia.

3.4.2. Mycobacterial isolation

The 40 granulomatous samples were collected from lungs and lymph nodes and processed for isolation of mycobacteria in accordance with Ameni *et al.* (2007) and OIE (2009). The specimens were sectioned using sterile blades, minced with scissors and homogenized with a sterile mortar and pestle under a biological safety cabinet level two (BSC_II). The homogenates

were decontaminated by adding an equal volume of 4% NaOH on the sample in order to remove contaminants and 3 ml distilled water or PBS. Then, the processed specimens were centrifuged at 3,000 rpm for 15 minutes to concentrate the mycobacteria. The supernatant was discarded and the sediment was neutralized by 1% (0.1 N) HCl using phenol red as an indicator. Neutralization was achieved when the color of the solution changed from purple to yellow (OIE, 2009). Next, 0.1 ml of suspension from each sample was spread onto a slant of Lowenstein Jensen (L-J) medium. Duplicate slants were used in which one was seeded on L-J enriched with sodium pyruvate and the other enriched with glycerol (OIE, 2009).

The cultures were incubated aerobically at 37⁰C and 5% CO₂ in slant position for one week with daily observation and 8–12 weeks in an upright position with weekly observation for growth of colonies. Positive cultures were confirmed with Ziehl Nelsen staining and preserved with freezing media while at the same time heat killed in water bath at 80⁰C for 45-60 minutes. The frozen and heat killed samples were stored at -20⁰C for further molecular typing.

3.5. Statistical analysis

The field collected data was entered, classified, filtered and coded using Microsoft Excel Spread Sheet (2010) and analyzed using the software package STATA 13 (Stata Corp, 2013) and IBM SPSS version 20. Prevalence was calculated as the proportion of suspected lesion positive animals from the total number of animals sampled (Thrusfield and Christley, 2018). Descriptive statistics was used for statistical summary. Univariable analysis and Chi-square (χ^2) test was performed to evaluate the statistical association of different risk factors with the result of gross and histopathology. Multivariable logistic regression analyses were used to analyze strength of association of the potential risk factors after their p-value was identified to be < 0.05 in univariable analysis. Effects were reported as statistically significant for the p-values less than 0.05. In cases of estimating the effect of different risk factors in terms of OR with corresponding 95% confidence interval, statistical significance was assumed.

4. RESULTS

4.1. Post-mortem and Gross Pathology

The cross-sectional study, by making use of pork inspection, in 556 slaughtered swine showed that 19.6% (109/556) were found to have tuberculous like lesions in parenchymatous organs of which 12% (69/556) were from lymph nodes, 5.7% (32/556) of the lungs, 1% (6/556) of the liver and 0.36% (2/556) of the spleen (fig 7). The tuberculous like lesions found in lymph nodes, particularly those of mesenteric and retropharyngeal, were statistically significant ($P < 0.05$) than in lungs and associated lymph node. The majority of the lesions were considered to be of typical tuberculous lesions characterized by central round, oval, or irregular, often coalescing areas of caseous necrosis and calcification. The calcified lesions were highly distributed in different lymph nodes and parts of the lungs (fig 8).

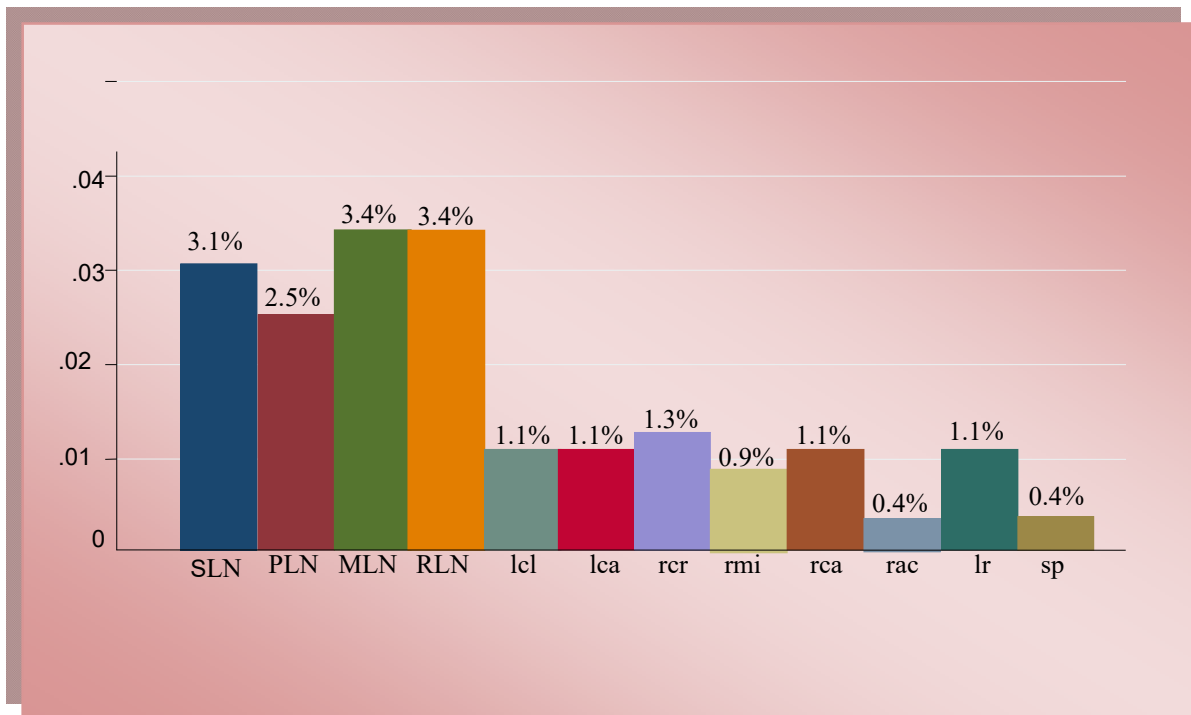


Fig 7. The mean prevalence of tuberculous like lesions in lymph nodes, lungs, liver and spleen
**SLN- submandibular lymph node; PLN- parotid lymph node; MLN- mesenteric lymph node;
RLN-retropharyngeal lymph node; lcr- left cranial; lca- left caudal; rcr- right cranial; rmi- right
middle; rca- right caudal and rac- right accessory lobes of lungs; lr-liver and sp-spleen

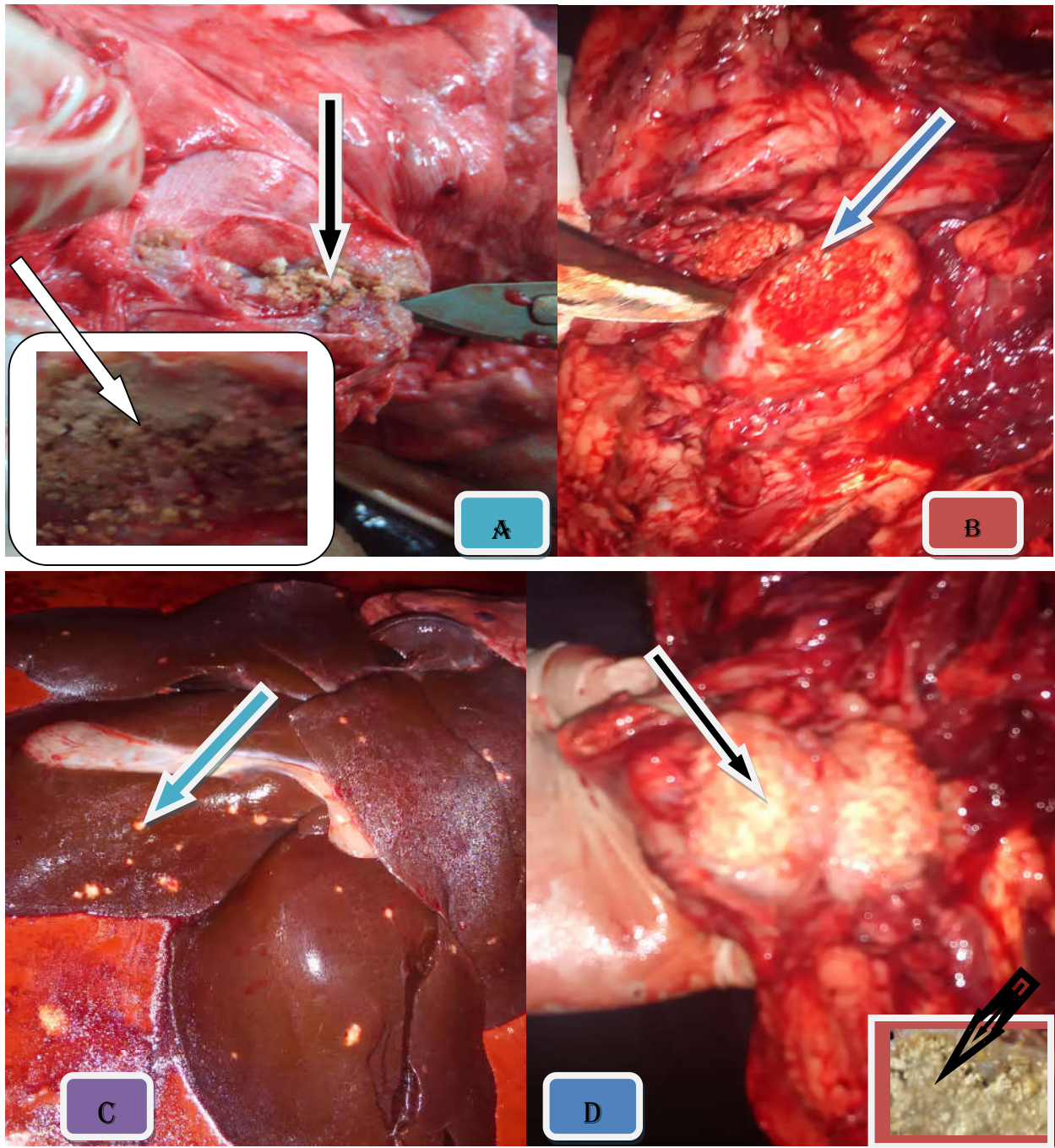


Fig 8. Gross TB like lesion in different organs. **Note:** The typical caseous calcified on left caudal lung (A), caseous exudates lesions in retropharyngeal lymph node (B), multifocal hepatic nodules (C), and typical caseous exudates in submandibular lymph node (D).

Pathological lesions were commonly seen in mesenteric lymph node 3.4% (19/556) and retropharyngeal lymph node 3.4% (19/556) (table 3). Among lung lobes found with TB like lesion right cranial, left caudal and left cranial lobes had 5 pathological lesion score with frequency 1.3% (7/556), 1.1% (6/556) and 1.1% (6/556) respectively (table 4).

