Thesis Reference No._____

EPIDEMIOLOGY AND PUBLIC HEALTH IMPLICATIONS OF AVIAN TUBERCULOSIS IN SELECTED DISTRICTS OF OROMIA, ETHIOPIA

MSc THESIS

BY

TESFAYE DEBELU BEDANE

Addis Ababa University College of Veterinary Medicine and Agriculture
Department of Clinical Studies, MSc program in Veterinary Epidemiology

JUNE, 2017
BISHOFTU, ETHIOPIA

EPIDEMIOLOGYAND PUBLIC HEALTH IMPLICATIONS OF AVIAN TUBERCULOSIS IN SELECTED DISTRICTS OF OROMIA, ETHIOPIA

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

BY

TESFAYE DEBELU BEDANE

JUNE, 2017

BISHOFTU, ETHIOPIA

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by: Tesfaye Debelu Entitled "Epidemiology and public health implications of avian tuberculosis in selected districts of Oromia, Ethiopia" and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Science in Veterinary Epidemiology.

Fikiru Regasa (DVM, MSc, Asso. Prof.)	_____	_____
Chairman	Signature	Date
Alemayehu Regassa (DVM, MSc, PhD, Asso. Prof.)	_____	_____
External Examiner	Signature	Date
Kebede Amenu (DVM, MSc, PhD, Asso. Prof.)	_____	_____
Internal Examiner	Signature	Date
Advisors		
1. Fufa Abunna (DVM, MSc, Asso. Prof.)	_____	_____
Main advisor	Signature	Date
2. Gezahegne Mamo (DVM, MSc, PhD Asso. Prof.)	_____	_____
Co- advisor	Signature	Date
3. Gobena Ameni (DVM, PhD, Prof.)	_____	_____
Co- advisor	Signature	Date

DEDICATION

This thesis is dedicated to my younger sister, Beyenech Debelu, whom I lost at her early age with mysterious case of ‘Tuberculosis’. Let God stay her life at heaven!

STATEMENT OF THE AUTHOR

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

Name: Tesfaye Debelu Signature: _____

Addis Ababa University College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: **June, 16/2017**

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the almighty God for making all this possible.

I would then like to express my deepest gratitude to my major advisor, Dr. Fufa Abunna, Department of Clinical Studies, AAU, CVMA, for his genuine advice and intellectual feedback throughout the preparation of this paper.

I would also express my deepest sense of regard to my co-advisors, Dr. Gezahegne Mamo, Department of Veterinary Microbiology, Immunology and Public Health, AAU, CVMA and Prof. Gobena Ameni, Aklilu Lemma Institute of Pathobiology, AAU, for their unreserved guidance and valuable suggestions during the preparation of this manuscript.

My deepest gratefulness also goes to Dr. Kassa Demissie, PhD fellow at AAU, CVMA, for his unreserved and unforgettable moral, technical and financial support throughout the preparation of this paper and Dr. Tilaye Demissie, AAU, CVMA, for his optimistic and unforgettable technical support in reading and interpretation of the histopathological slides. Without him, the histopathology results would not be realized.

I would also express my cordial appreciation to Mr. Debela Taweya, MSc fellow at AAU, CVMA, Mr. Tufa Bedane and Mr. Mahammad Seid, externship students at AAU, CVMA, for their unreserved support in facilitating the postmortem room and postmortem sample processing facilities and also assisting me in conducting the postmortem inspections during the postmortem investigation of the study animals.

I am grateful to the laboratory technicians, Mr. Aboma Zewde and Mr. Samson Tolosa, Aklilu Lemma Institute of Pathobiology, AAU and Mr. Tewodrose Arega and Mr. Solomon Getachew, National Animal Health Diagnostic and Investigation Center, Sebeta, for their unreserved technical support during the processing of samples at their respective laboratories.

My profound thanks also goes to Dr. Mahari Teklu, Ms. Tesfanesh Moges and Mr. Dereje Gudeta of AAU, CVMA for their optimistic and unreserved assistance in facilitating postmortem tissue sampling bottles.

I would also like to extend my heartfelt gratitude to Aklilu Lemma Institute of Pathobiology, AAU, for providing me avian PPD and laboratory facilities for culturing; and Addis Ababa university thematic research, for their financial support.

I would also like to acknowledge the office of Livestock and Fishery in the Gerar Jarso, Adea and Boset study districts, development agents in the selected peasant associations in the districts and farmers in the districts who allowed me their time and resources and assisted me in conducting the research work in their respective districts and peasant associations.

I am also thankful to my friends, Dr. Zerihun Asefa, Dr. Beksisa Urge and Dr. Kebede Shanko, for their extending support during the entire course of the study; especially on the area of data analysis and interpretation; where they shared me a lot.

My family also deserves my sincere appreciation for their patience and immense moral support to join the program in general and in the preparation of this manuscript in particular.

Lastly, I would like to acknowledge my MSc fellow Epidemiologists of 2016/2017 for the experiences they share me and unforgettable and lovely time we spent together.

TABLE OF CONTENTS

LIST OF TABLES

List of Tables **Pages**

LIST OF FIGURES

List of Figures **Pages**

LIST OF ANNEXES

List of Annexes **Pages**

ABBREVIATIONS

ATB	Avian Tuberculosis
CFT	Complement Fixation Test
CSA	Central Statistics Authority
ELISA	Enzyme Linked Immune Sorbet Assay
HPLC	High Performance Liquid Chromatography
IS	Insertion Sequence
MATR	*Mycobacterium avium* tandem repeat
MIRUS	Mycobacterial Interspersed Repetitive Units
M-MGIT	Manual- Mycobacteria Growth Indicator Tube
PCR	Polymerase Chain Reaction
PPD	Purified Protein Derivatives
RAT	Rapid Agglutination Test
RFLP	Restriction Fragment Length Polymorphism
rRNA	Ribosomal Ribonucleic Acid
VNTRs	**V**ariable Number Tandem Repeat Markers

ABSTRACT

Avian tuberculosis is a zoonotic disease which remains a problem in extensive poultry production systems under which chickens scavenge for survival in unhygienic environments. A cross-sectional study anticipated to generate additional epidemiological and public health information on the disease was carried out from November, 2016 to June, 2017 at Gerar Jarso, Ada'a, and Boset districts of Oromia, Ethiopia; located at high, mid and low altitudes respectively. Study animals were 273 village chickens of both sex and local, exotic and cross breeds aged 22 weeks and above. Single intradermal avian tuberculin test, postmortem inspection of positive reactors, mycobacteriological culturing, hitopathological examination and questionnaire survey were used to assess information on the epidemiology and public health implications of the disease. Consequently, avian tuberculin test revealed an overall apparent prevalence of 11.4% (31/273); and a specific prevalence of 6.8% (6/88) in the highland, 13.4% (13/97) in the midland and 13.6% (12/88) in the lowland study districts. Besides, it signified a significantly ($P<0.05$) higher odd of exposure in cross breed chickens as compared to locals. Moreover, 40.9% (9/22) of positive reactor chickens sacrificed for necropsy showed gross pathological lesions and acid fast stain evidenced the presence of acid fast bacilli. Similarly, histopathological examination revealed a granuloma characterized by central necrosis and peripheral mononuclear lymphocytes. Nevertheless, only 0.02% (2/120) of the cultured tissues had shown colonial growth due to unknown reasons. Besides, the questionnaire survey evidenced poor awareness of participant farmers; as only 11% (10/91) of them had well perceived the zoonotic risk of the disease. Generally, the study revealed decreasing prevalence of avian tuberculosis with increasing altitude and poor public perception on the zoonotic risk of the disease; demanding further studies on the epidemiology, cultural isolation and public health implications of the disease.

Key words: *Avian tuberculosis, Chicken, Epidemiology, Ethiopia, Public health*.

1. INTRODUCTION

Ethiopia is believed to have the largest chicken population in Africa. The total chicken population of the country is about 56.87 million; 95.86% of which are village chickens (CSA, 2015). Poultry play an important economic, nutritional and sociocultural role in the livelihoods of poor rural households in developing countries, including Ethiopia. In developing countries, chickens are most commonly owned by rural families. Many of these families have scarce resources and many may be headed by women. Increasing the productivity of their chickens would make a significant contribution towards increasing their food security and their ability to have secure livelihoods (Alders and Spradbrow, 2001).

Globalization and a growing demand for meat products in developing regions in recent years have led to rapid expansion of the livestock sector, particularly pork and poultry meat. With these changes come an increased threat of emerging zoonotic diseases and a need for improved food safety and the implementation of appropriate biosecurity measures and breed improvement (Prosser *et al.,* 2011). Apart from the emerging and re-emerging diseases which have public health implications, other infectious and chronic diseases such as avian tuberculosis are also the threat for the health of the public especially in village chicken production system where the concept of hygiene is poor (Une and Mori, 2007).

Avian tuberculosis, which is caused by MAC (*Mycobacterium avium* complex), is found worldwide, most commonly in small, backyard flocks and in zoo aviaries; it is rarely found in young flocks. Infected birds and contaminated water and soil are the main sources of infection since the *Mycobacterium* can survive for several months in the environment (Dhama *et al.,* 2008). *Mycobacterium avium* is very resistant; it can survive in soil for ≥4 years, in 3% hydrochloric acid for ≥2 hours, and in 2% sodium hydroxide for ≥30 minutes (Thoen, 2013).

The most common route of infection for susceptible birds is the alimentary tract. Respiratory tract is also suggested as a potential source of infection. The disease gets transmitted to the susceptible birds by ingestion of contaminated feed and water and

inhalation of aerosolized infectious organisms. Persistence within flocks and human exposure is associated with keeping older stocks without following adequate cleanliness and hygiene. Further, maintaining birds closely confined under stressful conditions provide favorable ways for the spread of the disease. The ability of the organism to persist in the environment for many years (about four years), especially in soil and litter favor the disease transmission to a great extent (Dvorska *et al*., 2007).

Avian TB has also a zoonotic significance particularly for children and immunocompromised individuals such as HIV patients (Une and Mori, 2007). A study conducted at Shashemene district of Ethiopia indicated that for chicken kept in extensive production system, there exist a close physical contact between the chicken and their owners and there could be a possibility of transmission of *Mycobacterium* between chicken and their owners. On top of that, the low perception of the owners about zoonotic TB including avian TB and high number of HIV patients, immunocompromised individuals, in the country could add up to the risk of transmission (Sultan *et al.,* 2015). In general, in Ethiopia, even though more than 95% of chickens are produced under backyard poultry production system, where there is poor hygienic standard, strong physical contact between chickens and their owners and high risk of acquiring zoonotic diseases like avian TB due to massive traditional slaughtering of chickens especially during major holidays; studies conducted on avian tuberculosis are very scant. Hence, it is crucial to generate additional epidemiological and public health information that helps to design appropriate control strategies of the disease.

General objective;

- To generate information on the epidemiology and public health implications of avian tuberculosis and associated risk factors in backyard poultry production system at selected districts of Oromia region, Ethiopia.

Specific objectives;

- To determine the apparent prevalence and risk factors of avian tuberculosis in domestic chickens at selected districts of Oromia region, Ethiopia;

- To isolate and characterize the causative agents of the disease from postmortem lesions of avian tuberculin test positive reactor chickens in the selected study districts ; and
- To assess the level of perception of backyard chicken owners at the selected study districts on the occurrence and public health implications of avian tuberculosis.

1. LITERATURE REVIEW

Tuberculosis occurs worldwide in birds as a contagious, chronic, bacterial disease caused by members of the *Mycobacterium avium* complex which currently consists of four subtypes namely: *M. avium* subsp *avium* (fully virulent for birds and small mammals), *M. avium* subsp *hominissuis* (found in the environment but some are virulent in birds), *M. avium* subsp *paratuberculosis* (affects ruminants and other animals), and *M. avium* subsp *silvaticum* (rarely found and can be virulent in birds) (Pattison, 2008). The disease is most often caused by *Mycobacterium avium* belonging to serotypes 1, 2, 3, and 6 (genotype IS901 and IS1245) and *M. genavense* (Dvorska *et al.,* 2007). Other species, such as *M. intracellulare*, *M. scrofulaceum*, *M. fortuitum*, *M. tuberculosis*, and *M. bovis* can also cause avian tuberculosis, but the incidences are rare. All species of birds can be affected, but susceptibility amongst the domestic species is in the following order: chickens, ducks, geese and turkeys. The disease is most commonly seen in older poultry due to the greater opportunity for infection with age (Pattison, 2008). Infections with *M. tuberculosis* are fairly common in pet psittacines. These infections are most likely due to contact with tuberculous owners (Dvorska *et al*., 2007; Dhama *et al.,* 2008).

Avian tuberculosis is found worldwide, most commonly in small, barnyard flocks and in zoo aviaries; it is rarely found in young flocks. *M. avium* is very resistant; it can survive in soil for ≥4 years, in 3% hydrochloric acid for ≥2 hours, and in 2% sodium hydroxide for ≥30 minutes (Thoen, 2013). Wild birds, such as cranes, sparrows, starlings, and raptors, have been found to be infected. Tuberculosis has been found in emus and other ratites. Infected birds with advanced lesions often excrete the organism in their feces; therefore, ingestion of contaminated feces is the most common route of transmission. Cadavers and offal may infect predators and cannibalistic flock mates. Rabbits, pigs, nonhuman primates, and mink are readily infected. Cattle exposed to contaminated feces may develop granulomatous lesions in lymph nodes associated with the gastrointestinal tract (GIT) and respond to *M. bovis* purified protein derivative (PPD) tuberculin and to PPD of *M avium*. *M avium* serovar 1 is often isolated from tuberculous free-living wild birds and birds in captivity. *Mycobacterium avium* subspecies and *M. genavense* have been isolated from

immunocompromised people; however, these organisms only rarely cause disease in immunocompetent individuals (Thoen, 2013).

The most important source of the organism is the infected host. Also infectious are the items contaminated with the droppings of infected birds, such as litter, pastures and pens, equipment and implements, and also the hands, feet and clothing of workers. Eggs are of a minor importance in the epidemiology of the disease, and chicks hatched from eggs laid by tuberculous hens are not infected. Lack of hygiene influences the appearance of the disease due to the organism being highly resistant in the environment. It is relatively resistant to many antimicrobials and disinfectants, but sensitive to ionic detergents (Pattison, 2008). In any avian species, stress factors appear to enhance the development of the disease and this is particularly important in case of birds living in captivity ((Dhama *et al.,* 2007). The disease is more prevalent in places with high population density and poor sanitation and hygienic conditions. The practices of allowing birds to roam freely and keeping the breeders for several years are highly conducive to the spread of tuberculosis (Dvorska *et al.*, 2007; Shitaye *et al.*, 2008; Kazda *et al.*, 2009).

In poultry, the disease follows a slow course through the flocks. The classical presentation is characterized by chronic and progressive wasting and weakness. Avian tuberculosis in domestic birds is primarily an intestinal and hepatic disease with dissemination to other organs including the lungs, air sacs, spleen, bone marrow, and skin (Dhama *et al*., 2007; Thomas, 2007). Similarly, avian tuberculosis reported in free living birds including raptors were presented with the disseminated form involving the digestive tract, liver and spleen (Heatley *et al.,* 2007; Millan *et al*., 2010). The disease has a long incubation period and a protracted course and if appreciable, the symptoms can prolong for weeks or months. Because of the chances to become established through a longer exposure, the disease is less prevalent in young fowls and lesions are less severe in them when compared to adult birds. Usually the losses are experienced more in older stocks of age group 18–20 months. The disease process can be divided into three phases: latency, lesion development, and period of cachexia. During cachexia, massive tubercles with large numbers of bacilli develop. In the classic form of infection the tubercles or granulomas develop in multiple organs; a second form is manifested with lesions in the intestinal tract; a third

type of infection often experienced as a nontuberculous one, mainly seen in finches, canaries, and psittacines. Some birds show respiratory signs and sudden death may occur, dyspnoea is less common, and granulomatous ocular lesions and skin lesions have been reported (Dhama *et al.,* 2007).

In most cases, an infected bird without overt clinical signs may serve as carrier; those results in the persistence of infection in flocks. In commercial broiler production units, generally avian tuberculosis is uncommon primarily due to the short life span and in layers and breeders; the infection is a matter of much concern. Mortality over a short period may be insignificant, but the intermittent loss of adult birds in valuable breeding stock and decreased egg production in layers are detrimental. Occasionally, heavy losses may occur in pullets on multiage sites where the infection is endemic and the hygienic standards are poor (Nazmul, 2014).

Clinical signs are not pathognomonic in avian TB and vary depending on the organs involved. Birds with the intestinal form of tuberculosis often present with chronic wasting disease. In majority of cases of tuberculosis in birds, especially in the initial phase of infection, clinical signs are not grossly observable. However, in advanced cases, birds may develop symptoms like progressive weight loss, depression, white diarrhea with soiled feathers, increased thirst, respiratory distress, fatigue, and decreased egg production (Dhama *et al*., 2007). Feathers are often dull or ruffled and comb, wattle, and earlobes often appear pale, thinner and dry. Birds eventually become lethargic and emaciated with marked atrophy of breast muscles manifested as "knife edged" keel. In extreme cases, the body fat disappears, and the face of the bird appears smaller than normal. If a jerky hopping gait is observed due to unilateral lameness, then it should be assumed that there could be the presence of tubercular lesions in bone marrow of the leg bones or joints. Some birds may adapt a sitting position. Tuberculous arthritis can even lead to paralysis. Fatal results often occur due to massive hemorrhage caused by ruptured liver or spleen. In this case, occasionally birds may die suddenly in good bodily condition and yet show advanced lesions of tuberculosis. The body temperature of the affected bird remains normal, even in severe cases (Tell *et al.,* 2002).

The primary lesions of avian tuberculosis in birds are nearly always in the intestinal tract. Such lesions take the form of deep ulcers filled with caseous material containing many mycobacterial cells, and these are discharged into the lumen and appear in the faeces. Before the intestinal tract is opened, the ulcerated areas appear as tumour-like masses attached to the gut wall, but when the intestine is opened, the true nature of the mass becomes evident. Typical caseous lesions are nearly always found in the liver and spleen, and these organs usually are greatly enlarged because of the formation of new tuberculous tissue. The lungs and other tissues are ordinarily free from lesions even in advanced cases. Findings of tuberculous lesions that involve other organs such as liver, spleen, lungs, etc. are rare, usually occurring at the advanced stage of the disease (Shitaye *et al.,* 2008).

Among domestic animals (mammals), domestic pigs, cattle, deer, sheep, goats, horses, cats, dogs, and exotic animals can be infected by avian tuberculosis; of which pigs are the most susceptible and cattle are highly resistant (Shitaye *et al.,* 2009). These animals are usually asymptomatic reactors and tuberculous lesions are detected only on meat inspection in head, mesenteric and occasionally in liver lymph nodes. It is also essential to bear in mind that all members of *M. avium* complex and *M. genavense* are capable of giving rise to a progressive disease in humans that is refractory to treatment, especially in immunocompromised individuals. Hence the handling of infected birds in farms or live cultures of *M. avium* in laboratories should be carried out with adequate care (Shitaye *et al.*, 2009).

1.1. Diagnostic Techniques of Avian Tuberculosis in Chickens

The diagnosis of *M. avium* infection is based on clinical signs, postmortem gross lesions, and by demonstrating the acid-fast bacilli in crushed lesions using microscopy, which is sufficient for a positive diagnosis (Pattison, 2008). If acid-fast bacilli are not found, but typical signs or lesions are present in the birds, culture of the organism must be attempted. In live birds tuberculin test and serological tests such as ELISA and RAT and molecular techniques such as PCR can also be utilized (OIE, 2010).

2.1.1. Antemortem diagnosis

Antemortem diagnosis of avian tuberculosis in birds is problematic and typically unrewarding. The clinical presentation is highly variable and practical diagnostic tools which are highly sensitive and specific in all bird species are not available. Newer techniques involving amplification and identification of species-specific mycobacterial DNA fragments hold much promise for antemortem diagnosis and for epidemiological investigations. The problem with utilizing this technology is the intermittent shedding of mycobacterial organisms and the difficulty in targeting tissues to test (OIE, 2001).

2.1.1.1. Microscopical examination

Microscopical examination is rapid, inexpensive and provides immediate results, but organisms can be easily missed if numbers of mycobacteria are low. The limit of detection for cytological specimens is estimated to be 10, 000 bacilli/ml; by comparison, culture has a detection limit of 100 bacilli/ml. Debris in the sample can further hamper cytological identification of these organisms; consequently faecal samples, whether from clinically normal birds shedding mycobacteria or from diarrhoeic birds, may be negative on cytological examination. Therefore, fecal acid-fast stains serve as a poor screening tool (Aranaz *et al.,* 1997).

The Ziehl-Neelsen stain is the standard method for identification of acid-fast organisms. A modified Ziehl-Neelsen stain utilizes peanut oil with xylol at the beginning of the staining process. The addition of peanut oil limits damage to the lipid layer of the mycobacterial cell wall. Mycobacteria appear pink-red with the Ziehl-Neelsen stain. *Mycobacterium tuberculosis* is rod-shaped, while *M. avium* can appear almost coccoid or as long, beaded rods (Tell *et al*., 2002). Aranaz *et al.,* suggest that fluorescent dyes, such as auramine or acridine orange, are superior to the conventional fuschin stains because the mycobacteria are rendered more visible and a larger area of the smear can be examined in a shorter time (Aranaz *et al.,* 1997).

A suggestive diagnosis of avian mycobacteriosis may be based on identification of the organism in cytologic samples; however, the acid-fast stain is not a specific test, because non-pathogenic mycobacteria can be transient gastrointestinal inhabitants or environmental contaminants. Therefore, positive acid-fast cytology should be confirmed by culture, histopathology or DNA probe analysis. Depending on the chronicity and severity of the infection, these acid-fast organisms may be found either intracellularly (within tissue macrophages or epithelioid cells) or extracellularly in clinical samples (Tell *et al*., 2002).

According to a study conducted on layer chickens in Bangladesh, histopathological staining using Hematoxyline and Eosin staining of the section of affected areas of liver, spleen, heart, kidney and intestine and microscopic examination of the specimens showed multi-focal accumulation of macrophages, epitheloid cell, lymphocytes and fibrous connective tissue. The epithelial lining of affected ileum was also necrotized and sloughed off and there were accumulation of macrophages and lymphocytes in the mucosa (Nazmul, 2014)**.**

2.1.1.2. Immunological techniques

In live birds, during life time, besides the culture and isolation techniques, immunological tests, namely, tuberculin test; whole blood agglutination test or rapid agglutination test (RAT) and enzyme-linked immunosorbent assay (ELISA) are also valuable diagnostic tools. ELISA which is reported to be less specific than tuberculin test can detect specific antibodies and thereby help determine exposure to *M. avium.* However, false positives may be common in ELISA (OIE, 2001).

RAT has been used with good results, especially in both domestic and ornamental waterfowl. The test involves combining a drop of prepared antigen with a drop of blood or serum on a white tile. The presence and degree of agglutination is subjectively scored within the first thirty to sixty seconds, while gently agitating the tile. This test has the advantages of requiring only one blood sample (and thus, single handling of the birds), being rapid and simple to perform, requiring no sophisticated or expensive equipment, and providing an immediate result. RAT test is especially useful for screening large flocks for immediate culling, and therefore has advantages

over the tuberculin test for the control of the disease, even in domestic fowl. It has also been claimed that in domestic fowl it is more reliable than the tuberculin test (Keavska *et al.,* 2010). The identification of immunogenic proteins of *M. avium* may favor the development of more precise sero-diagnostic tools (Amador *et al.,* 2010). According a study conducted by Shitaye *et al*. (2008) in naturally infected hens, RAT positivity was significantly higher in hens affected by advanced avian tuberculosis with concurrent tuberculous lesions in contrast to moderately and slightly affected hens.

2.1.1.3. Culturing

Culture of the organism must be attempted if acid-fast bacilli are not found, but typical signs or lesions are present in the birds. In necropsy, liver or spleen is usually the best organ to use, but if the carcass is decomposed, bone marrow may prove more satisfactory as it could be less contaminated. In live birds, cultural examination using feces or tracheal swabs is necessary to isolate and identify the etiological agent. But usually a definitive diagnosis is performed by culturing the organism in suitable media, namely, Dorset's or Herrold's egg yolk medium, Lowenstein-Jensen medium, Middlebrook 7H10 and 7H11, or Coletsos medium with 1% sodium pyruvate (OIE, 2010). For growth of *M. avium*, media containing whole egg or egg yolk is desirable and the incubation temperature should be 37–40°C. Growth may be confined to the edge of the water of condensation. Cultures should be incubated for at least 8 weeks.

Typically, *M. avium* produces "smooth" colonies, within 2–4 weeks; rough variants do occur; smooth transparent colonies are virulent for chickens while variants with smooth domed or rough colonies are avirulent. The colonies, observed only after 10–21 days of incubation, are small, slightly raised, discrete, and grayish white in appearance (Dhama *et al.,* 2007). Colonies are larger if the medium contains glycerin. Shorter incubation times can be achieved using the liquid culture BACTEC system.
Some strains of *M. avium* have been identified to have special requirement of mycobactin as a growth factor. For *M. genavense*, the optimal solid medium is Middlebrook 7H11 medium acidified to pH 6 and supplemented with blood and charcoal. The incubation period at 37°C should be extended for at least 6 months (Shitaye *et al.,* 2010).