Table 3: Frequency of lesions in lymph nodes, liver and spleen based on pathological score

Lymph nodes	Total pathological score	Frequency	Percent (%)
SLN	3	17	3.1
PLN	2	14	2.5
MLN	4	19	3.4
RLN	4	19	3.4
Lr	-	6	1.1
Sp	-	2	0.4

*SLN-sub-mandibular lymph node; PLN-parotid lymph node; MLN-mesenteric lymph node; RLN-retropharyngeal lymph node; Lr-liver and Sp-spleen

Table 4: Frequency of lesion in different lobes of the lung based on pathological score

Lobes of lung	Total pathological score	Frequency	Percent (%)
Lcr	5	6	1.1
Lca	5	6	1.1
Rcr	4	7	1.3
Rml	4	5	0.9
Rca	5	6	1.1
Rac	4	2	0.4

* Lcr- left cranial; Lca- left caudal; Rcr- right cranial; Rml- right middle; Rca- right caudal and Rac- right accessory.

Based on the stages of granuloma, 4.7% (26/556) lesions were calcified granulomas, 4.1% (23/556) lesions were necrotized, 3.6% (20/556) lesions were pyogranulomatous and 2.5% (14/556) lesions were resulted granulomas without calcsification (table 5).

Table 5: Frequency of different types of lesions in lymph nodes, lungs, liver and spleen

Types of lesions	Frequency	Percent (%)
CG	26	4.7
N	23	4.1
PG	20	3.6
G	14	2.5

***Note:** CF-calcified granuloma; G-granulomas without calcsification; N-necrotized lesions and PG-pyogranulomatous

4.2. Association of Different Risk Factors Considered with Gross Lesions

The univariable and multivariable logistic regression analyses of the different putative risk factors considered for tuberculous like lesion positivity were indicated in table 6 and 7 respectively. Swine with poor body condition had higher odd (likelihood) to develop TB like lesions (OR=0.21, 95% CI, 0.11-0.38) than good body conditioned swine. Old aged swine were positive to have tuberculous like lesions (OR=3.14, 95% CI, 1.62-6.09) than younger ones.

Table 6: Univariable logistic regression of different risk factors to postmortem lesion findings.

Variables	No. Swine	No. positive	Prevalence of TB (%)	OR	χ^2	P-value
Sex						
<i>Female</i>	297	43	14.47		0.1	0.750
<i>Male</i>	259	40	15.44	1.07		
BCS						
<i>poor</i>	264	21	7.9		26.81	0.000*
<i>Moderate</i>	174	28	16.09	0.47		0.010*
<i>Good</i>	118	34	28.81	0.21		0.000*
Age (in year)						
1	254	26	10.23		27.58	
2	212	28	13.20	1.33		0.319
3	72	19	26.38	3.14		0.001*
4	18	10	55.56	10.96		0.000*

BSC = body condition score; χ^2 =Chi-square; OR = odds ratio (crude); * = statistically significant

Table 7: Multivariate analysis of risk factors associated with postmortem lesion findings.

Variables	No. Swine	χ^2	OR	95% CI for OR		P-value
				Lower	Upper	
BCS						
		26.81				0.000*
<i>Poor</i>	264					
<i>Medium</i>	174		0.47	0.26	0.83	
<i>Good</i>	118		0.21	0.11	0.38	
Age (in year)						
		27.58				0.000*
1	254		1			
2	212		1.3	0.75	2.35	
3	72		3.14	1.62	6.09	
4	18		10.95	3.97	30.22	

BSC =body condition score; CI =Confidence interval; χ^2 =Chi-square; OR =odds ratio (adjusted); * = statistically significant

4.3. Histopathological Lesions

The most characteristic microscopic lesion was tubercle granuloma with central necrosis and calcification. The central area is made of necrotic cellular debris, calcium deposits, and connective tissue capsule walled off the granuloma from the surrounding tissue. The next layers from center to outer were made up of lymphocytes, macrophages, epitheloid macrophages distributed under connective tissue layers (fig 9A). The frequency of characteristic tubercle granuloma was 4.7% (26/556) (fig 9B). The presence of concomitant pyogranulomatous and granulomatous lesions in different organs was observed in 7.1% (40/556). Some granulomas were characterized by necrotic foci and intense calcification and fibrosis with absence of epithelioid cells. Multiple small granulomas in the lymph node (fig 9C) with less dense lymphocyte at periphery and epithelioid cells surrounding the deep outer lymphatic layer of the granuloma.

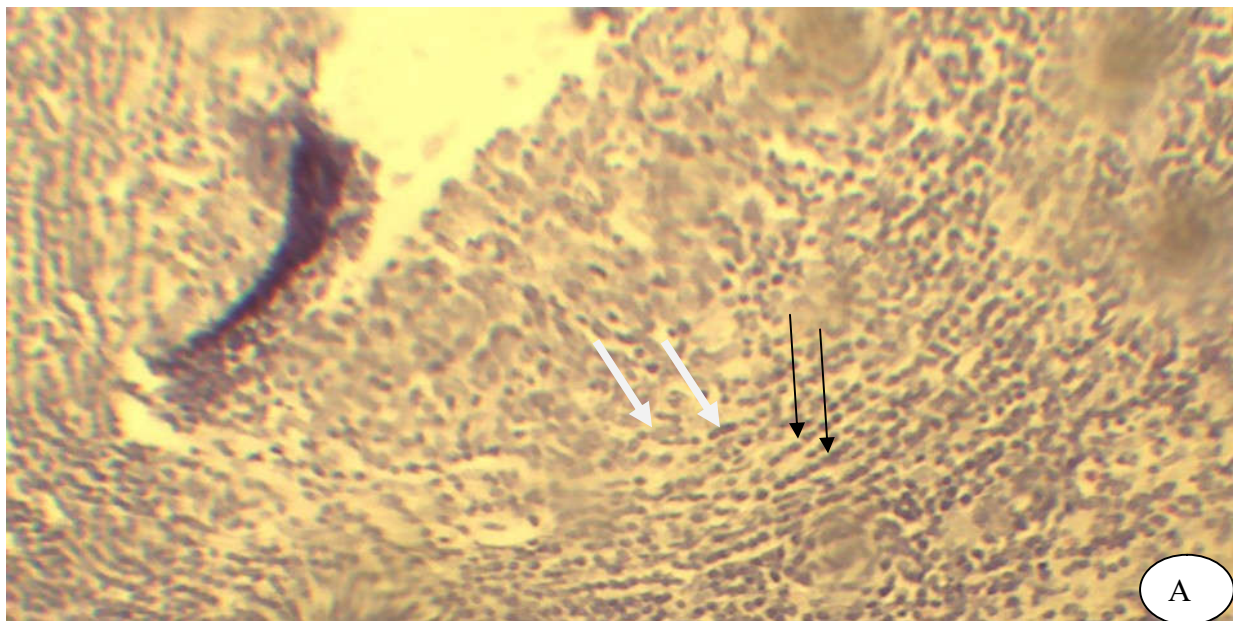


Fig 9A. Characteristic epithelioid macrophages in the lymph nodes. **Note;** The epithelioid macrophages shown as elongated (white arrow) and the lymphocytes in the next layers (black arrows). H & E stains (10x).

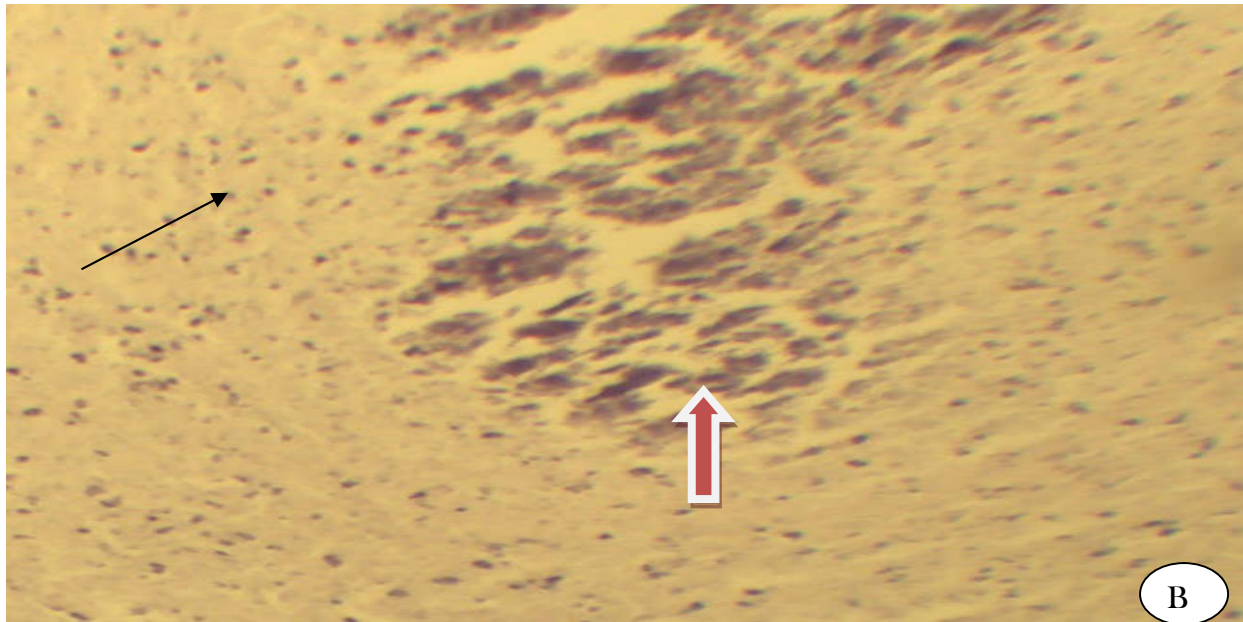


Fig 9B. The characteristic granulomas in the lymph node with calcium deposits (red arrow) at the center, followed by sever necrosis and cellular debris (black arrow) immediate to calcium deposits. H & E stain (10x)