Comparison of the different recent methods namely, the conventional culture method (solid Herrold's and Stonebrink media and liquid Sula medium) and newly developed liquid culture systems, the manual mycobacteria growth indicator tube (M-MGIT), and the fully automated BACTEC MGIT 960 system (A-MGIT), for the detection of *M. avium* subsp. *avium* (MAA) in naturally infected hens revealed overall detection rates to be 60, 70, and 76%, with the mean time of mycobacteria detection being 32.6, 17.6, and 14.6 days, respectively (Shitaye *et al*., 2009).

2.1.1.4. Tuberculin test

The tuberculin test is most widely used test in domestic fowl, and the only test for which an international standard for the reagent exists (Dhama *et al.,* 2007). Using standard purified protein derivative (PPD) of heat-treated culture of *M. avium*, tuberculin test can be performed in the wattle, which is considered as the test of choice in domestic fowl. This test is less useful in other species of birds. Birds are tested by intradermal inoculation of 0.05 ml or 0.1 ml tuberculin (2000 IU) using a very fine needle of approximately 10 mm × 0.5 mm and the test is read after 48 hours. A positive reaction is identified as a hot and oedematous swelling at the site or by the presence of a small firm nodule of approximately 5 mm in diameter (Dhama *et al.,* 2007). It serves as a means of identifying birds infected with or sensitized to the same species of tubercle bacillus. Tuberculin test has 80% accuracy in detecting infective birds relative to gross lesions but in an advanced stage of infection birds may give no reaction. Tuberculin testing of the wattle in turkeys is much less reliable than in the domestic fowl. Inoculation in the wing web has been recommended as being more efficient, but this is still not as good as for domestic fowl. Other birds may also be tested in the wing web, but results are not generally satisfactory (Dhama *et al.*, 2007).

2.1.1.5. Molecular techniques

Molecular techniques to identify specific antigens or gene sequences have revolutionized the detection and species identification of pathogenic mycobacteria in clinical samples. Many variations exist, but the basic process involves release of nucleic acids from the mycobacterial cells, amplification using PCR or another technique, and identification of target antigens or gene sequences. Commercial

nucleic acid hybridization probes have become a "gold standard" for distinction between *M. avium, M. intracellulare*, and *M. genavense* (Soini, *et al*., 1996). For intra-species genotyping, pulsed-field gel electrophoresis of large DNA restriction fragments has proved to be highly sensitive. The PCR approach, using species-specific primers is also capable of specifically detecting DNA fragments of *M. avium* genome, thus acting as a diagnostic alternative to the conventional procedures (Yamamoto *et al*., 2008). Also, a multiplex PCR method has been developed for the determination of the subspecies within *M. avium* species, and for differentiating *M. avium* from *M. intracellulare* and *M. tuberculosis* complex (Yamamoto *et al*., 2008). Efficient differentiation of MAC species and subspecies by use of five-target multiplex PCR, designed to amplify a 16S rRNA gene target common to all *Mycobacterium* species, has been proved to be rapid, reliable, and simple (Shin *et al.*, 2010). Recently, Nazmul (2014) reported that PCR technique detected *Mycobacterium avium* by making use of 16S rRNA from the tissues obtained from clinically infected layer chickens.

Insertion sequences (*IS*) in DNA molecule have likewise been identified for differentiation of various mycobacterial species. *IS* 901 and *IS* 1245, which are virtually *M. avium* specific, has been shown to be the most discriminative for the analysis of various strains based on PCR-RFLP (Dvorska *et al.*, 2007). Generally, the PCR-RFLP analysis of suspected tissue samples like liver, spleen, and gonads can be performed targeting 16*S*-rRNA gene for *Mycobacterium* spp., *IS*6110 for *M. tuberculosis*, *IS*1245 for MAC, *IS*901 for *M. avium* subsp. *Avium,* and hsp65 for *M. genavense* (Ikonomopoulos *et al.*, 2009; Manarolla *et al*., 2009; Shitaye *et al.*, 2008). 16*S* rRNA and hsp65 sequencing may also be used to differentiate between mycobacterial strains and for distinguishing the *M. avium* subsets (Fatima, 2009). The real-time TaqMan PCR assay targeting the hsp65 gene of *M. genavense* and MAC subsp. may provide a useful tool for evaluating clinical samples for DNA from mycobacteria species that most commonly infect birds. Slana *et al.* (2010) has recently developed a real-time quantitative PCR for the identification and quantification of *Mycobacterium avium* subsp. *avium* and *M. a. hominissuis*. Other novel tests like IFN-γ assay, GenoType assay, and DNA microarrays that are used to diagnose human tuberculosis may also be useful in diagnosing the infection in birds (Inagaki *et al.*, 2009; Neonakis *et al.*, 2008). More recently, the use of molecular

techniques for species identification brought to light the prominent role of nonculturable mycobacteria, primarily *M. genavense*, in several cases of avian tuberculosis in pet birds (Manarolla *et al.,* 2009).

Utilization of new variable number tandem repeat markers (VNTRs) of genetic elements called mycobacterial interspersed repetitive units (MIRUs) for typing *Mycobacterium avium* subsp. *paratuberculosis* and *M. avium* strains (*M. avium* subsp. *avium*, *M. avium subsp. hominissuis*, and *M. avium subsp. Silvaticum*) has been reported to be fast typing method, and which in combination with other methods, might prove to be optimal for PCR-based molecular epidemiological studies (Thibault *et al.,* 2007).

MATR (*Mycobacterium avium* tandem repeat—MATR)-VNTR typing method (MATR-VNTR) to be having excellent discriminatory power compared with MIRU-VNTR and *IS*1245-RFLP typing; and its concomitant use with *IS*1245-RFLP typing increases the discriminatory power. MATR-VNTR typing is inexpensive and easy to perform and thus could be very useful for epidemiological studies (Radomski *et al*., 2010).

2.1.1.6. Animal inoculation

Pathogenicity tests are performed for knowing the likelihood of the etiological agent in case the typing facilities are not available which should be carried out on the species of bird being investigated, but failing that, domestic fowl or Japanese quail may be used. Young adult birds are best, and when inoculated intravenously with 1 mL of the suspension (culture at 0.1 mg/mL) the bird will die in 5–6 weeks if the organism is virulent, or, by that time, the bird will have extensive lesions filled with acid-fast bacilli (Thoen *et al*., 1998).

2.1.2. Postmortem examination

M. avium prevents the fusion of phagosomes with lysosome after entering the host, and the subsequent bacteremia provides a generalized distribution of lesion. The gross lesions are characterized by the presence of epithelioid cells /tissue macrophage

containing large numbers of organisms that may either diffusely infiltrate the organ or form discrete granulomas. There is presence of tubercular nodules in intestine, liver, spleen, ovaries, testes, and bone marrow but the pulmonary lesions, which are a striking feature of tuberculosis in other species, are rarely observed in birds (Dvorska *et al.*, 2007). Pulmonary avian tuberculosis is only seen occasionally as in case of tuberculosis of pigeons and water fowl. The principal lesions of tuberculosis in birds are seen in intestine, where infection often presents with studded greyish-white to greyish-yellow nodules. Before the intestinal tract is opened, the ulcerated areas appear as tumour-like masses attached to the gut wall, but when the intestine is opened, the true nature of the mass becomes evident. The nodules bulge from the serosal surface of the intestine and can be palpated. Due to this, spleen takes irregular "knobbly" appearance. Lesions evident as deep ulcers filled with caseous material discharges the organism into the intestinal lumen and get excreted via the droppings. Typical caseous lesions, without calcification, are always found in the liver and spleen with considerable enlargement of the organs (Dvorska *et al.*, 2007)). Nodules are firm but can be incised easily since mineralization is rare in avian TB (this is in contrast to leucosis, in which lesions cannot be enucleated from the surrounding tissue). The bone marrow of the long bones frequently contains tubercular nodules. Some exotic bird species may have lesions in the liver and spleen without intestinal involvement. Microscopically, lesions consist of granulomas with a central necrosis, either coagulative or caseous, and multinucleate giant cells. Acid fast bacilli are numerous in the central or necrotic zone of the tubercle (Dhama *et al.*, 2007).

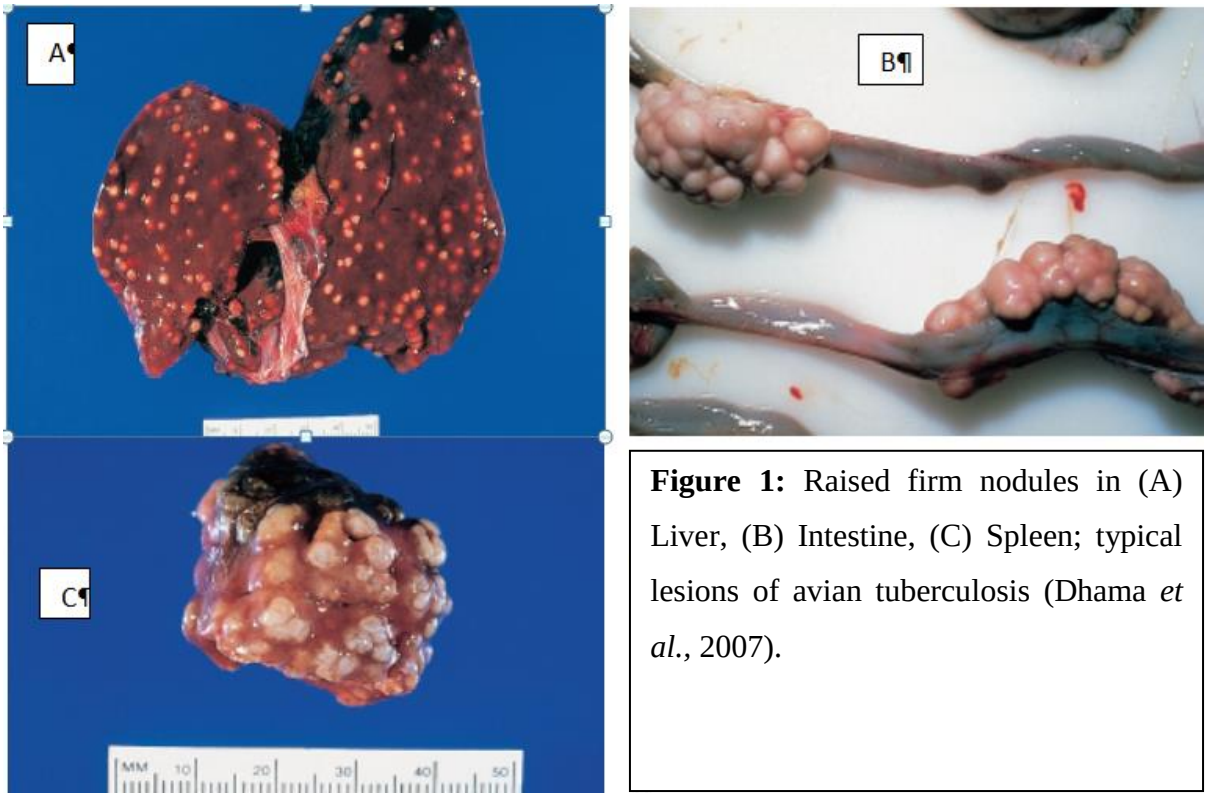


Figure 1: Raised firm nodules in (A) Liver, (B) Intestine, (C) Spleen; typical lesions of avian tuberculosis (Dhama *et al.*, 2007).

Avian tuberculosis should be differentially diagnosed from those diseases that are known to develop tumorous or granulomatous lesions in gastrointestinal tract and other visceral organs. Diseases that are to be differentially diagnosed are pseudotuberculosis (common in ducks and turkeys caused by Yersinia pseudotuberculosis), Coligranuloma (Hjarre's disease-*Escherichia coli*), neoplasia due to lymphoid leucosis (Retrovirus) or Marek's disease (Herpes virus), fowl cholera (*Pasteurella multocida*), Pullorum disease (*Salmonella Pullorum*), and enterohepatitis, Black head /*Histomonas meleagridis* (Thoen, 1998).

2.2. Economic Importance

Avian tuberculosis can result in both direct and indirect economic losses. Direct losses include reduced production as a result of mortality or decreased egg production (Quaranta *et al*., 1996), condemnation at slaughter in poultry and the costs of treatment and/or control programmes (e.g. hygienic measures, renovation of facilities, culling and restocking, surveillance programs). Direct economic losses to the small producer can be severe (Radkowski *et al.*, 1996).

Indirect losses may be less obvious, but can be far-reaching. These include loss of valuable, rare, or endangered species from collections or breeding programmes, such as the white-winged wood duck captive breeding programme (Cromie *et al*., 1992). The disease may even cause a negative impact on tourism in wildlife reserves. For example, an epidemic of avian tuberculosis in lesser flamingos *(Phoenicopterus minor)* along the shores of Lake Bogoria and Lake Nakuru in Kenya contributed to the deaths of more than 18,500 flamingos over a three-month period in 1993 (Koek *et al.*, 1999). Another cause of indirect economic loss is transmission of mycobacteria from infected birds to swine and cattle. In swine, MAC organisms can cause clinical disease. In cattle, such organisms can cause non-specific positive reactions to tuberculin, thus compounding problems in eradication and surveillance programmes for bovine tuberculosis.