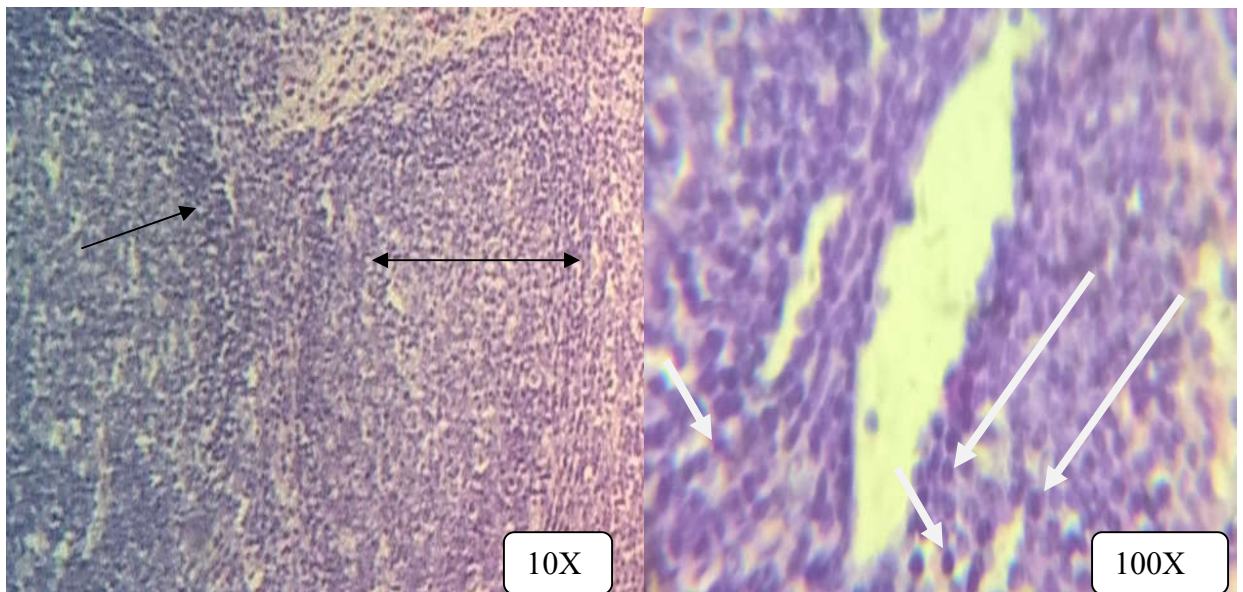


Fig 9C. Multiple small granulomas in the lymph node with central necrosis, layer of dense lymphocyte at periphery (double headed black arrows) admixed with epithelioid cells (white arrow). H & E stains.

Multifocal hepatonecrosis with total removal of hepatocytes and infiltration of inflammatory cells (mixed mononeuclear and polymorphoneuclear leukocytes) (fig 9D). At the periphery of this central necrosis are huge infiltrations of inflammatory cells (eosinophils, neutrophils and lymphocytes) in the portal triad region. Some foci of hepatic degeneration with swollen hepatocytes.

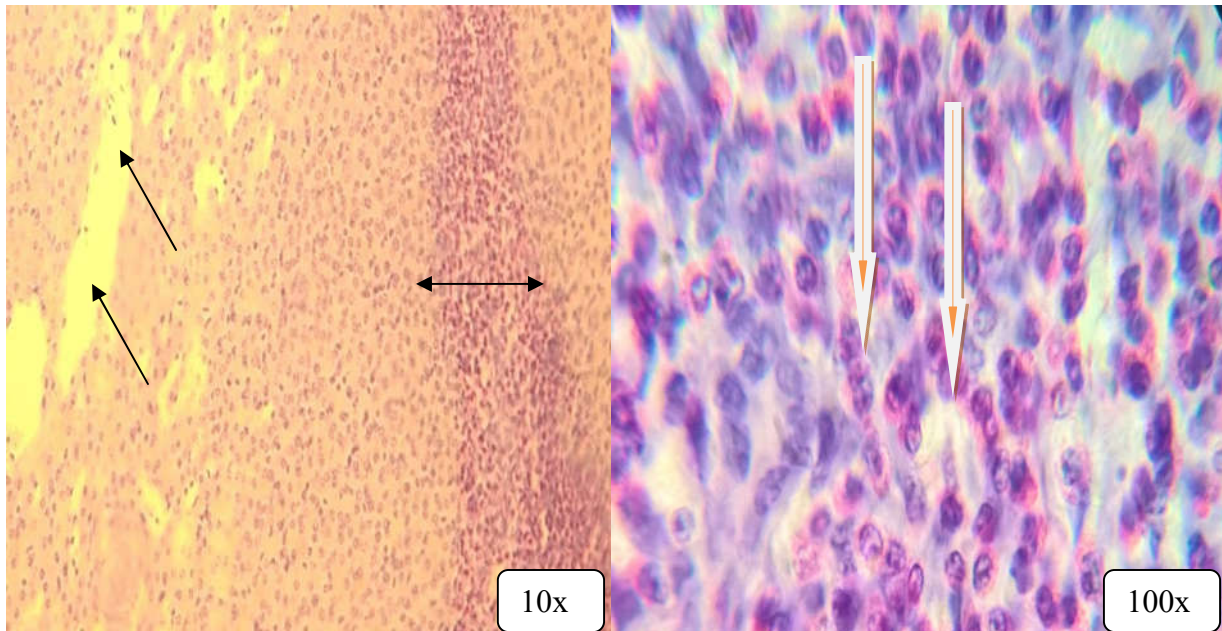


Fig 9D. Multifocal hepatonecrosis with total removal of hepatocytes (black arrows), infiltration of inflammatory cells into portal triads (double head arrows) and infiltration of inflammatory cells (white arrows). H & E stains

4.3. Mycobacterial Isolation and Characterization

Mycobacterial culturing was conducted from characteristic tubercle lesions of which 7.5% (3/40) showed growth on primary culture media. Of those cultures which showed visible colony, 5% (2/40) were seen on L-J media enriched with glycerol and the rest 2.5% (1/40) were grown on L-J media enriched with pyruvate. Three grown cultures were subjected to Zeihl-Neelsen staining technique in order to check the presence of acid fast bacilli. 5% (2/40) of the cultures were found to be acid fast positive. The observed colonial morphology was smooth whitish or yellowish

color, sticky, off-white and breaks apart easily (fig 10). The results attained of ZN staining were cocci, short and some long rod shaped and also found in single and clumps (fig 11).

Table 8: Bacterial culture result from tissues suspected tuberculosis lesions.

Sample type	Growth on				Z-N stain
	L-J media with pyruvate		L-J media with glycerol		Positive (%)
	Total	Positive (%)	Total	Positive (%)	
SLN	10	1(2.5)	10	-	-
PLN	7	-	7	-	-
MLN	10	-	10	1(2.5)	1(2.5)
RLN	8	-	8		
Lung	5	-	5	1(2.5)	1(2.5)
Total	40	1(2.5)	40	2(5)	2(5)

*SLN-sub-mandibular lymph node; PLN-parotid lymph node; MLN-mesenteric lymph node; RLN-retropharyngeal lymph node

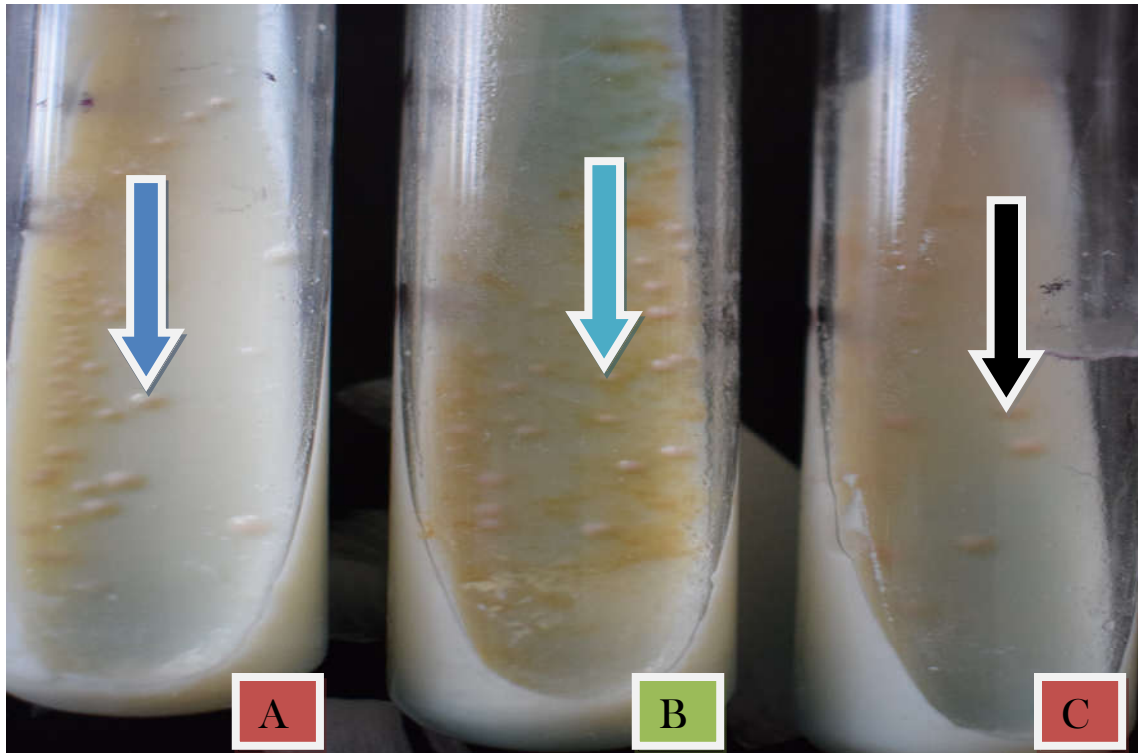


Fig 10. Grown bacilli on L-J culture media.

Note: (A & B) Colonies growth on L-J medium supplemented with glycerol; (C) Colony growth on L-J medium supplemented with pyruvate (Arrows indicate colony growth which is characterized as smooth, whitish or yellowish in color).

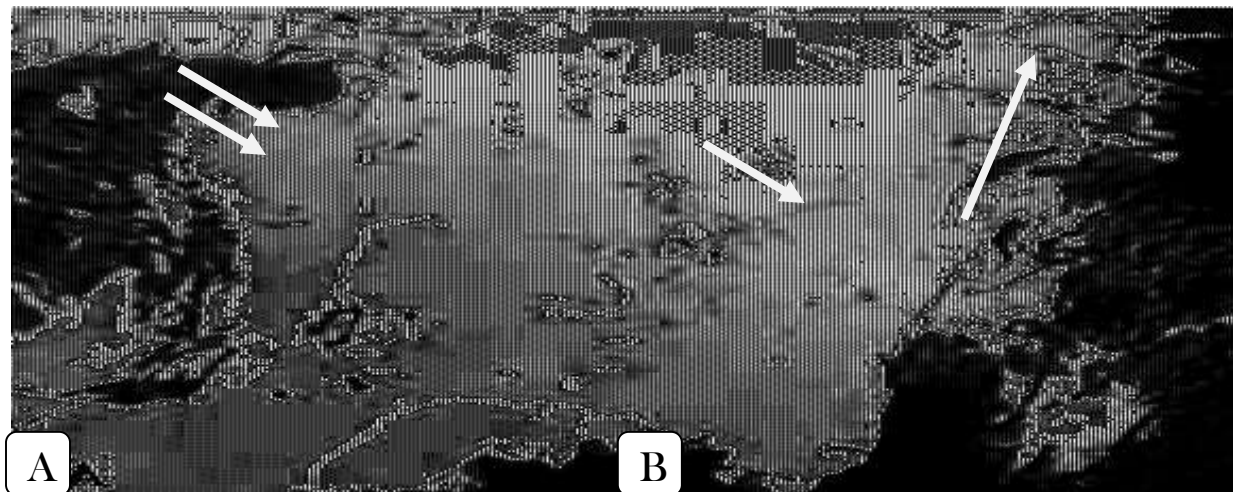


Fig 11. AFB staining positive from mycobacteriological culture colony growth.

Note: AFB stained smear from positive culture collected and seeded from mesenteric and lung TB lesions (arrows indicate acid fast bacilli with a short, and rod shaped arranged in the field).

5. DISCUSSION

The recognition of lesions of swine tuberculosis during meat inspection is of paramount importance for the surveillance and control of TB infection. Domingo *et al.* (2014) indicated that failure to detect a lesion during abattoir meat inspection has the greatest significance in cattle with single lesion; once the lesion is missed there is no further chance of detecting the disease in the animal. This idea also supports the chance of missing small sized lesions while inspecting swine carcasses at abattoir. Furthermore, the early slaughtering of swine before development of tuberculosis lesions in different organs and inadequate illumination in an inspection room of the abattoirs are the major factors determining the detection of tuberculous lesion/s. The slaughtered swine at an early age without sufficient time for lesion development and progression in the present study (45.6% (254/556)), was in agreement with the findings of the Domingo *et al.* (2014).

According to Corner *et al.* (1990) detection of swine tuberculous lesions in particular depends on the work load, time and the thoroughness of the inspector conducting the examination. Moreover Corner *et al.* (1990) pointed out that, detection of tuberculous lesions in abattoirs can be affected by early infection or parasitic lesions, non-specific reactions due to infection other than *M. bovis* and other irregularities of abattoir meat inspections.

The present study revealed tuberculous like lesions in 19.6% (n = 109) of swine carcasses inspected which was comparable to 19.8% reported by Cleaveland *et al.* (2007) in cattle slaughtered in rural Tanzania. The current result was higher than 5.8% reported by Arega *et al.* (2013) in Addis Ababa and Bishoftu abattoir in swine, 7.96% reported by Regassa (1999) in Wolaita abattoir in cattle, 8.8% reported by Biffa *et al.* (2009) in Hawassa municipal abattoir in cattle, 9% reported by Nemomsa *et al.* (2014) in Butajira abattoir in cattle and 11.50% reported by Abdurohaman (2009) in Butajira abattoir in cattle. On the other hand, the finding of this study was lower than 24.7% reported by Biffa *et al.* (2009) and 24% by Mamo (2007) in cattle at Adama municipal abattoir. The higher prevalence recorded in the present study could be due to the fact that swine feed mainly poultry meal and their offal's. Furthermore, the variations in prevalence could be attributed to the possible differences in the epidemiology of the disease in

different species of animals, origin, housing, age, body condition scores of the animals and types of production system. The majority of swine under this study were originated from intensive poor management production system. According to Ameni *et al.* (2007), Radostits *et al.* (2007) and Mamo *et al.* (2013) the intensive livestock management system could contribute to the development of mycobacterial infections than the extensive livestock management system which is in line with the current finding.

The difference in the prevalence of gross lesions between sexes was statistically insignificant ($\chi^2 = 0.1$ and $P > 0.05$). The overall gross lesion prevalence in different organs of the both sex was 19.6% (109/556). This study result is higher than the previous studies by Teklu *et al.* (2004) who reported 4.53% in cattle in Hossana, Shitaye *et al.* (2006) who reported 3.6% in swine in Addis Ababa, Arega *et al.* (2013) who reported 5.8% in swine in Addis Ababa, and to some extent consistent with the report by Cleaveland *et al.* (2007) who reported 19.8% in cattle in Tanzania. The possible reason for this discrepancy might be due to the difference in lesions distribution of female and male animals, in which it was higher in females, sample size and study methodology. Less number of male swine and relatively more number of adult female swine as a means of culling from the breeding stock were presented for slaughter to the study abattoirs during the current study duration.

There was statistically significant difference in this study ($\chi^2 = 27.58$ and $P < 0.05$) between age and lesions in different organs (lungs, lymph node, spleen and liver). This result is in consistent with the reports of Gebremedhin *et al.* (2014) who reported 2.6% in Dilla Municipal Abattoir in cattle and Nemomsa *et al.* (2014) who reported 9% ($p < 0.05$) in Butajira abattoir in cattle. The older swine (OR = 10.95) were nearly eleven times positive to have the gross pathological lesions than the younger ones. The results of the current finding also agreed with the findings of Barwinnek and Taylor (1996), Ameni *et al.* (2007), Regassa *et al.* (2010) and Biffa *et al.* (2011) in cattle who indicated as the age of the animal increases the probability of acquiring TB infection also increases. O'Reilly and Daborn, (1995) pointed out that, the protective capability is declining in aging animals due to a weaker immune system. Furthermore, Humblet *et al.* (2009) indicated stresses, malnutrition and immunosuppression increase with age. The findings

of all these authors supported the current result. The possible reason of difference between ages in current study will be the number of animals considered in each category is not proportional.

The effect of difference in the prevalence of swine TB among swine having different body condition scores was statistically significant ($\chi^2 = 26.81$; $P < 0.05$). The prevalence was highest in swine with poor body condition (47%) as compared to swine with medium body condition (21%) and good (8%) body conditions. This finding is in agreement with study reported by Nemomsa *et al.* (2014) who reported the higher prevalence of tuberculosis lesions in poor body condition and good body condition animals ($\chi^2 = 10.38$; $p < 0.05$) in Butajira abattoir in cattle. This could be due to the weak protective immune response in poor body conditioned swine when compared to swine with good body condition. Moreover, TB in poor body conditioned swine may result in extensive lesions and wasting of the body due to its chronic nature. The present result is in consistent with previous reports of Collins and Grange (1994), Radostits *et al.* (1994), and Radostits *et al.* (2007) in that animals with good body condition have relatively good immunological response to the infectious agent than animals with medium and poor body condition scores. On the other hand, the animals with poor body conditions and in nutritional deficiency have reduced resistance to TB which is also in consistence with the report by Doherty *et al.* (1995).

A significant difference between the lungs and lymph node ($P < 0.05$) with lesions was associated with the route of infection. In addition, observation of tuberculous lesions in 3.4% (19/556) of the mesenteric and retropharyngeal lymph nodes may also imply that, the occurrence of intestinal tuberculosis was found to be more frequent than respiratory form. This finding is in agreement with the report described by Shitaye *et al.* (2006) who observed tuberculous lesions in 33% (5/15) in mesenteric lymph node of swine that dictated that the occurrence of intestinal tuberculosis is more frequent than respiratory tuberculosis. However, the current finding is in disagreement with Corner (1994), Neill *et al.* (1994), Collins (1996), Whipple *et al.* (1996) and Teklu *et al.* (2004) who reported that greater than 84% TB lesions due to *M. bovis* occurred in the respiratory system indicating inhalation/aerosolization in cattle to be the predominant route of transmission. Charles (2017) described on the similar that ingestion of contaminated poultry

faeces and offals and shared grazing with infected animals and/or ingestion of contaminated dairy products are the major sources of TB infection in swine.

Granuloma with calcification was the lesion most frequently detected in the examined organs and occurred in 4.7% (26/556) of the cases (fig 9) in the current histopathological finding. In consistence to this finding, Shitaye *et al.* (2006) reported 3.4 % (3/69) granulomatous lesions typical to swine TB. Granulomatous lesions typical to swine tuberculosis, manifesting granulomas with central necrosis surrounded by epitheloid cells distributed under connective tissue layers, were observed in 7.1% (40/556). Consistent to this finding, Whipple *et al.* (1996) described that manifestation of typical granulomatous lesions in tissues with gross lesions was evident histologically.

The growth rate of mycobacteria on culture media in the current study was 7.5% (3/40). Amanfu (2006) and Cleaveland *et al.* (2007) pointed out the poor growth rate of *M. bovis* on standard L-J medium. However, Teklu *et al.* (2004) explained *M. avium* and *M. tuberculosis* to be well grown on standard L-J medium. Furthermore, Teklu *et al.* (2004) indicated the presence of caseous and/or calcified lesions may not be truly tuberculous lesions; Pritchard, (1988) and Diguimbaye-Djaibe *et al.* (2006) also implied that viable mycobacteria may not be present in calcified lesions. The 7.5% culture positivity from the lesion current finding, was lower than 47% reported by Ameni *et al.* (2010), 35% reported by Müller *et al.* (2008), 32% reported by Shimeles (2008), 31.4% reported by Woyessa *et al.* (2014) and 23.6% reported by Araujo *et al.* (2014), in slaughtered cattle, and 30.6% by Arega *et al.* (2013) in swine. However, the present finding was higher than that of Aknaw *et al.* (2016) who reported 2.9% in slaughtered cattle at Bishoftu abattoir. This findings justify that the types of culture media and weakly developed and/or trace number of tubercle bacilli in swine tissue may result in lower growth rate.

Mycobacteria in this study were detected by ZN staining in culture positive samples. This finding was consistent with a previous report by Gracey (1986). The result will be further justified with molecular techniques. *M. bovis* are often low in bovine specimens and they can be visualized by ZN only if a limited quantity (at least 5×10^4 mycobacteria/ml) of mycobacteria are present (Quinn *et al.*, 1994).

6. CONCLUSION AND RECOMMENDATIONS

The results of histopathological analysis followed by cultural isolation and ZN staining indicated that swine can serve as a source of infection of tuberculosis to human and/or other animal species. The molecular diagnostic method for further identification of swine tuberculosis at species level is on the process and will be included for publication. However, comparison of cultural isolation of *Mycobacterium* to pathological lesions indicated culture positivity to be smaller than lesions. This is due to the fact that lesions may be caused by other infectious agents rather than *Mycobacterium* species. Therefore, the diagnosis of mycobacteriosis in swine on a herd basis is important and usually directed to detect lymph nodes on the first hand and lungs as the second option from swine at slaughter. Isolation and identification methods including molecular and immunological diagnostic tests should be in use. Further investigation to elucidate the significance of swine TB in relation to public health and proper identification of its potential risk factors of infection and transmission among livestock and at animals-human-wildlife interface are evocative.

Based on the above conclusion the following recommendations are therefore forwarded:

- ✚ Detailed abattoir pork inspection should be implemented
- ✚ The epidemiological, economic and public health significance of swine tuberculosis should be studied in detail in the national swine herd of Ethiopia.
- ✚ Further research including screening via tuberculin skin test, culturing media and molecular diagnostic techniques should be warranted.