Avian tuberculosis in domestic poultry have recently been declined due to changes in poultry husbandry practices, namely, integrated poultry farming, emphasizing all-pullet flocks rather than older hens and maintaining one-age flocks, all in all out farming system, along with better hygiene, disinfection, and biosecurity practices. However, the occurrence of avian TB in birds in zoo aviaries or parks is still an economically important affair perhaps because of inadequate cleaning and disinfection of pens (Nazmul, 2014).

2.3. Public Health Significance

Exposure of humans to infected birds with the *Mycobacterium avium* subsp. *avium* organisms may cause a zoonotic infection. Infected animals and their products (mainly eggs) often come from small household production pose a risk for human health (Kríz *et al*., 2010). Although infectivity for humans is very low, it is progressive and often fatal disease that is particularly severe in children and immunocompromised individuals. The situation worsens due to the spread of HIV infection in developing countries (Une and Mori, 2007).

Human beings are generally resistant to *M. avium* infection unlike *M. tuberculosis;* but occasionally they can get infected. Human pulmonary tuberculosis due to avian tubercle bacilli has been reported during the early 1940's. High incidence of

sensitivity to avian tuberculin in man has also been identified. It is essential to bear in mind that *M. avium*, *M. intracellulare,* and *M. genavense* are of public health concern mostly in immunocompromised hosts. Infections of humans and animals caused by this *M. intracellulare* agent are expected to rise. The *M. avium* infection, seen in many AIDS patients, is a progressive disease that is refractory to treatment (Biet *et al*., 2005). This is especially true in cases of exposure to large numbers of organisms. In humans, *M. avium* is capable of inducing localized primary lymphadenitis, pulmonary disease, and a disseminated form of infection particularly in case of immunosuppressed individuals or patients under transplant therapy. *M. avium* also causes local wound infections with swelling of regional lymph nodes. In adults, the organism frequently affects the lungs, producing respiratory signs and in children, the cervical lymph nodes are often involved (OIE, 2014).

M. avium causes a serious disseminated bacterial infection in up to 40% of patients with advanced HIV infection. In AIDS patients, the main route for *M. avium* infection is the gastrointestinal tract and *M. avium* is naturally tolerant to the low pH that exists in stomach (Bodmer *et al.,* 2000). The transmission occurring via aerosols results in pulmonary infections as the organism frequently affects the lungs with endobronchial lesions. During the infection, *M. avium* can be demonstrated *in vivo* in lymph nodes, bone marrow, urine, and sputum (Fukuoka *et al*., 2003). Primarily, the serotype-1 of *M. avium* subsp. *avium* has been isolated from such individuals, clearly pointing the role of birds in acquiring infection (Aranaz *et al.,* 1997). It should also noteworthy that *M. avium* is a pathogen that infects several hosts including birds, humans, cattle, and pigs. They are also encountered in environmental sources like soil and water, having considerable ability to overcome adverse and competitive conditions (Slana *et al.,* 2010).

Contaminated food originating from pig or other livestock is identified as potential source of human infection. During a study from 1953 to 1968, in cattle and swine of Great Britain, 13% of the total tubercle bacilli were typed as *M. avium* and in pig population it was an astonishingly 81%. This should be correlated with the ever increasing number of recorded cases of tuberculosis in man caused by *M. avium* (Mobius, *et al*., 2006). *M. avium* can infect and cause disease in some domestic mammals but lesions usually are localized and less severe. When domestic farm

animals are infected, particularly cattle and pigs, tuberculous lesions are commonly found localized to the head and intestinal lymph nodes (Dvorska *et al.*, 2007). *M. avium* isolates from swine represent the major threat to human beings. The similarity of the *IS*1245 RFLP patterns of the human and porcine isolates indicates close genetic relatedness, suggesting that *M. avium* is transmitted between pigs and humans (Tirkkonen *et al.*, 2007).

The handling of infected birds in farms should be carried out with adequate care, and manipulation of material from infected birds or open live cultures of *M. avium* in laboratories must be performed with appropriate biohazard containment. Hence, it is considered prudent to keep infected birds away from humans, particularly the elderly, and individuals with poor immune status (OIE, 2010). Healthy individuals with normally functioning immune system have a high resistance to this infection. However, it is recommended to take proper precautions and avoid contact or exposure to infected birds or their carcasses.

3. STATUS OF AVIAN TUBERCULOSIS IN ETHIOPIA

Ethiopia has a large population of chickens which is estimated to be 56.87 million CSA (2015), of which 95.86% are raised under the traditional backyard system of management (Tadelle *et al.,* 2003). Nevertheless, despite the potential future expansion of the poultry industry in the country, infectious diseases such as avian tuberculosis which has direct impact on the sector has not been well studied (Sultan *et al.,* 2015). A study conducted by Tadesse *et al.* (2004) on avian TB in central Ethiopia has indicated that the disease is prevalent in traditionally managed local chickens and reported to have a prevalence of 6.3%. Antemortem surveillance conducted using avian tuberculin test at Shashemene district of Ethiopia also showed a 4.24% apparent prevalence of avian TB. Postmortem examination of birds during the same study also signified varied distribution of tuberculous lesions in liver, lung, spleen and intestine that were associated with simultaneous exposure to different routes of infection (Sultan *et al.,* 2015).

Sufficient evidence suggests that in Ethiopia, avian tuberculosis is found at significant levels in domestic cattle; despite the paucity of information. For example, MAC-like organisms were isolated on culture from dairy cattle in East Shoa of Oromia region, Ethiopia (Tadelle, 1998). Furthermore, the report by Tadesse *et al., (*2004*)* is also in agreement with Tadelle (1998), with the highest numbers of MAC isolation in chickens from the same area, East Shoa and a large association of chickens with other domestic animals, including cattle, sheep, and goats but not pigs. This study substantiates that pigs are not reared in most parts of Ethiopia because of religious and cultural taboos. Therefore, pigs have no importance in the epidemiology of MAC in Ethiopia, leaving cattle and other ruminants in second line for transmission.

Sultan *et al.,* (2015) indicated sex variation on the occurrence of avian TB, with slightly higher occurrence in female adult chickens than male chickens. He also suggested the reason as due to the fact that female chickens are allowed to live longer than their male counterparts as they are needed for long term egg production. This gives the bacilli a better chance to establish over a long period of time and to be shed in the external environment. Thus, female older hens could act as a source of infection to other members in the flock (Sultan *et al.,* 2015).

According to a study conducted in three selected agro ecological zones of Ethiopia ,namely Debre Berhan, Sebeta and Adama, experimental infection with the strains from culture resulted in death of 10 (83.3%) of 12 inoculated chickens 56 to 110 days after inoculation; indicating that the isolates was virulent strains of MAC. On postmortem examination, the experimentally infected chickens showed similar tuberculous lesions to natural infection that was confined at the site of injection, on the liver, spleen and small intestine (Tadesse *et al.,* 2004).

In Ethiopia, millions of chickens are coming from the rural backyard domestic chicken raisers and are extensively used for human consumption especially during major holydays such as Christmas, Easter and Moulid. However, attention given to zoonotic diseases such as avian tuberculosis seems unrewarding.

A preliminary survey conducted by Sultan *et al.* (2015*)* indicated that chickens kept in extensive production system experienced a close physical contact with their owners especially during the night. Hence, there could be a possibility of transmission of mycobacteria between chickens and their owners. On top of this, the low perception of the owners about zoonotic TB including avian TB and high number of HIV patients/ immunocompromised individuals in the country could add up to the risk of transmission (Sultan *et al*., 2015).

4. MATERIALS AND METHODS

4.1. Description of the Study Areas

The study was conducted from November, 2016 to June, 2017 at three selected districts of Oromia, Ethiopia, namely Gerar Jarso, Ada'a, and Boset; located at high, mid and low altitudes respectively. From the three agro-ecologies in the region, one district was purposively selected and their respective peasant associations were selected based on chicken population and accessibility to seasonal and /or all weather roads. Generally, according to Hailu (2014), areas located between 500-1500, 1500-2300, and 2300-3200 meters above sea level are considered as lowland, midland and highlands respectively.

Gerar Jarso district is located in north Shewa zone of Oromia regional state; 114 km north of Addis Ababa at 8.54^0-10.23^0 N and 37.56^0-39.24^0 E. The total area of the district is 42,763 hectares; of which 52%, 41% and 7% are highland, midland and lowland, respectively. The minimum altitude of the district is 1080m and the maximum is 3541m above sea level. The average minimum and maximum annual rainfall are 793mm and 1443mm, respectively; while the average minimum temperature is 10^0c and maximum temperature is 32^0c. The total population of the district is 67,298 (34,462 males and 32,836 females). There are 99,000 chickens in the district of which 84,000 (84.8%) are local and 15,000 (15.2%) are exotic breeds. (Gerar Jarso district office of livestock and fishery development, 2016). Hence, for this study PAs at highland areas of Gerar Jarso were selected.

Ada'a district is found 47 km southeast of Addis Ababa, the capital of Ethiopia. About 90% of the district belongs to the sub-tropical agro-climatic zone having an altitude ranging from 1500 to over 2000 m above sea level. The district receives an annual rainfall of 851mm with annual minimum and maximum temperature of 11 and 29°C, respectively. Though the district is most known for cereal crops (mainly teff and wheat) and legumes, livestock production is an integral part of the system. Cattle, small ruminant, poultry and equines are the major livestock species kept with fast growing smallholder dairy production. The district has a total livestock population of 264,310 and a total poultry population of 107,554; of which 72,541 are exotic and the

remaining 16,700 and 18,313 are local and cross breeds, respectively (Ada'a district office of livestock and fishery development, 2017).

Boset is one of the districts in the Oromia Region of Ethiopia. It is part of east Shewa Zone located in the Great Rift Valley. The district is bordered on the south by the Arsi Zone, on the west by the Awash River which separates it from Adama, on the north by the Amhara Region, and on the east by Fentale. The administrative center of the district is Welenchiti. This district is predominantly level land with undulating features; almost 90% is less than 1500 meters above sea level; Boset Guddo is the highest point. A survey of the land in this district shows that 26.2% is arable or cultivable, 30% pasture, 15.8% forest, and the remaining 28% is considered barren, degraded or otherwise unusable. Fruits and vegetables are important cash crops of the district. Boset has 97 kilometers of dry-weather and 103 of all-weather road, for an average of road density of 136.8 kilometers per 1000 square kilometers. About 87% of the urban, 36% of the rural and 45% of the total population have access to drinking water. The 2007 national census reported a total human population of 142,112, of whom 73,925 were men and 68,187 were women; 26,514 or 18.66% of its population were urban dwellers (Wikipedia, 2014). The total chicken population of Boset district is 107,095; of which 95,070 are local and 12,025 are exotic (Boset district office of livestock and fishery development, 2017).

.

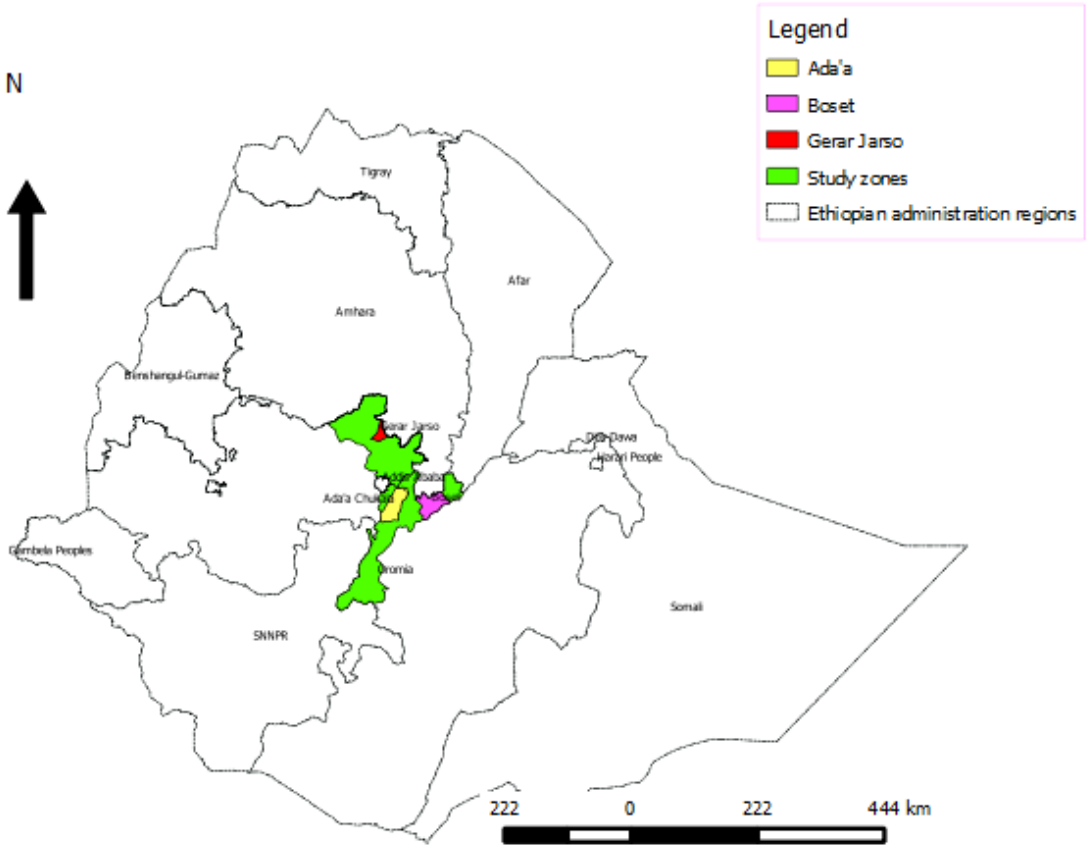


Figure 2: Locations of the study districts on the map of Ethiopia

4.1.1. Study animals

The study animals were adult chickens of 22 weeks and above old, both sexes, local, exotic and cross breeds reared under backyard production system. Information about the age and breeds of chickens was obtained from farmers themselves. Accordingly, pullet chickens which had begun laying were considered to be greater than 22 weeks old; and young cokes which were in the same batch with the laying pullets were also considered in the same manner. Concerning the breed of chickens, using their local language, Afan Oromo, owners categorized indigenous breeds as 'Habesha', exotic breeds as 'Matika', and cross breeds as 'Sanyi maka'.

4.2. Study Design and Sampling Methodology

A cross-sectional study design was used primarily to determine the apparent prevalence of avian tuberculosis in selected districts of Oromia region, Ethiopia. Three districts, namely, Gerar Jarso, Ada'a, and Boset were purposively selected from high, mid and low altitudes of the region, respectively. Moreover, multistage sampling method was used to identify peasant associations (PAs) within the districts, households in the PAs and study animals in the households. Thus, from each district, three PAs were selected based on their chicken population and road accessibility; and then, 10 farmers who had chickens and willing to participate in the study were selected from different villages of each PA. Moreover, study animals in the households were purposively selected based on their age and physical appearance. In the selected households where the number of chickens was ten and below, all chickens aged 22 weeks and above were used for avian tuberculin test. Nevertheless, in those households which had more than ten chickens, only ten chickens which were relatively older and with poor physical appearance, poor feather quality and/ or repeated history of disease were selected and used for the test. A sampling frame of households who possess chickens was established with the help of livestock development agents. The unit of sampling was an individual bird.

4.2.1. Sample size determination

The desired sample size (n) was calculated according to the formula given by Thrusfield, (1995); considering 6.3% prevalence from previous study undertaken in central Ethiopia by Tadesse *et al*, (2004), 95% confidence level and 5% precision.

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

Where, n= required sample size, P_{exp}= expected prevalence, d= desired absolute precision. Substituting values in the formula yielded the following result:

$$\frac{1.96^2 \times 0.063 (1-0.063)}{(0.05)^2} = 91$$

Thus, the estimated sample size was 91 chickens. However, to increase precision and maximize the efficiency of the study, 91 chickens were considered to be the share of each district and the total sample size was 273 chickens; which is three times the calculated sample size.

4.2.2. Questionnaire survey

A questionnaire survey was conducted to assess the perception of chicken owners at the study districts regarding avian tuberculosis, associated risk factors and public health implications of the disease. This was achieved by a structured questionnaire on which a total of 91 willing individuals who were rearing chickens under the traditional production system and proportionally divided among the study districts were involved. Moreover, the farmers were consulted for their willingness to conduct avian tuberculin test on their chickens, the objective of the test and the benefit that they could obtain from the test. Subsequently, they understood the prime intent of the test and were became volunteers for the single intradermal avian tuberculin test that was conducted on their adult chickens irrespective of sex and breed.

4.2.3. Avian tuberculin test

Single intradermal avian tuberculin test was performed on selected chickens of volunteer farmers in the study districts by following the principles described by (Fulton and Thoen, 2003). Before injecting the avian purified protein derivatives (PPD), the wattle of a chicken to be injected was thoroughly inspected for any abnormality that could create confusion with the reaction of PPD. Then after, 0.1ml of 2500 IU/ml avian PPD was injected intradermally on the right wattle of selected chickens using a sterile insulin syringe of 1ml capacity, and the sites of injection were marked with permanent marker. Moreover, in circumstances when the number of chickens in the flock was more than ten, a cord was tied on one of the legs of tested chickens to differentiate them from the remaining untested chickens. The test result was appreciated for the presence of allergic reactions 48 hours after the injection of avian PPD. Based on the principle described by (Tell *et al.*, 2001; Dhama *et al.*, 2008), a positive reaction was identified as a hot and edematous swelling at the site of

inoculation or by the presence of a small firm nodule of approximately 5 mm or above in diameter which was clearly observed by comparison with uninoculated left wattle which was used as a control. In test negative chickens which had a tie on their legs before inoculation, the cords were removed and left only on test positive chickens in the flock which later were purchased and sacrificed for postmortem examination.

4.2.4. Postmortem examination

Avian tuberculin test positive chickens were purchased from farmers and transported to College of Veterinary Medicine and Agriculture, Addis Ababa University postmortem room; and humanely sacrificed for postmortem examinations to look for lesions related to avian tuberculosis. After the chickens were made unconscious by dislocating their neck, feather covering around the entire ventral part of the chickens was removed to avoid the risk of contamination of internal organs with external contaminants on chickens' feather. Subsequently, incision was made from beak to anus following the ventral midline of the chickens and the underlying soft and boney tissues were dissected with sterile scissors and bone shears to expose the internal organs for postmortem examination. This is in accordance with the procedures of Zander and Mallison (1991).

All internal organs at necropsy with special attention to liver spleen and intestine were examined and any observed gross lesions were recorded. Subsequently, the gross lesions were characterized and their distributions were described. A pair of any suspected organs and tissues such as liver, spleen and intestine of all slaughtered chickens with visible or invisible gross pathological lesions was sliced and sampled using sterile surgical blades to minimize the possibility of missing obscure tubercular lesions. To avoid the risk of cross contamination and false culture positivity of sampled tissues and organs, a separate sterile surgical blade was used for each and every organs and tissues sampled. Meanwhile, the tissue samples were kept in universal bottles filled with approximately 5ml of phosphate buffered saline solution and 10% buffered formalin for mycobacteriological culturing and histopathological examinations, respectively. Then, the tissue samples were transported using an ice box with ice packs to College of Veterinary Medicine and Agriculture (CVMA) Addis Ababa University bacteriology laboratory. Tissue samples that were collected for

mycobacteriological culturing were stored at -20^{0}C until transported to Aklilu Lemma Institutes of Pathobiology (ALIPB) TB laboratory for cultural isolation and characterization of MAC and other suspected *Mycobacterium* species; while tissue samples preserved in 10% buffered formalin were kept at room temperature until transported to National Animal Health Diagnostic and Investigation Center (NADIC) for histopathological examination.

4.2.5. Histopathological examination

Tissues collected for this purpose from organs showing pathological gross lesions and samples of liver, spleen and intestines suspected for obscure lesions and preserved in 10% buffered formalin were transported to National Animal Health Diagnostic and Investigation Center at Sebeta. The tissues were dehydrated in different grades of ethanol (70%, 95% and 100%), cleared in xylene and refixed with formalin in an automatic tissue processing machine for about 20 hours and 30 minutes. Then after, the tissues were embedded in paraffin using an embedding machine and cut into thin sections of 4 to 5 µm using a microtome; following the procedures described by (Thoen, 1997). Subsequently, the tissue sections were stained with haematoxylin and eosin and prepared for microscopic examination (Bancroft and Cook, 1994).

4.2.6. Culturing

Tissue samples were transported in a cold chain using an ice box packed with ice block to Aklilu Lemma Institute of Pathobiology (ALIPB) TB laboratory. Following the protocols of OIE (2004) for isolation of mycobacteria from tissues, the samples were sectioned into pieces using sterile scissors, and homogenized by pestle and mortar. The homogenate was dispensed into the falcon tubes, decontaminated by adding an equal volume of 4% NaOH and centrifuged at 3000 rpm for 15 minutes. After centrifugation, the supernatant was discarded whereas the sediment was neutralized by 10% HCl using a drop of phenol red as an indicator. Neutralization was achieved when the color of the solution changed from purple to yellow. Then after, 0.1ml of suspension was spread onto a slope of Lowenstein Jensen (LJ) medium. For each sample, duplicates of LJ medium; one enriched with sodium pyruvate, the other

enriched with glycerol were used to increase the chance of growth of both *Mycobacterium tuberculosis* and non-tuberculosis mycobacterium species (NTM) such as mycobacterium species under *mycobacterium avium* complex (MAC). Cultures were incubated at 37^{o}C in a slant position for one week and in upright position for 12 weeks; with weekly observation for mycobacterial growth, dehydration and over hydration of the culture.

4.2.7. Acid fast staining

Ziehl Neelsen staining was performed on two positive cultures to confirm the presence of acid-fast bacilli following the protocols described by (Quinn *et al*., 1994) and (WHO, 1998).

4.2.8. Data analysis

Descriptive statistics, Fisher's exact test, univariable and multivariable logistic regression were performed to analyze the data using Stata version 13.0 software packages and statistical package for social sciences (SPSS) version 20.0. For all analysis that was performed, 95% CI and *P*-value < 0.05 was set for statistical significance of an estimate.

4.3. Ethical Considerations

Ethical clearance certificate with Ref.no. VM/ERC/008/03/09/2016 was obtained from the animal research ethical review committee of the College of Veterinary Medicine and Agriculture (CVMA) evidencing that humane and humble handling of the chickens was used to minimize unnecessary suffering (Annex 3).

5. RESULTS

5.1. Epidemiological Investigation of Avian Tuberculosis

A total of 273 chickens, from Gerar Jarso, Ada'a and Boset study districts were inoculated with avian PPD at their right wattle to investigate the epidemiology of avian tuberculosis in the districts. Among them, 31 chickens, 6 from Gerar Jarso (highland), 13 from Ada'a (midland) and 12 from Boset (lowland), were identified as positive reactors to avian tuberculin test (Table 1 and Figure 3 and 4).

Table 1: Summary of the spatial distribution of chickens tested with avian tuberculin test in the present study and prevalence of avian TB with sex, breed and altitude

Variables	Categories	Number of chickens tested with avian PPD % (N)	Number positive % (N)	Prevalence of avian TB % (N)	95% Confidence interval
Sex	Male	15.4 (42)	6.5 (2)	4.8 (2/42)	[0.6, 16]
	Female	84.6 (231)	93.5 (29)	12.6 (29/231)	[9,18]
Breed	Local	70.7 (193)	90.3 (28)	14.5 (28/193)	[10, 20]
	Exotic	26.7 (73)	3.2 (1)	1.4 (1/73)	[0.03, 7]
	Cross	2.6 (7)	6.5 (2)	28.6 (2/7)	[4, 71]
Altitude	Highland	32.2 (88)	19.4 (6)	6.8 (6/88)	[3, 14]
	Midland	35.6 (97)	41.9 (13)	13.4 (13/97)	[7, 22]
	Lowland	32.2 (88)	38.7 (12)	13.6 (12/88)	[7, 23]
Total		100 (273)	100 (31)	11.4 (31/273)	[8, 16]

Figure 3: Avian intradermal tuberculin test positive chicken with edematous welling on the right wattle at the site of inoculation of avian PPD observed in this study (indicated by an arrow).

Figure 4: Firm erythematic nodular swelling on the right wattle of avian intradermal tuberculin test positive chicken (indicated by an arrow).

To evaluate the spatial distribution of the disease, a total of nine peasant associations from the three study districts, 3 from Gerar Jarso, 3 from Ada'a, and 3 from Boset were included in the present study. Accordingly, one or more chickens were identified as avian tuberculin test positive in eight of them; except one peasant association in Gerar Jarso district where no single positive reactor chicken for avian tuberculin PPD was recorded. Hence, the current study revealed that avian tuberculosis is well prevalent in the selected peasant associations in the study districts.

The effect of host related and environmental risk factors such as breed, sex and altitude as predisposing factors for avian tuberculosis in chickens were also evaluated using univariable logistic regression model. In view of that, the comparison between

breeds as the risk factor for the occurrence of the disease revealed a twofold higher odd of exposure of cross breed chickens (OR= 2.36, 95% CI: 0.44, 12.75) as compared to local breed chickens involved in the present study. However, this difference was not statistically significant (P>0.05) (Table 2).