7. REFERENCES

- Abdu, S. and Gashaw, A. (2010): Production system dynamism and parasitic interaction of swine in and around Holetta Ethiopia: *Ethiop Vet J.* **14(1)**:71-81.
- Abdurohaman M. (2009): Cross sectional study of bovine tuberculosis in Butajira municipal abattoir, South West Ethiopia. *J. Global Vet.*, **6**: 172-179.
- Abubakar, U.B., Ameh, J.I., Abdulkadir, I.A., Silasu, I., Okaiyeto, S.O. and Kudi, A.C. (2011): Bovine tuberculosis in Nigeria: *Vet Res.* **1** 24-27.
- Akinosoglou, K.S., Karkoulis, K. and Marangos, M. (2013): Infectious complications in patients with lung cancer. *Eur Rev Med Pharmacol Sci*, **17**:8-18.
- Aknaw, W., Tilaye, D., Gazhagne, M. and Gobena, A. (2016): Bovine tuberculosis lesion description with molecular characterization of *Mycobacterium* species from cattle slaughtered at ELFORA and municipal abattoir, Bishoftu, Ethiopia. *MSc Thesis*.
- Ali, M. (2006): The status of bovine tuberculosis in selected area of north Gondar administrative zone, MSc Thesis, Ethiopia. Pp: 18-23.
- Amanfu W. (2006): The situation of tuberculosis and tuberculosis in animal of economic interest. *Tuberculosis*, **86**: 330-335.
- Ameni, G., Aseffa, A., Engers, H., Young, D., Hewinson, G. and Vordermeier, M. (2006): Cattle husbandry in Ethiopia is a predominant factor for affecting the pathology of bovine tuberculosis and gamma interferon response to *mycobacterial* antigens. *Clin Vaccin Immunol.* **13**:1030-1036.
- Ameni, G. and Erkihun, A. (2007): Bovine tuberculosis on small-scale dairy farms in Adama town, central Ethiopia, and farmer awareness of the disease. *Rev Sci Tech Off Int Epiz*, **26(3)**:711-719.
- Ameni, G, Aseffa, A, Engers, H, Young, D, Gordon, S, Hewinson, G. and Vordermeier 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 Vaccine Immunol*, **14(10)**:1356-1361.

- Ameni, G., Bekele, S. and Tolosa, T. (2010): Preliminary study on the impact of Bovine Tuberculosis on the reproductive efficiency and productivity of Holstein dairy cows in central Ethiopia, *B. of Ani. H and Prn in Afr.* **58**: 223-228.
- Ameni, G., Vordermeier, M., Firdessa, R. and Aseffa, A. (2011): *Mycobacterium tuberculosis* infection in grazing cattle in central Ethiopia, *the Vet. J.* **188**: 359-361.
- Ameni, G., Tadesse, K., Hailu, E. and Deresse, Y. (2013): Transmission of *Mycobacterium tuberculosis* between Farmers and Cattle in Central Ethiopia. *PLOS one.* **8**:1-10.
- Antonelli, L., R., V. Rothfuchs, A., G. and Gonçalves, R. (2010): Intranasal poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen permissive monocyte/macrophage population. *J Clin Invest.* **120**:1674-1682.
- Araújo, C.P., Osório, A.A.R., Jorge, K. and Ramos, C.A.N. (2014): Direct detection of *Mycobacterium tuberculosis* complex in bovine and bubaline tissues through nested-PCR. *Brazilian J Micro.* **2**: 633-640.
- Arega, S.M., Josef, F.C. and Ameni, G. (2013): Prevalence of tuberculosis in swine slaughtered at two abattoirs in Ethiopia and molecular characterization of *Mycobacterium tuberculosis* isolated from tuberculous-like lesions in swine. *BMC. Veterinary Research*, **9**:97.
- Arentz, M. and Hawn, T.R. (2007): Tuberculosis infection. Insight from immunogenomics. *Drug Discovery Today: Disease Mechanisms*, **4**: 231-236.
- Ashford, D.A., Whitney, E., Raghunathan, P. and Cosivi, O. (2001): Epidemiology of selected *mycobacteria* that infect humans and other animals, *Scientific and Technical Review, OIE.* **20**: 325-337.
- Ayana, T., Tafessa, K., Mamo, G., Tessema, T.S. and Ameni, G. (2013): Isolation and molecular characterization of non-tuberculosis mycobacteria from skin positive reactors and pathological lesions of cattle at Bako, W/Ethiopia. *AJMR*, **20**: 2190-2197.
- Bailey, S.S., Crawshaw, T.R., Smith, N.H. and Palgrave, C.J. (2013): *Mycobacterium bovis* infection in domestic swine in Great Britain. *Vet J.* **198**: 391-397.
- Barwinnek F. and Taylor N. M. (1996): Assessment of the socio-economic importance of bovine tuberculosis in Turkey and possible strategies for control or eradication. Turkish-German Animal Health Information Project. General directorate of protection and control, Ankara. Eschborn: Deutsche Gesellschaft fur Technische Zusammenarbeit, Pp. 3-45.

- Beamer, G.L. and Turner, J. (2009): Murine models of susceptibility to tuberculosis. *Arch Immunol Ther Exp*, **53**: 469-483.
- Bekele, M. and Belay, I. (2011): Evaluation of Routine Meat Inspection Procedure to Detect Bovine Tuberculosis Suggestive Lesions in Jimma Municipal Abattoir, South West Ethiopia. *Glob Veterinaria*. **2**: 172-179.
- Berg, S., Garcia-Pelayo, M. C., Müller, B., Hailu, E., Asiimwe, B., Kremer, K. and Dale, J. (2011): African, a clonal complex of *Mycobacterium bovis* epidemiologically important in East Africa, *J Bact.*, **193**: 670-678.
- Betancourt, S.L., Martinez-Jimenez, S., Rossi, S.E., Truong, M.T., Carrillo, J. and Erasmus. J.J. (2010): Lipoid pneumonia: spectrum of clinical and radiologic manifestations. *AJR Am J Roentgenol*, **194(1)**:103-109.
- Biffa, D., Inangolet F., Oloya J., Asseged B., Badaso M., Yilkal A. and Skjerve E., (2009): Prevalence of bovine tuberculosis in Ethiopian slaughter cattle based on post-mortem examination. *Trop. Anim. Health Pro.*, **41**: 755–765.
- Biffa, D., Inangolet F., Bogale A., Oloya J., Djonne B., Skjerve E. (2011): Risk factors associated with prevalence of tuberculosis-like lesions and associated mycobacteria in cattle slaughtered at public and export abattoirs in Ethiopia. *Trop. Anim. Health Pro*, **43**: 529-538.
- Bilal, S., Iqbal, M., Murphy, P. and Power, J. (2010): Human bovine tuberculosis–remains in the differential. *J Med Mic*. **59**: 1379–1382.
- Brosch, R., Gordon, S.V., Marmiesse, M. and Brodin, P. (2002): A new evolutionary scenario for the *Mycobacterium tuberculosis* complex; *Proceeding of the National Academy of Science USA*, **99**: 3684-3689.
- Cambier, C.J., Takaki, K.K., Larson, R.P., Hernandez, R.E., Tobin, D.M. and Urdahl, K.B. (2013): Mycobacteria manipulate macrophage recruitment through coordinated use of membrane lipids. *Nature*, **505**: 218-222.
- Central statistical agency (CSA) (2017): Population and Housing Census of Ethiopia. Addis Ababa, Ethiopia.
- Charles O. Thoen, (2017): Overview of Tuberculosis and other Mycobacterial Infections DVM, PhD, Professor, Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University.

- Chukwu, I.D., Chukwu, C.O. and Kandakai-Olukemi, Y.T. (2013): Detection of *MTBC* in Lung Specimen of Slaughtered Cattle and Goats by a DNA Based multiplex PCR and Ziehl-Neelsen Methods In Jos, Nigeria. *Br Mic. Res J.* **3**: 550-556.
- 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. *Tuberculosis*, **87**: 30 - 43.
- Collins C.H. and Grange J.M. (1994): The bovine tubercule bacillus. *J.Appl.Bacteriol.*, **55**:13-29.
- Collins J.D. (1996): Factors relevant to *M. bovis* eradication. *Irsh Vet. J.*, **49**: 241-243.
- Comas, I, Chakravarti, J, Small, P., M, Galagan, J, Niemann, S. and Kremer, K. (2010): Human T cell epitopes of *Mycobacterium tuberculosis* are evolutionarily hyperconserved. *Nat Genet* **42**: 498-503.
- Contzen, M, Sting R, Blazey, B. and Rau, J. (2011): *Corynebacterium ulcerans* from diseased wild boars. *Zoonoses. Public. Health.* **58**: 479-488.
- Cooper, A., M. (2009): Cell-mediated immune responses in tuberculosis. *Annu. Rev. Immunol* **27**: 393-422.
- Corner, L.A., Merville, L., McCubbin, K., Small, K.I., McCormick, B.S., Wood, P.R. and Rothel, J.S. (1990): Efficacy of inspection procedures for detection of tuberculous lesions in cattle, *Austr Vet J.* **67**: 338-392.
- Corner L.A. (1994). Post mortem diagnosis of *Mycobacterium bovis* infection in cattle. *Vet. Microbiol.*, **40**: 53-63.
- Cosivi, O., Meslin, F.K. and Dabron, C.J. (1998): Zoonotic tuberculosis due to *M. bovis* in developing countries. *Emer. inf. Dis.* **4**: 1-17.
- Cowie, C.E., Hutchings, M.R., Barasona, J.A., Gortazar, C., Vicente, J. and White, P.C.L. (2016): Interactions between four species in a complex wildlife: livestock disease community: implications for *Mycobacterium bovis* maintenance and transmission. *European Journal of Wildlife Research*, **62**: 51-64.
- Cronan, M.R. and Tobin, D.M. (2014): Fit for consumption: zebrafish as a model for tuberculosis. *Dis Model Mech.* **7**:777-784.
- Dannenber, Jr. A. (1994): Roles of cytotoxic delayed-type hypersensitivity and macrophage-activating cell-mediated immunity in the pathogenesis of tuberculosis. *Immunobiology*, **191**: 461-473.