In addition, within a breed, the statistical estimation of the effect of sex as the predisposing factor of chickens to avian tuberculosis (ATB) showed the odd of exposure of female chickens (OR=2.87, 95% CI: 0.66, 12.5) to be nearly three times the odd of exposure of males. Nevertheless, the difference was not statistically significant (P>0.05) (Table 2).

Likewise, the study also assessed the effect of altitude as the predisposing factor for avian TB in chickens. Consequently, the result connoted a twofold increment in the odds of exposure of chickens in the mid altitude (OR= 2.12, 95% CI: 0.77, 5.83) and low altitude (OR=2.16, 95% CI: 0.77, 6.04) to the disease as compared to chickens in the high altitude of the districts selected for this particular study; though, the difference was not statistically significant (P>0.05) (Table 2).

Table 2: Univariable logistic regression output of host related and environmental risk factors and their odds of exposure of chickens to avian tuberculosis

Variables	**Categories**	**Number of chickens tested % (N)**	**Number Positive % (N)**	**Odds ratio**	**P-value**	**95% Confidence Interval**
Breed	Local*	70.7 (193)	14.5(28)	1		
	Exotic	26.7 (73)	1.4 (1)	0.08	0.013	[0.01, 0.61]
	Cross	2.6 (7)	28.6 (2)	2.36	0.319	[0.44, 12.75]
Sex	Male*	15.4 (42)	6.5 (2)	1		
	Female	84.6 (231)	93.5(29)	2.87	0.160	[0.66, 12.5]
Altitude	Highland*	32.2 (88)	19.4 (6)	1		
	Midland	35.6 (97)	41.9(13)	2.12	0.148	[0.77, 5.83]
	Lowland	32.2 (88)	38.7(12)	2.16	0.143	[0.77, 6.04]

Furthermore, the data were re-analyzed using multivariable logistic regression to identify the most contributing variables to the odd of exposure of chickens to avian TB as compared to other risk factors of the disease. For this purpose, environmental and host related risk factors such as breed, sex and altitude was included as putative risk factors of the disease. Accordingly, the result indicated, a statistically significant ($P<0.05$) effect of breed as compared to sex and altitude. Hence, a 24 fold odds of exposure of cross breed chickens (OR= 24.7, 95% CI: 3.4, 182.1) to avian TB as compared to local breeds was observed (Table 3). Nevertheless, the wider confidence interval observed indicates a weaker probability of such wider differences between the odds of exposure of the two breeds. The result also revealed a twofold increment in the odds of exposure of chickens at midland altitude as compared to highland chickens; but, this difference was not statistically significant ($P>0.05$)

.

Table 3: Multivariable logistic regression output of host and environmental related risk factors and their odds of exposure of chickens to avian tuberculosis

Variables	Categories	Odds Ratio	P-value	95% CI
Sex	Male	1		
	Female	4.02	0.087	[0.8, 19.7]
Breed	Local	1		
	Cross	24.74	0.002	[3.4, 182.1]
	Exotic	0.08	0.013	[0.1, 0.6]
Altitude	Highland	1		
	Midland	2.07	0.175	[0.7, 5.9]
	Lowland	1.56	0.422	[0.5, 4.6]

5.2. Necropsy Findings

Post mortem examination was carried out on avian tuberculin test positive chickens. From the total of 31 positive reactor chickens, 22 chickens were sacrificed for necropsy. Nevertheless, the remaining 9 positive reactor chickens were not obtained for necropsy due to reasons like reluctance of owners to sell broody chickens, death

and disposal of test positive chickens; because of other acute and subacute diseases such as New castle disease before handing over them for necropsy.

Postmortem gross lesions such as organomegally, organ atrophy, milliary nodules and diffused or petechial hemorrhages were observed on liver and/ or spleen and intestine in the 22 sacrificed chickens; among which 40.9% (9/22) of them showed these lesions (Table 4).

Table 4: Postmortem gross pathological lesions in positive chickens with their breed, sex and origin

Variables	Category	Number of positive reactor chickens to avian PPD	Number of reactor chickens positive for postmortem gross lesions
Sex	Male	6.5 (2)	11.1(1)
	Female	93.5 (29)	88.9 (8)
Breed	Local	90.3 (28)	77.8 (7)
	Exotic	3.2 (1)	11.1 (1)
	Cross	6.5 (2)	11.1 (1)
Altitude	Highland	19.4 (6)	22.2 (2)
	Midland	41.9 (13)	33.3 (3)
	Lowland	38.7 (12)	44.4 (4)

The milliary nodular lesions observed on a liver of local breed female chicken in the present study were grayish-yellow to grayish-white, pin-point to irregularly round, and few to numerous; approximately measuring up to 2 cm in diameter and raised above the surface of the affected organ (Figure 5).Nevertheless, no calcification or caseation was visualized in the nodules.

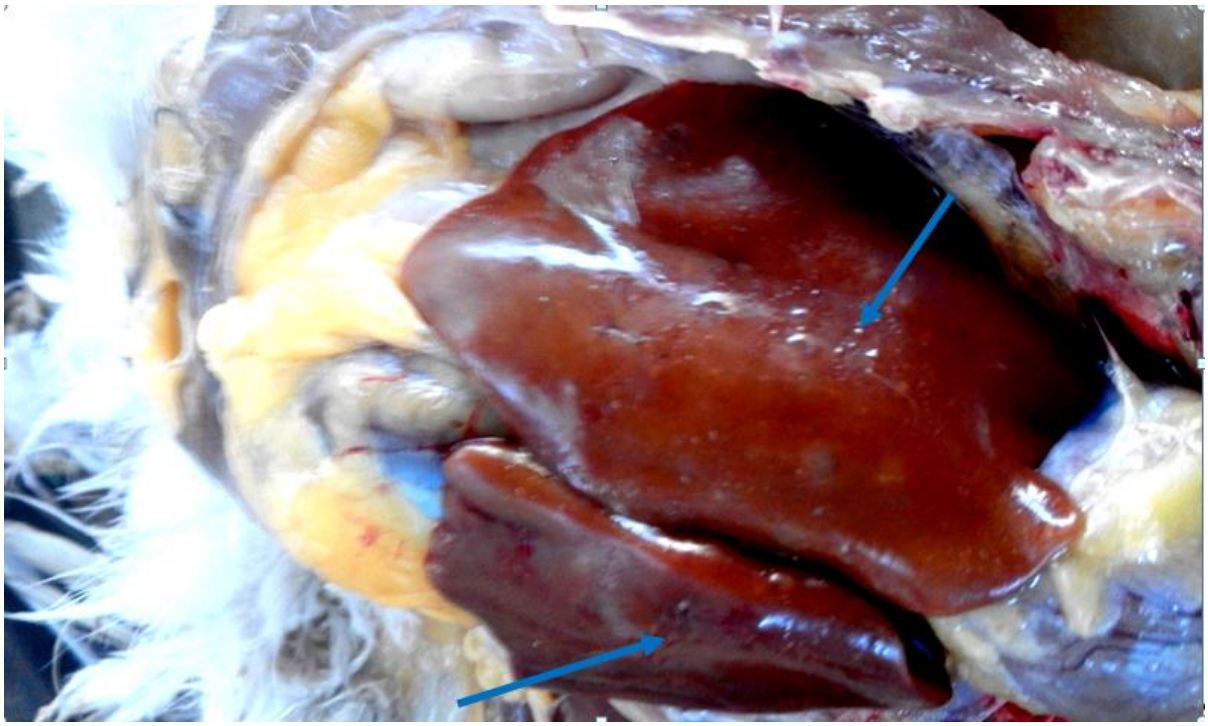


Figure 5: Arrows showing grayish-yellow to grayish-white nodular swelling on the liver tissue of avian intradermal tuberculin test positive chicken observed upon postmortem examination.

Postmortem gross lesions were observed on the 9 postmortem examined chickens in the present study. Moreover, one chicken showed spleenomegaly and an exotic female chicken from Gerar Jarso showed pale and highly atrophied liver and paired ceca filled with air. Besides, the atrophied part of the cecal wall of intestine in this chicken was devoid of epithelial cells and connective tissue; except a thin fascia which accounts for most of the cecal part of the intestine (Figure 6). However, upon ante mortem examination, this exotic chicken had a very good body condition and shiny feathers with good physical appearance.

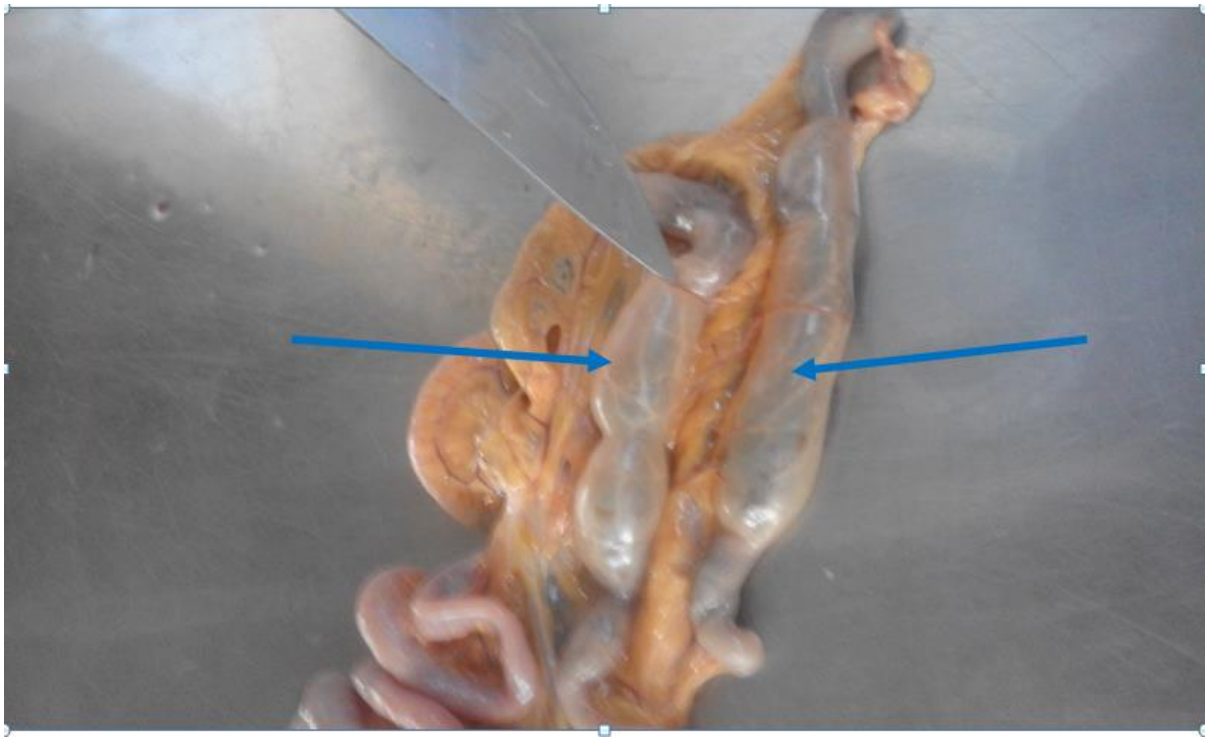


Figure 6: Arrows showing paired ceca of avian intradermal tuberculin test positive chicken filled with air and cecal walls devoid of epithelial cells and connective tissue as observed upon postmortem examination

The other striking feature that was visualized during postmortem examination of a well matured positive reactor male chicken with good body condition and feather quality was a remarkable wasting of breast muscle, a skeletal muscle attached to the keel bone, leathery appearance of the skin covering the breast muscle and a prominent keel bone having a knife edged appearance (Figure 7).

Figure 7: Arrow showing prominent keel bone and wasting of breast muscle observed on avian intradermal tuberculin test positive chicken upon postmortem examination.

5.3. Mycobacteriological Culture Results

A total of 60 tissue samples collected from different organs including liver, spleen, intestine, lungs and heart were cultured on a pair of Lowenstein Jensen medium (LJ) slants; one enriched with sodium pyruvate and the other with glycerol (60 tissue samples were seeded on sodium pyruvate enriched and 60 glycerol enriched media). Of these, only 0.02% (2/120) of them had shown colonial growth up to 12 weeks of incubation and both were seen on sodium pyruvate enriched LJ slants. However, no

more than a single slight colony was appreciated on both LJ slants; and hence, after smearing for Ziehl Nielsen staining, it was not sufficient for molecular typing of the isolates. Nonetheless, the samples are still under process to see colonial growth for molecular typing of the isolates.

5.4. Acid Fast Staining

Ziehl Nielsen staining technique performed on the colonies from the two culture positive LJ slants revealed that the organisms were acid fast rods suggesting that they are under the genus *Mycobacterium*.