- De la Rúa Domenech, R., Goodchild, A.T., Vordemeier, H.M., Hewinson, R.G., Christiansen, K.H. and Clifton-Hadley, R.S. (2006): Ante mortem diagnosis in cattle: A review of the tuberculin tests, gama-interferon assay and other ancillary diagnostic techniques. *Res. Vet. Sci.* **81**: 190-21.
- De Lisle, G.W. (1994): Mycobacterial infections in swine. *Surveillance*, **21**: 23-5.
- Di Marco, V, Mazzone, P, Capucchio, M.T, Boniotti, M.B, Aronica, V and Russo M. (2012): Epidemiological significance of the domestic black pig (*Sus scrofa*) in maintenance of bovine tuberculosis in Sicily. *J. Clin. Microbiol.* **50**: 1209-1218.
- Diguimbaye-Djaïbe, C., Vincent, V., Schelling, E., Hilty, M., Ngandolo, R. and Mahamat, H.H. (2006): Species identification of non-tuberculous *Mycobacteria* from humans and cattle of Chad. *Schweiz Arch Tierheilkd*, **148**: 251-256.
- Divangahi, M, Behar, S.M. and Remold, H. (2013): Dying to live: how the death modality of the infected macrophage affects immunity to tuberculosis, vol 783, New York, pp. 103-120.
- Doherty, M., Basset, H., Quinn, P., Davis, W., Kelly, A. and Monaghan, M. (1996): A sequential study of the bovine tuberculin reaction. *Immunology*, **87**: 9-14.
- Domingo, M., Vidal, E. and Marco, A. (2014): Pathology of bovine tuberculosis, *Research in Veterinary Science* **97**: 20–29.
- Elkington, P, Shiomi, T, Breen, R, Nuttall, K, Ugarte-gil, C.A and Walker, N.F. (2011): MMP-1 drives immunopathology in human tuberculosis and transgenic mice. *J Clin Invest.* **121**: 1827-1833
- Ewnetu, L., Melaku, A. and Birhanu, A. (2012): Bovine Tuberculosis Prevalence in Slaughtered Cattle at Akaki Municipal Abattoir Based on Meat Inspection Methods. *Glob Vet.* **5**: 541-545.
- Fabri, M, Stenger, S, Shin, D.M, Yuk, J.M, Liu, P.T. and Realegeno S. (2011): Vitamin D is required for IFN-mediated antimicrobial activity of human macrophages. *Sci Transl Med.* **3**:104ra102–104ra102
- Fentahun, T. and Luke, G. (2012): Diagnostic Techniques of Bovine Tuberculosis: A Review, *Afr. J Ba App Scie.* **4**: 192-199.
- Firdessa, R. Berg, S. Hailu, E. Schelling, E. Gumi, B. Erenso, G. Gadisa, E. and Kiros, T. (2013): Mycobacterial Lineages Causing Pulmonary and Extra pulmonary Tuberculosis, Ethiopia. *Emer Inf Dis.* **19**: 460-464.

- Flynn, J.L, Gideon, H.P, Mattila, J.T. and Lin, P.L. (2015): Immunology studies in non-human primate models of tuberculosis. *Immunol Rev*, **264**: 60-73
- Gebremedhin, G., Gebremedhin, R. and Gobena, A. (2014): Prevalence Study of Bovine Tuberculosis and Genus Typing of its Causative Agents in Cattle Slaughtered at Dilla Municipal Abattoir, Southern Ethiopia. Pp: 103-109.
- Gemechu, A. Giday, M. Worku, A. and Ameni, G. (2013): In vitro Anti-mycobacterial activity of selected medicinal plants against *M. tuberculosis* and *M. bovis* Strains. *BMC*. **13**: 291.
- Gideon, H.P., Phuah, J., Myers, A.J., Bryson, B.D., Rodgers, M.A. and Coleman, M.T. (2015): Variability in tuberculosis granuloma T cell responses exists, but a balance of pro- and anti inflammatory cytokines is associated with sterilization. *PLoS Pathog*. 11, e1004603.
- Gómez-Laguna, J., Carrasco, L., Ramis, G., Quereda, J.J., Gómez. S., Pallares, F.J. (2010): Use of real-time and classic PCR assays for the diagnosis of porcine tuberculosis in formalin-fixed, paraffin- embedded tissues. *J.Vet. Diagn. Invest*. **22**: 123-127.
- Gracey, J.F. (1986): Meat hygiene. 8th edition. London: *Bailliere-Tindall*; pp, 350–360.
- Grange, J. M. (1995): Human aspect of *mycobacterium bovis* infection. In: *mycobacterium bovis* infection in animals and humans. Ames: Iowa state university, Pp. 29-46.
- Heitmann, L., Abad Dar, M., Schreiber, T., Erdmann, H., Behrends, J. and Mckenzie, A.N. (2014): The IL-13/IL-4R is involved in tuberculosis-associated pathology. *J Pathol*. **234**: 338–350.
- Higgins, J., Camp, P., Farrell, D. Bravo, D., Pate, M. and Robbe-Austerman, S. (2011): Identification of *Mycobacterium* spp. of veterinary importance using rpo B gene sequencing. *BMC Vet Res*. **7**:77.
- Huang, Z.Y.X., de Boer, W.F. and van Langevelde, F. (2013): Dilution effect in bovine TB: risk factors for regional disease occurrence in Africa. *Proc R Soc B*. **280**: 1-7.
- Humblet, M.F., M.L. Boschioli and C. Saegerman, (2009): Classification of worldwide bovine risk factors in cattle: a stratified approach. Review Nottingham University Press, Nottingham, UK, article *Veterinary Research*, **40(50)**: 1-24.
- Hunter, R.L, Actor, J.K, Hwang, S.A, Karev, V. and Jagannath, C. (2015): Pathogenesis of post primary tuberculosis: immunity and hypersensitivity in the development of cavities. *Ann Clin Lab Sci*, **44**: 365-387.

- Hunter, R.L. (2016): Tuberculosis as a three-act play: A new paradigm for the pathogenesis of pulmonary tuberculosis, **97**: 8-17.
- Igor, K. and Gillian, B. (2016): Mouse models of human TB pathology: roles in the analysis of necrosis and the development of host-directed therapies. *Semin Immunopathol*, **38**: 221-237.
- Jovan, B., Božidar, S., Renata, R., Katarina, T., Tihomir, P., Dragan, R., and Ivan, P. (2011): The Case of Bovine Tuberculosis at the Slaughter House. *Bulletin UASVM*. **68**: 1843-5378.
- Kamerbeek, J., L. Schouls, A. and Kolk, M. (1997): Simultaneous detection and strain differentiation of *M. tuberculosis* for diagnosis and epidemiology. *J Clin Microbiol*. **35**: 907-914.
- Kramnik, I., Dietrich, W.F., Demant, P. and Bloom, B.R. (2000): Genetic control of resistance to experimental infection with virulent *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA*, **97**: 8560-8565.
- Kremer, K. (2005): Discriminatory power and reproducibility of novel DNA typing methods for *Mycobacterium tuberculosis* complex strains. *J Clin Microbiol*. **11**: 28-38.
- Kubica, T., Agzamova, R., Wright, A., Rakishev, G., Rüsç-Gerdes S. and Niemann, S. (2006): *Mycobacterium bovis* Isolates with *M. tuberculosis* Specific Characteristics. *Emerging Infectious Diseases*, Vol., 12, No. 5, May 2006, pp: 763-765.
- Kubler, A., Luna, B., Larsson, C., Ammerman, N.C, Andrade, B.B. and Orandle M. (2015): *Mycobacterium tuberculosis* dysregulates MMP/TIMP balance to drive rapid cavitation and unrestrained bacterial proliferation. *J Pathol*, **235**: 431-444.
- Lara, G.H., Ribeiro, M.G., Leite, C.Q., Paes, A.C., Guazzelli, A. and da Silva, A.V. (2011): Occurrence of *Mycobacterium* spp. and other pathogens in lymph nodes of slaughtered swine and wild boars (*Sus scrofa*). *Res. Vet. Sci*. **90**: 185-188.
- Lilenbaum, W. and Fonseca, L. (2006): The use of ELISA as a complementary tool for bovine tuberculosis control in Brazil. *Braz. J. Vet. Res. Anim. Sci*. **43**: 256-261.
- Lilenbaum, W., Schettinni, J., Souza, G.N., Ribeiro, E.R. and Moreira, E.C. (1999): Comparison between a gamma-IFN assay kit and intradermal tuberculin test for the diagnosis of bovine tuberculosis on field trials in Brazil. *J. Vet. Med. B, Infect. Dis*. **46**: 353-358.

- Machackova, M., Matlova, L. and Lamka, J. (2003): Wild boar (*Sus scrofa*) as a possible vector of mycobacterial infections: review of literature and critical analysis of data from Central Europe from 1983 to 2001. *Vet Med Czech*, **48**: 51-65.
- Majlessi, L., Prados-Rosales, R., Casadevall, A. and Brosch, R. (2015): Release of mycobacterial antigens. *Immunol Rev*, **264**:25-45.
- Mamo, B. (2007): Efficiency of abattoir inspection to detect tuberculosis lesions in cattle slaughtered at Adama municipality abattoir, central Ethiopia, DVM thesis, Addis Ababa, Ethiopia. Pp: 8-15.
- Mamo, G., 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 Inter*. **10**: 1-8.
- Mamo, G., Abebe, F., Worku, Y., Hussein, N., Legesse, M., Tilahun, G., Medhin, G. Bjune, G. and Ameni, G. (2013): Bovine tuberculosis and its associated risk factors in pastoral and agro-pastoral cattle herds of Afar Region, Northeast Ethiopia. *JVMAH*. **6**: 171-179.
- Marassi, C.D., Medeiros, L. and Lilenbaum, W. (2010): The use of a Gamma-Interferon assay to confirm a diagnosis of bovine tuberculosis in Brazil. *Acta Trop*. **113**: 199-201.
- Marshall, B.G., Wangoo, A., Cook, H.T. and Shaw, R.J. (1996): Increased inflammatory cytokines and new collagen formation in cutaneous tuberculosis and sarcoidosis. *Thorax*, **51**: 1253-1261.
- Martín-Hernando, M.P., Höfle, U., Vicente, J., Ruiz-Fons, F., Vidal, D. and Barral, M. (2007): Lesions associated with *Mycobacterium tuberculosis* complex infection in the European wild boar. *Tuberculosis*. **87**: 360-367.
- Mathema, B. Natalia, E. K., Pablo, J. B. and Barry, N. K. (2006): Molecular Epidemiology of Tuberculosis: Current Insights. *Clin Microbiol Rev*. **4**: 658.
- Matlova, L., Dvorska, L., Ayele, W.Y., Bartos, M., Amemori, T. and Pavlik, I. (2005): Distribution of *Mycobacterium avium complex* isolates in tissue samples of swine fed peat naturally contaminated with Mycobacteria as a supplement. *J Clin Microbiol*, **43**: 1261-1286.
- Medeiros, L., Marassi, C.D., Figueiredo, E. and Lilenbaum, W. (2010): Potential application of new diagnostic methods for controlling bovine tuberculosis. *Braz. J. Microbiol*.