5.5. Histopathological Findings

Histopathological examination was carried out on samples collected from gross pathological lesions. From a total of ten histopathological slides prepared from strongly suggestive gross pathological lesions on liver spleen and intestines of seven positive reactor chickens, 40% (4/10) of them revealed tubercular granuloma characterized by central necrosis and peripheral inflammatory cells; which were mainly mononuclear lymphocytes and macrophages. Moreover, lymphoid depletion of the cortical part of spleen with empty cystic structure at the sites of lymphoid depletion, a characteristic feature of diseases caused by *Mycobacterium* species, was also visualized (Figure 8). These histopathological findings were obtained from postmortem tissues of three different positive reactor female chickens. Furthermore, out of the four post mortem tissues which had shown tubercular granuloma, 50% (2) of them were intestines and the remaining two were liver and spleen.

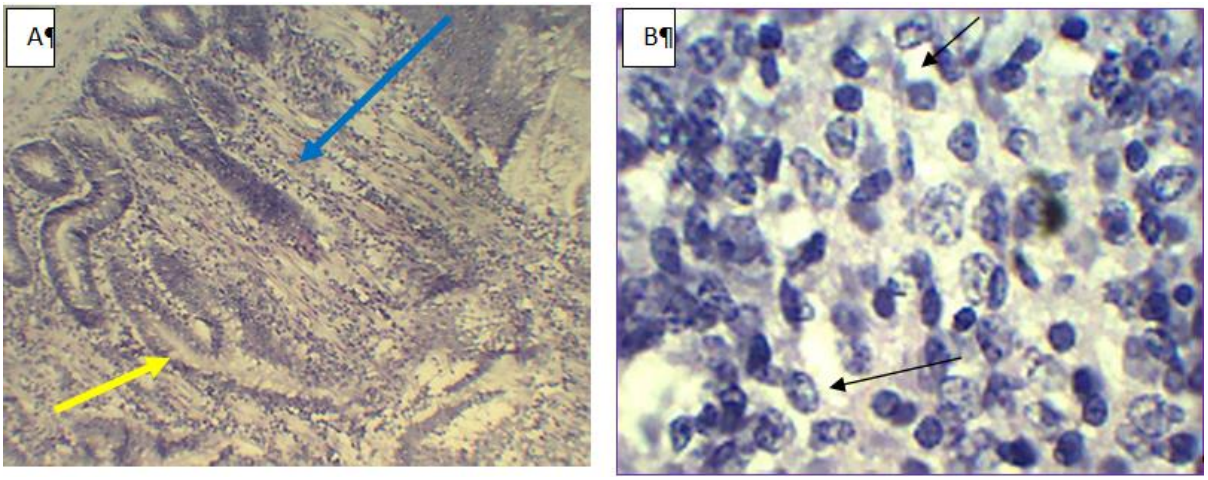


Figure 8: Tubercular granuloma on intestinal tissue (A) characterized by central necrosis indicated by blue arrow, and peripheral inflammatory cells, shown by the yellow arrow; and lymphoid depletion on spleen (B) indicated by the black arrows

5.6. Public Health Implications

A questionnaire survey was conducted to investigate the perception of farmers on avian tuberculosis in chickens and its public health implications. For this purpose, a total of 91 chicken owners, 53 males and 38 females, were included in the study. Of the interviewed households, only 11% (10/91) of them had the awareness of the occurrence of the disease in chickens and its zoonotic implications. Hence, the remaining 89% (81/91) of them had no awareness of the disease and its public health consequences.

There was significant awareness difference among farmers of different age groups in general; apart from the smaller proportion of people responded to have better perception of the disease and its zoonotic impact. Better awareness was observed in younger age groups aged between 19 to 30 years. Nonetheless, there was no significant awareness difference ($P>0.05$) among farmers at different educational level, altitude and the two gender categories participated in this study (Table 5 and Figure 9).

Table 5: Summary of the major host related and environmental risk factors and their effect on the awareness of farmers involved in the study about the occurrence of avian tuberculosis and its zoonotic implications

Variables	**category**	**Number of farmers participated on the study % (N)**	**Number aware of avian TB in chickens and its zoonotic implications % (N)**	**Fisher's exact value**
Age	19-30 years	24.2 (22)	31.8 (7)	0.003
	31-64 years	72.5 (66)	4.5 (3)	
	65-75 years	3.3 (3)	0 (0)	
Gender	Male	41.8 (53)	7.5 (4)	
	Female	58.2 (38)	15.8 (6)	0.310
Edu.level	Illiterates	46.2 (42)	7.1 (3)	
	Adult edu.	11 (10)	0 (0)	
	Elem. edu.	30.8 (28)	14.3 (4)	0.135
	H.sch-edu.	9.9 (9)	22.2 (2)	
	Co-edu.	2.2 (2)	50 (1)	
Altitude	Highland	33 (30)	20 (6)	
	Midland	34.1 (31)	9.7 (3)	0.119
	Lowland	33 (30)	3.3 (1)	

Where, Edu=education, Elem=elementary, H.sch=high school and Co=college

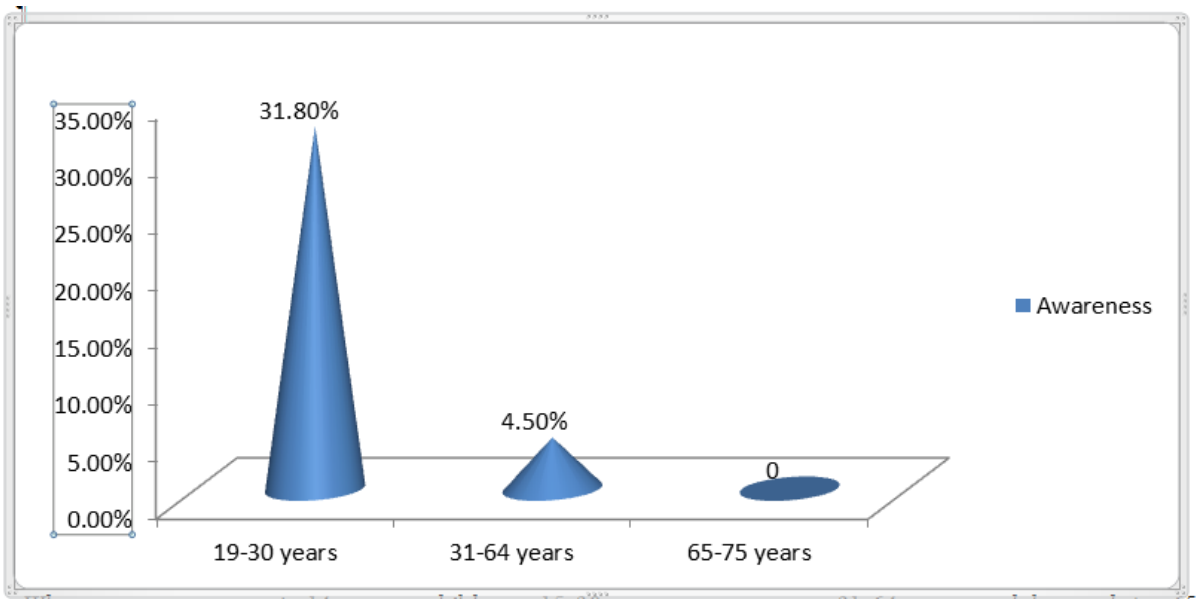


Figure 9: The trend of awareness in different age groups of farmers on the occurrence and public health implications of avian tuberculosis

The data was also analyzed using Fisher's exact test to evaluate the experience of farmers participated on the study; on chicken housing and chicken house cleaning activities; in relation to their perception on avian TB and its zoonotic implications. Accordingly, 46.2% (36/91) of the farmers were responded that they were keeping their chickens in a separate house at night. However, only 14.3% (6/36) of them were aware of avian TB and its zoonotic risk. In addition, a larger proportion of the participants responded their experience of sharing their home with chickens by keeping them on a perch or a carton in their home at night. Hence, the result revealed that there was no significant association ($P>0.05$) between chicken housing, house cleaning and farmers' awareness of the disease and its public health implications (Table 6).

Table 6: Summary of the Fisher's exact test result on chicken housing and house cleaning experience of farmers participated on the study; and their perception on avian TB and its zoonotic implications

Variables	Categories	Number of farmers participated on the study % (N)	Number aware of avian TB in chickens and its zoonotic implications % (N)	Fisher's exact value
Chicken housing	Separate	46.2 (36)	14.3 (6)	0.616
	W-human	22 (20)	15 (3)	
	W- shoats	2.2 (2)	0 (0)	
	W-cattle	2.2 (2)	0 (0)	
	On trees	27.5 (25)	4 (1)	
Chicken house cleaning interval	Daily	39.6 (36)	13.9 (5)	0.715
	E-2-3dd	24.2 (22)	9.1 (2)	
	Weekly	3.3 (3)	0 (0)	
	E-2-3wks	3.3 (3)	33.3 (1)	
	Rarely	29.7 (27)	7.4 (2)	

Where, W=with, E=every, dd=days

6. DISCUSSION

The present study attempted to investigate the epidemiology and public health implications of avian tuberculosis in selected districts of Oromia region, Ethiopia. It revealed that the overall apparent prevalence of avian tuberculosis in scavenging domestic chickens in the area was 11.4%. It also connoted the specific prevalence of the disease in the three study districts as 6.8% in Gerar Jarso (highland), 13.4% in Ada'a (midland) and 13.6% in Boset (lowland); implying that the prevalence of the disease decreases with increasing altitude. The study is also the first of its kind to explore the prevalence of avian tuberculosis in the central highland of Ethiopia. Nevertheless, these figures seem a little bit bigger as compared to similar study conducted in central Ethiopia which reported a 6.3% prevalence of the disease from Adama town (Tadesse *et al.,* 2004). This discrepancy could be due to the temporo-spatial disparity between the two studies, which implies that, the prevalence of avian tuberculosis in central Ethiopia and hence its public health implications in traditional chicken producers of the area could differ over time and location. Another study conducted on domestic chickens at Shashemene district also reported the prevalence of avian tuberculosis in the district as low as 4.23% (Sultan *et al.,* 2015). This variation could also be related to the variation in the spatial distribution of the disease and other host related factors in the area that negatively affected the prevalence of the disease during that particular study.

Recently, a study conducted on domestic chickens in selected sites of Ethiopia also revealed the prevalence of avian tuberculosis in semi-intensive exotic chickens in Bishoftu town as 5.85% (Kindu and Getaneh, 2016). This result also varies from the results of this study probably due to the difference between the management systems under which the chickens were kept; because in semi-intensive production system the farm hygiene and other husbandry practices are better than the backyard production system where the awareness of farmers regarding the hygienic and other management systems are more of traditional. Hence, these hygienic and management problems could strongly hamper the health and productivity of the traditional chicken production system in our country and favor the prolonged existence and transmission of the environmentally resistant bacteria, MAC, which are the etiologic agents of the disease in chickens and the risk of zoonosis for humans as well. According to Dhama

et al., (2008)*,* wild birds such as sparrows, crows, and pigeons may be infected with *M. avium* and may spread it to poultry flocks. Consequently, since scavenging chickens are in unlimited interaction with wild birds, they could easily transmit the disease to the scavenging domestic chickens. Moreover, Sultan *et al.* (2015) also reported that most owners give irregular grain supplements to their chickens which could attract the wild birds and increase the risk of contact between wild birds and domestic scavenging chickens. Hence, there is a strong likelihood that scavenging chickens may acquire the infection from the soil in a contaminated environment with the droppings of chronic carrier chickens and wild birds (Tell *et al.,* 2001). Thus, it is logical to see increased prevalence of avian tuberculosis in scavenging chickens as compared to semi-intensively managed ones.

Univariable and multivariable statistical evaluation of the major host related and environmental risk factors of avian tuberculosis in chickens also revealed breed as the major host related risk factor of the disease as compared to sex and spatial location of chickens. Accordingly, though the disease was well prevalent in all breeds of the traditionally produced domestic chickens in the study districts, multivariable logistic regression analysis revealed a statistically significant ($P<0.05$) odds of exposure of cross breed chickens (OR=24.36, 95% CI: 3.4, 182.1) as compared to the odd of exposure of local breeds involved in the study. This finding was also in agreement with the finding of a study conducted on semi-intensive poultry farms of Bishoftu town and village chickens at Bahir Dar and its surrounding; by Kindu and Getaneh (2016) who reported a significantly ($P<0.05$) higher rate of avian tuberculin positivity of exotic breed chickens as compared to local breeds. This implies that breed is a putative risk factor which predominantly predisposes chickens to avian tuberculosis. Thus, exotic and cross breed chickens have shown higher risk of exposure to the disease as compared to local breed.

Multivariable logistic regression result also connoted a significantly ($P<0.05$) reduced odd of exposure of exotic breed chicken as compared to local. Such controversial result to the previously documented scientific information concerning the innate resistance of local and exotic breeds could be due to disproportionate number of local and exotic breeds involved in the study.