- Mekibeb, A., Fulasa, T.T., Firdessa, R. and Hailu, E. (2013): Prevalence study on bovine tuberculosis and molecular characterization of its causative agents in cattle slaughtered at Addis Ababa municipal abattoir, Central Ethiopia. *TAHP*. **45**:763-769.
- Menzies, F.D. and Neill, S.D. (2000): Cattle-to-cattle transmission of bovine tuberculosis. *The Veterinary Journal* **160**: 92-106.
- Miranda, C., Matos, M., Pires, I., Correia-Neves, M., Ribeiro, P. and Alvares. S. (2012): Diagnosis of *Mycobacterium avium* complex in granulomatous lymphadenitis in slaughtered domestic swine. *J. Comp. Pathol.* **147**: 401-405.
- Mohamed, A.M., El-Ella, G.A.A. and Nasrm, E.A. (2009): Phenotypic and molecular typing of tuberculous and nontuberculous *Mycobacterium* species from slaughtered swine in Egypt. *J Vet Diagn Invest*, **21**: 48-52.
- Montoya, D., Inkeles, M.S., Liu, P.T., Realegeno, S., Teles, R.M.B. and Vaidya, P. (2014): IL-32 is a molecular marker of a host defense network in human tuberculosis. *Sci Transl Med.* **6**:250ra114–250ra114.
- Müller, B., Dürr., S., Alonso., S. and Hattendorf, J. (2008): Zoonotic *Mycobacterium bovis* induced Tuberculosis in Humans. *Eme Infec Dis.* **19**: 899-908.
- Mustafa, T., Leversen, N.A., Sviland, L. and Wiker, H.G. (2014): Differential in vivo expression of mycobacterial antigens in *Mycobacterium tuberculosis* infected lungs and lymph node tissues. *BMC Infect Dis* **14**:535.
- Nahar, Q., Pervin, M., Islam, M.T. and Khan, M.A. (2011): Application of PCR for the detection of bovine tuberculosis in cattle. *J. Bangladesh Agril. Univ.* **9**: 73-78.
- Neill, S.D., Pollock, J.M., Bryson, D.B. and Hanna, J. (1994): Pathogenesis of *M. bovis* infections in cattle. *Vete. Mic.*, **40**: 41-52.
- Neill, S.D., Bryson, D.G. and Pollock, J.M. (2001): Pathogenesis of tuberculosis in cattle. *Tuberculosis*, **81**: 79-86.
- Nemomsa, B., Gebrezgaber, G., Birhanu, T., Tadesse, G. and Getachew, B. (2014): Epidemiology of bovine tuberculosis in Butajira, southern Ethiopia. A cross sectional abattoir based study. *AJMBR.* **33**: 3112-3117.
- NMSA (2015): National Meterological Agency of Ethiopia.
- Office of international des epizootics (OIE) (2009): Bovine Tuberculosis. World organization for animal health manual of diagnostic tests and vaccines for terrestrial animals (mammals).

- Office International des Epizooties (OIE), (2014): OIE terrestrial manual. Paris, Pp: 5-26.
- O'Garra, A., Redford, P.S., McNab, F.W., Bloom, C.I., Wilkinson, J. and Berry, M.P.R. (2013): The immune response in tuberculosis. *Annual Reviews in Immunology*, **31**: 475-527.
- Oliveira, M., Barroco, C., Mottola, C., Santos, R., Lemsaddek, A. and Tavares, L. (2014): First report of *Corynebacterium pseudotuberculosis* from caseous lymphadenitis lesions in Black Alentejano pig (*Sus scrofa domesticus*). *BMC. Vet. Res.* **218**: 1-5.
- Ong, C.W.M., Elkington, P.T., Brilha, S., Ugarte-Gil, C., Tome-Esteban, M.T. and Tezera, L.B. (2015): Neutrophil-derived MMP-8 drives AMPK-dependent matrix destruction in human pulmonary tuberculosis. *PLoS Pathog.* 11, e1004917.
- O'Reilly L.M. and Daborn C.J. (1995): The epidemiology of *Mycobacterium bovis* infections in animals and man: a review. *Tubercle. Lung Dis.*, **76**: 1-46.
- Orme, I. and Cooper, A. (1999): Cytokine chemokine cascades in immunity to tuberculosis. *Immunology Today*, **20**: 307-312.
- Palomino, J. C., Martin, A., Portaels, F. (2007): Rapid drug resistance detection in *Mycobacterium tuberculosis*: a review of colourimetric methods. *Clin Microbiol Infect.* **13**: 754-762.
- Pavlik, I., Matlova, L., Dvorska, L., Shitaye, J.E. and Parmova, I. (2005): Mycobacterial infections in cattle and swine caused by *Mycobacterium avium* complex members and atypical mycobacteria in the Czech Republic during 2000–2004. *Vet Med Czech*, **50**: 281- 290.
- Pavlik, I. (2006): Control and eradication of bovine tuberculosis in central Europe. In *Mycobacterium bovis* infection in animals and humans. 2nd edition. Edited by Thoen CO, Steele JH, Gilsdorf MJ. Iowa: Blackwell Publishing; Pp: 136-148.
- Phillips, C.J., Foster, C.R., Morris, P.A. and Teverson, R. (2003): The transmission of *Mycobacterium bovis* infection to cattle. *Res Vet Sci*, **74**: 1-15.
- Pollock, J.M. and Neill, S.D. (2002): *Mycobacterium bovis* infection and tuberculosis in cattle. *Vet. J.* **163**: 115-127.
- Pollock, J.M., Welsh, M.D. and McNair, J. (2005): Immune responses in bovine tuberculosis: towards new strategies for the diagnosis and control of disease. *Vet Immunol Immunopathol.* **108**: 37-43.

- Pritchard D.G. (1988): A century of bovine tuberculosis, 1888-1988: conquest and controversy. *J. Comp. Pathol.*, **99**: 357-387.
- Quinn, P.J., 1994. Clinical Veterinary Microbiology. Spain: Mosby, pp: 158-169.
- Quinn, P. J., Carter, M.E., Markey, B., and Carter, G.R. (1999): *Mycobacterium* species. In: clinical veterinary microbiology. London Philadelphia. Pp: 157-170.
- Radostits, O.M., Blood, D.C. and Gay, C.C. (1994): Diseases caused by *Mycobacterium*. In: veterinary medicine, a text book of disease of cattle, sheep, swine, goats and horses. 8th ed. London, Bailliere tindu. Pp: 748-785.
- Radostits, O.M., Gay, C.C., Hincheliff, K.W. and Constabel, P.D. (2007): Veterinary Medicine. A text book of the disease of cattle, sheep, pig, goat and horses. 10th ed., Elsevier, London. Pp: 1007-1040.
- Rainy, F., Klattle, S., Krappenstedf, R. and Stackebrandet, E. (1995): Ditzia, a new genus including Ditziamaliscomb.nov. Formerly *Rhod...**Intern. J. of system. Bact.*, **45**: 32-36.
- Ramakrishnan, L. (2013): The zebrafish guide to tuberculosis immunity and treatment. *Cold Spring Harb Symp Quant Biol*, **78**: 179-192.
- Reece, S.T., Loddenkemper, C., Askew, D.J., Zedler, U., Schommer- Leitner, S., Stein, M. (2010): Serine protease activity contributes to control of *Mycobacterium tuberculosis* in hypoxic lung granulomas in mice. *J Clin Invest*, **120**: 3365-3376.
- Regassa A. (1999): Preliminary study on bovine tuberculosis in Wolaita-Sodo, South Ethiopia. DVM Thesis. Faculty of Veterinary Medicine, Addis Ababa University, Debre-Zeit, Ethiopia.
- Regassa, A. (2005): Study on *Mycobacterium bovis* infection in humans and animals in northwest shoa zone, Oromia national regional state, *Msc. Thesis*, Ethiopia.
- Regassa, A., Tassew, A., Amenu, K., Megersa, B., Abuna, F., Mekibib, B., Marcotty, T., and Ameni, G. (2010): Across sectional study on bovine tuberculosis in Hawassa town and its surroundings, Southern Ethiopia, *TAHP*. **42**: 915-920.
- Rekha, V.B., Gunaseelan, L., Pawar, G., Nassiri, R. and Bharathy, S. (2015): Molecular detection of *Mycobacterium tuberculosis* from bovine milk samples. *J Adv Vet. Anim Res*. **2**: 80-83.

- Repasy, T., Martinez, N., Lee, J., West, K., Li, W. and Kornfeld, H. (2015): Bacillary replication and macrophage necrosis are determinants of neutrophil recruitment in tuberculosis. *Microbes Infect*, **17**: 564-574.
- Russel, D.G. (2003): Highlighting the parallels between human and animal tuberculosis. *J of vet med edu*. **30**: 140-142.
- Sakamoto, K. (2012): The Pathology of *Mycobacterium tuberculosis* infection. *Vet Patho*. **3**: 423-439.
- Salgame, P., Geadas, C., Collins, L., Jones-Lopez, E. and Ellner, J.J. (2015): Latent tuberculosis infection. Revisiting and revising concepts. *Tuberc Edinb*, **95(4)**:373-384.
- Samuel, K. A. (2010): Molecular characterization of *mycobacterial* species isolated from tuberculous lesions seen in bovine carcasses at postmortem: a case study of three abattoirs in the Accra Region of Ghana. *A thesis Master of Science*, Ghana.
- Santos, N., Geraldés, M., Andreia, A., Almeida, V. and Correia-Neves, M. (2010): Diagnosis of tuberculosis in the wild boar (*Sus scrofa*): A comparison of methods applicable to hunter-harvested animals. *PloS ONE*. **5(9)**.
- Seifert, B. (1996): *Formica paralugubris* nov.spec. –a sympatric sibling species of *Formica lugubris* from the western Alps (Insecta: Hymenoptera: Formicoidae: Formicidae). *Reichenbachia*, **31**: 193-201.
- Sharifipour, E., Nasiri, M. J., Farnia, P. Mozafari, M. Irani, S. and Velayati, A.A. (2014): Evaluation of Molecular Diversity of *Mycobacterium tuberculosis* Strains by Polymorphisms in RD Regions. *J Mycobac Dis*. **4**: 3.
- Shimeles, S.B. (2008): Bovine tuberculosis. Epidemiologic aspects and public health implications in and around Debre Birihan, MSc thesis, Ethiopia.
- Shitaye, J.E., Getahun, B., Alemayehu, T., Skoric, M., Fictum, P., Vrbas, V. and Pavlik, I. (2006): A prevalence study of bovine tuberculosis by using abattoir meat inspection and tuberculin skin testing data, histopathological and IS6110 PCR examination of tissues with tuberculous lesions in cattle in Ethiopia, *Vet. Med*, **51**: 512- 522.
- Shitaye, J., Horváthová, A., Bartošová, L. Morávková, M., Kavevska, M., Donnelly, N. and Pavlík, I. (2009): Distribution of Non-Tuberculosis *Mycobacteria* in Environmental Samples from a Slaughterhouse and in Raw and Processed Meats. *Czech J Food Sci*. **27**: 194-202.

- Sibhat, B., Kassahun, A., Kassa, D., Gelagay, A., Gezahegne, M. and Gobena A. (2017): Bovine tuberculosis in Ethiopia: A systematic review and meta-analysis. *Journal of Preventive Veterinary Medicine*, **147**: 149-157.
- StataCorp. (2013): Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.
- Supply, P. (2001): Automated high-throughput genotyping for study of global epidemiology of *Mycobacterium tuberculosis* based on mycobacterial interspersed repetitive units. *J Clin Microbiol.* **10**: 3563-71.
- Tadayon, K., Mosavari, N. and Mehdi, F.M. (2013): An epidemiological perspective on bovine tuberculosis spotlighting facts and dilemmas in Iran, a historically zebu-dominant farming country. *Iranian j Mic.* **5**: 1-13.
- Talukder, S. (2007): Histopathology techniques: tissue processing and staining. www.talukd.com
- Tamura, A., Hebisawa, A., Fukushima, K., Yotsumoto, H. and Mori, M. (1998): Lipoid pneumonia in lung cancer: radiographic and pathological features. *Jpn J Clin Oncol*, **28**:492-6.
- Teklu A., Asseged B., Yimer E., Gebeyehu M. and Woldesenbet Z. (2004): Tuberculous lesions not detected by routine abattoir inspection: the experience of the Hossana municipal abattoir, Southern Ethiopia. *Rev. Sci. Tech.*, **23**: 957-964.
- Tessema, B., Beer, J., Emmrich, F., Sack, U. and Rodloff, A.C. (2011): Rate of Recovery of *Mycobacterium tuberculosis* from Frozen Acid-Fast-Bacillus Smear-Positive Sputum Samples Subjected to Long-Term Storage in Northwest Ethiopia. *J CLIMICR.* **49**: 2557–2561.
- Toen, C.O. (2005): Tuberculosis and other mycobacterial infections. In The Merck veterinary manual. 9th edition. Edited by Kahn CM, Line S. New Jersey: Merck & Co.,Inc:549-553.
- Toen, C.O., P. Lo Bue, D.A. Enarson, J.B. Kaneene and I.N. de Kantor. (2009): Tuberculosis: a re-emerging disease of animals and humans. *Veterinaria Italiana.* **45**: 135-81.
- Thrusfield, M.V. and Christley, R. (2018): Veterinary epidemiology. 4rd Ed. Published by Black Well science Ltd. Edinburgh, UK. Pp: 276.
- Varello, K., Pezzolato, M., Mascarino, D., Ingravalle, F., Caramelli, M. and Bozzetta, E. (2008): Comparison of histologic techniques for the diagnosis of bovine tuberculosis in the framework of eradication programs. *J. Vet. Diagn. Invest.* **20**: 164-169.

- Volkman, H.E., Pozos, T.C., Zheng, J., Davis, J.M., Rawls, J, F. and Ramakrishnan, L. (2010): Tuberculous granuloma induction via interaction of a bacterial secreted protein with host epithelium. *Science (New York, NY)*, **327**: 466-469.
- Wangoo, A., Johnson, L., Gough, J., Ackbar, R., Inglut, S. and Hicks, D. (2005): Advanced granulomatous lesions in mycobacterium bovis-infected cattle are associated with increased expression of type I procollagen, $\gamma\delta$ (WC1+) T cells and CD 68⁺ cells. *Journal of Comparative Pathology*, **133**: 223-234.
- Welsh, M.D., Cunningham, R.T., Corbett, D.M., Girvin, R.M., McNair, J., Skuce, R.A., Bryson, D.G. and Pollock, J.M. (2005): Influence of pathological progression on the balance between cellular and humoral immune responses in bovine tuberculosis. *Immunology*. **114**: 101-111.
- Whipple L.D., Boline A.C. and Miller M.J. (1996): Distribution of lesion in cattle infected with mycobacterium bovis. *J. Vet. Diagn. Invest.*, **8**: 351-354.
- Wood, P.R. and Jones, S.L. (2001): BOVIGAM™: An in vitro cellular diagnostic test for bovine tuberculosis. *Tuberculosis*. **81**: 147-155.
- Wood, P.R. and Rothel, J.S. (1994): In Vitro Immunodiagnostic Assays for Bovine Tuberculosis. *Veterinary Microbiology*, **40**: 125-135.
- World health organization (WHO) (2014): Global tuberculosis report. Geneva, Switzerland. WHO press. Pp: 1-147.
- Woyessa M., Jibril Y., Ameni G. and Duguma R. (2014): Molecular Epidemiology of *Mycobacterium Tuberculosis* Complex at Nekemte Municipality Abattoir, Western Ethiopia. *Sci. Tech. Arts Res.*, **3**: 167-173.
- Yan, B., S, Pichugin, A., V, Jobe, O, Helming, L, Eruslanov, E., B. and Gutierrez-Pabello, J., A. (2007): Progression of pulmonary tuberculosis and efficiency of bacillus Calmette-Guerin vaccination are genetically controlled via a common sst1-mediated mechanism of innate immunity. *J Immunol*, **179**: 6919–6932
- Zeru, F., Romha, G. and Ameni, G. (2013): Gross and Molecular Characterization of *Mycobacterium tuberculosis* Complex in Mekelle Town Municipal Abattoir, Northern Ethiopia. *Glob Vet*. **5**: 541-546.
- Zeweld, S.W. (2014): Cultural and molecular detection of zoonotic tuberculosis and its public health impacts in selected districts of Tigray region, Ethiopia. *Sokoto J Vet Sc*. **12**:1-12.

APPENDICES

Annex I: Histopathological procedures (Talukder, 2007)

1. Fixation of tissue by 10% neutral buffered formaldehyde
2. Trimming part of the tissue in a way that the lesion we require be included or not missed and to fit standard histological processing tissue cassettes (5mm thickness).
3. Tissue specimen processing: Dehydrating tissue by increasing alcohols concentration, clearing of tissue by xylene and impregnation of tissue by paraffin wax.
Formalin-I 2hr → Formalin-II 2hr → 70% Alcohol 1hr → 95% Alcohol 1hr → 100% Alcohol-I 1hr → 100% Alcohol-II 2hrs → 100% Alcohol-III 2hrs → Xylene I 1:30hrs → Xylene-II 1:30hrs → Xylene-III 1:30hrs → Paraffin-I 2hrs → Paraffin-II 3hrs. Full programme time 20 hrs and 30 minutes
4. Embedding of processed tissue: impregnated tissue is placed in a mould with their labels and then fresh melted wax (54-60c°) is poured and allowed to settle and solidify.
5. Sectioning: sectioning of tissue in 3-5 micron thickness and put on water bath to straighten the ribbon, and then adhere on the surface of frost ended and clear slide. Later label and put in an incubator over night.
6. Staining: Hematoxyline-eosine stain procedure

Annex II: Hematoxyline Eosine stain procedure (Talukder, 2007)

1. Deparaffinize slides in 3 changes of xylene for 3 minutes each.
2. Hydrate slides in 100% alcohol and 95% alcohol, 2 changes for 3 minutes each, and rinse in distilled water until ripples disappear from slides.
3. Place in Hematoxylin for 8 - 15 minutes.
4. Rinse in tap water until water runs clear.
5. Decolorize in 1% acid alcohol, 3 - 6 quick dips. Check differentiation microscopically: Nuclei should be distinct; Cytoplasm should be uncolored.

6. Rinse in tap water until ripples disappear from slides.
7. Dip in Bluing Agent, 3 - 5 long dips.
8. Wash in lake-warm tap water for 5 minutes (37-40°C.)
9. Stain in Eosin for 30 seconds - 2 minutes.
10. Dehydrate in 95% alcohol and 100% alcohol, 3 changes each for 2 minutes.
11. Clear in 3 changes of xylene for 2 minutes each.
12. Mount cover glass using Canada Balsum or D.P.X (Deapistix)

Annex III: Media preparation, procedures of culturing inoculums and staining (OIE, 2014).

Media preparation

- ❖ Measure 4.17 g LJ media for each Pyruvate and Glycerol labeled flasks
- ❖ Then pour 67.5 ml distilled water and mix by heating as well as stirrer
- ❖ Then add 1.23g Pyruvate in “P” labeled flasks and add 1.35ml glycerol in “G” labeled flasks
- ❖ Then cover using aluminum and autoclave the solution at 121°C for 30 minutes
- ❖ Then cool at room temperature
- ❖ Then prepare 112.5ml homogenized whole egg (2-2.5egg) for each flask after soaking with alcohol. And filter the solution.
- ❖ Then mix with the autoclaved media in a well manner to prevent bubble formation
- ❖ Then pour around 8-10ml of the media into sterile screw capped test tubes
- ❖ Then put the media in autoclave at 85°C for 45minute in slanted position for drying purpose.
- ❖ Then the media was dated and incubated at 37°C for 24hrs to check for sterility.

Then for culturing the sample was taken from the deep freeze, trimmed, homogenized the tissue using pistol and mortal, add 5ml saline water in the falcon tube having the homogenized tissue, add 5ml NaOH for decontamination, centrifuge at 3000rpm for 15 minute, discard the supernatant and use the sediment by adding a drop of phenol red as indicator, add a drop of HCl to see any color change (from red-yellow), then pour 1ml of the sample to the prepared media.

Inoculation

- ✚ The inoculum is distributed evenly over the surface of the slants.
- ✚ The tubes are allowed to remain in a slanted position at 37°C for approximately 1 week with screw caps loose.
- ✚ The tubes are returned to a vertical position when the free moisture has evaporated from the slants.
- ✚ The lids are tightened and the tubes are placed in baskets in an incubator at 37°C.
- ✚ The slants are incubated for at least 2 months and observed weekly for the presence of any growth from the week one onwards.
- ✚ Cultures will be considered positive based on the colony characteristics and presence of acid fast bacilli in the smear.

Staining

- Fix the smear of the specimen over the glass slide by heating.
- Then pour carbol fuschin over smear and heat gently until fumes appear and allow it to stand for 5 minutes and then wash it off with water.
- Then pour methanol (acid alcohol), wait for one minute and keep on repeating this step until the slide appears light pink in color. Wash off with water.
- Then pour methylene blue, wait for two minutes, again wash with water
- Allow it to air dry and examine under oil immersion lens.