Evaluation of the sex of chickens as host related trait indicated that 29 of the 31 chickens (93.6%) that were positive for avian tuberculin test were females. Hence, the univariable logistic regression model revealed a nearly threefold higher odd of exposure of female chickens to avian TB as compared to their male counterparts (OR=2.87, 95% CI: 0.66, 12.5). Nevertheless, the difference was not statistically significant (P>0.05). This result also agrees with similar studies in domestic chickens which were conducted at Adama, Sebeta and Debre Berhan towns of central Ethiopia by Tadesse *et al.,* (2004) and at Shashemene district of east Shewa zone of Oromia by Sultan *et al.,* (2015); who reported sex variation on the occurrence of avian TB with slightly higher occurrence in female adult chickens than male sex counterparts. This might be due to the fact that farmers keep female chickens for a prolonged period of time for egg production as compared to males and this longer production time could increase the risk of exposure of female chickens to the etiology of the disease, MAC, which is highly resistant to environmental extremes and can survive in the environment for a prolonged period of time, usually for ≥ 4 years (Thoen, 2013). Moreover, the number of male chickens (N=42) included in the study was also by far less than the number of female chickens (N=231); because most farmers usually keep only 1 male chicken in their breeding females or others might not keep them at all. Hence, this disproportionate involvement of male and female chickens in the intradermal avian tuberculin test in this particular study could also cause deviation of the true effect of sex as the risk factor of avian tuberculosis.

The study also revealed the effect of altitude as a risk factor for the occurrence of avian TB in chickens. Accordingly, from the three agro-ecological zones compared in this study, the odds of exposure of chickens at midland (OR=2.12, 95% CI: 0.77, 5.83) and lowland (OR=2.16, (OR=2.12, 95% CI: 0.77, 6.04) altitudes to avian TB was observed to be increased by two folds as compared to the odd of exposure of chickens at high altitude; but this difference was not statistically significant (P>0.05).The result was also in agreement with similar studies on domestic chickens conducted at Bishoftu town, Bahir Dar and its surrounding by Kindu and Getaneh (2016) and at Adama, Sebeta and Debre Berhan towns of central Ethiopia by Tadesse *et al.,* (2004) who reported the contribution of mid and low altitudes as a risk factor for the occurrence of avian tuberculosis in chickens to be higher as compared to high altitude from where they had identified no positive reactor chicken to the avian

intradermal tuberculin test conducted in their respective studies. This implies that, even though MAC is known to resist and survive in all environmental extremes except direct sun light, the hot and less humid environment of the mid and low altitudes might better favor the growth and survival of the organisms, which are the major etiology of avian tuberculosis in chickens, and hence increase the risk of exposure of chickens in the area as compared to high altitudes; where the environment is colder and humid.

According to some scholars, postmortem examination of gross lesions have been commonly used for diagnosis of avian TB concurrently with tuberculin test (Fulton and Thoen, 2003; OIE, 2010; Tell *et al.,* 2001). In this study, the postmortem detection rate of tuberculous lesion from the tuberculin test positive chickens was 40.9% (9/22). However, gross lesions were not detected on the remaining 59.1% (13/22) of chickens and this might be associated with the possibility of missing the lesions of recent infection and existence of obscure lesions which are common in mycobacterial infections (Gallagher, 1998).

Necropsy finding in the present study also evidenced gross pathological lesions which were suggestive for avian tuberculosis such as knife edged appearance of the keel bone and sloughing and disappearance of the wall of the cecal part of intestine; except a thin facia which had filled with air; in a chicken with a very good physical appearance and shiny feathers upon antemortem inspection. This implies that, a chicken which is performing well and having good physical appearance could be positive for avian tuberculosis and be a risk factor for the health of consumers. According to Dhama *et al,* (2011), in most cases, an infected bird without overt clinical signs may serve as carrier; that results in the persistence of infection in flocks. Hence, this result is also in agreement with the report of this scholar.

Nevertheless, the culture of post mortem tissue samples with gross or suspected avian TB lesions collected from liver, spleen, intestine and lungs of reactor chickens to avian tuberculin test was hardly responding; though the process is still underway. From a total of 60 tissue samples cultured in pair on 60 pyruvate enriched and 60 glycerol enriched Lowenstein Jensen medium slants, which totals 120, only 0.02% (2/120) had shown culture positivity up to 12 weeks of incubation at 37^0C.This could

be either due to poor specificity of the test to rule-in the true positive chickens to avian tuberculosis or due to problems with the efficiency of the media used for culturing, Lowenstein Jensen medium, to support MAC or the prolonged time it requires for the growth of these organisms. It could also be due to problem with the temperature at which the culture was incubated; which is lower than the average body temperature of domestic chickens (41^0C). This finding is also in agreement with the result of Kindu and Getaneh (2016) who reported that from the total of 34 samples taken from different organs of 12 slaughtered tuberculin reactor chickens and cultured on LJ slants, 17 were inoculated on sodium pyruvate enriched and the other 17 on glycerol enriched LJ slants, none of them have shown colonial growth up to 8 weeks of incubation. They also suggested the reason for the failure of MAC growth as inappropriateness of the incubation temperature (37°C); since it does not fit the natural hosts' internal body temperature.

According to Thoen *et al.* (1978), who compared the efficiency of four culture media for the isolation of *Mycobacterium avium* complex from 197 porcine tissues with or without microscopic granulomas and acid-fast bacilli, a significantly greater number of isolates ($P<0.05$) were obtained on Middle brook 7H10 medium with sodium pyruvate than on Stone brink medium, Herrold egg yolk agar medium, or Lowenstein Jensen medium. The time required to grow *M. avium* complex on Lowenstein Jensen medium was also significantly greater than the time required to observe growth on Stone brink, Middle brook 7H10, or Herrold egg yolk agar medium ($P<0.05$) (Thoen *et al.*,1978). This implies that, Lowenstein Jensen medium is not the medium of choice for culturing MAC to obtain better number of isolates within optimum period of time. On the other hand, Tadesse *et al*. (2004) reported culture positivity of 6.3% (6 out of 95) chickens' postmortem tissue samples cultured on pyruvate-enriched Lowenstein Jensen slants. Similarly, Sultan *et al*. (2015) also reported culture positivity of 50% (3 from 6) chickens with gross lesions on the same medium, Lowenstein Jensen medium. Hence, these controversial results signify the issue of MAC culturing to be an area for future research.

Histopathological examination of postmortem tissues collected from similar organs (liver, spleen and intestine) from which tissues for culturing were collected on the other hand revealed, a tubercular granuloma characterized by central necrosis and

peripheral inflammatory cells; mainly of mononuclear lymphocytes and macrophages. Moreover, lymphoid depletion of the cortical part of spleen with empty cystic structure at the sites of lymphoid depletion, which is also a characteristic feature of avian TB, was also visualized on the histopathological slides. This finding agrees with the findings of Tadesse *et al.* (2004) who reported the presence of granuloma characterized by caseonecrotic cores that were surrounded by a broad ring of palisading epithelioid cells, macrophages, multinucleate giant cells with a moderate mixture of heterophils, lymphocytes, and plasma cells. Hence, the result supports and confirmed the tuberculin test result and necropsy findings; but it contradicts the poor cultural response observed in this study; suggesting that poor culture positivity may be due to problems related to the nature of the bacteria and/ or poor efficiency of the culture media, LJ medium to support *Mycobacterium* species under *Mycobacterium avium* complex and the prolonged time it requires for the growth of MAC on LJ medium.

The other important component of this study was a questionnaire survey to investigate the public health implications of avian tuberculosis in the study districts. Accordingly, the result revealed that only 11% of the backyard chicken owners included in the survey responded that they had awareness of avian TB and its public health implications. However, 89% of them had no perception of the occurrence and zoonotic implications of the disease; in spite of their gender, educational status and spatial location. This result also agrees with the findings of Sultan *et al.* (2015) who reported the proportion of farmers who heard about avian TB and its zoonotic contribution as 13% and 2%, respectively from Shashemane district of East Shewa, Ethiopia. On the other hand, apart from the smaller proportion of people responded to have better awareness of avian TB and its zoonotic implications, there was significant ($P<0.05$) awareness difference among farmers of different age groups; with better awareness observed in younger age groups aged between 19 to 30 years; probably due to the access to formal education.

This study clearly indicated that lack of awareness of majority of the farmers involved in the survey regarding the occurrence and public health implication of avian tuberculosis and hence its source of infection and mode of transmission. Conversely, according to the report of OIE (2014) in humans, all members of *M. avium* complex

and *M. genavense*, are capable of inducing a progressive disease that is refractory to treatment, mostly in immunocompromised patients. In addition, the situation worsens due to the spread of HIV infection in developing countries (Une and Mori, 2007). So, it is considered prudent to keep infected birds away from humans, particularly the elderly, and individuals with poor immune status (Tadesse *et al.,* 2004).

The study also assessed the experience of farmers participated on the study; on chicken housing and chicken house cleaning activities. Consequently, the result showed that, 46.2% (36/91) of the participant farmers on the study were keeping their chickens in a separate house at night; and 22% (20/91) of them share their home with chickens. However, there was no significant awareness difference ($P>0.05$) among farmers of different age groups, gender category, and at different educational level and altitude concerning chicken housing and house cleaning. The result of the current study also agrees with the findings of Tadesse *et al.* (2004) and Sultan *et al.* (2015) from different parts of Ethiopia who reported that, most of the chicken owners share their home with chickens; a situation which creates conducive environment for transmission of mycobacterial infection from chicken to humans. This multifaceted effect of lack of awareness and strong association of farmers with their chickens, especially in those farmers who share their home with chickens, could terribly predispose them to zoonotic diseases such as avian TB.

The other epidemiological risk factor that was noticed during this study which possibly favor the occurrence and chance of transmission of avian tuberculosis was a situation in which farmers use the same night shade for chickens and domestic animals such as shoats and cattle. Consequently, 2.2% of the farmers involved on the survey were responded that they were keeping chickens with shoats and the other 2.2% of them keep chickens with cattle in the same night shade; of which none of them had awareness of the disease and its public health implications. In such instances, there may be the risk of cross transmission of the disease between chicken and other domestic animals and hence become an immediate source of the disease for each other and humans as well. This finding also agrees with the findings of Tadesse *et al.* (2004) and Sultan *et al.* (2015), a study conducted at selected towns of central Ethiopia and Shashemene district of east Shewa, who reported a large association of chickens with domestic animals and human beings which is stressful for the chicken

and also create conducive environment for the transmission of mycobacterial infection from chickens to other species including human and vice versa. In addition Martin and Schimmel (2000) also identified contaminated food originating from pig or other livestock as potential source of human infection.

According to the farmers involved on the study, consumption of chickens' meat and egg was a common practice in the area and chicken slaughtering is commonly practiced at home. Nevertheless, Kr´ız*et al.* (2010) reported that infected animals and their products, mainly eggs, which often come from small household production, are a risk for human health. Hence, in a community where the awareness of zoonotic diseases such as avian tuberculosis is poor and slaughtering is practiced under unhygienic conditions, these traditional practices could potentially affect the health of the public. This logic also holds true for urban dwellers in our country, Ethiopia; where chickens originated from the traditional chicken producing areas of the country are extensively slaughtered at home especially during major holidays such as Christmas, Easter and Moulid. Hence, the situation urges for critical intervention programs in order to increase the level of perception of the society and device alternative chicken slaughtering sites.

7. CONCLUSION AND RECOMMANDITIONS

The current epidemiological investigation in village chickens conducted using avian tuberculin test revealed an overall increment of the apparent prevalence of avian tuberculosis and its decrease with increasing altitude. Besides, it signified a relative breed and sex variation in the risk of acquiring the disease; with cross breeds and female chickens having a higher odds of exposure. However, an attempt made to isolate and characterize the species of mycobacteria from postmortem samples of positive reactor chickens during this particular study was hardly successful. Nevertheless, it is still underway to obtain isolates for molecular typing.

The questionnaire survey to investigate the perception of farmers in the study area on avian tuberculosis in chickens and its public health implications evidenced poor awareness of farmers irrespective of their gender, educational status and spatial location. Nevertheless, better awareness was observed in younger age groups aged 19-30 years. However, in Ethiopia, where millions of chickens coming from the rural backyard, chicken raisers are extensively used for human consumption, and their products such as eggs and meat are one of the crucial sources of revenue for poultry farmers in the country, including the rural chicken owners, attention given to avian tuberculosis seems unrewarding. Therefore, based on the above conclusive remarks the following points are recommended:

- This study and some other previous studies revealed a number of complexities in cultural isolation of MAC on conventional culture media; especially LJ medium. Therefore, the most efficient culture media which is time intensive and economical should be investigated for better isolation and characterization of MAC.
- Medical professionals should give due considerations to the zoonotic risk of avian tuberculosis in diagnosing and treating multi-drug resistant TB and extra pulmonary TB in human patients; because tuberculosis due to *M. avium* is mostly intestinal form and hence extra pulmonary; which is also refractory to treatment.
- Government should device a system which encourages chicken slaughter in the abattoir and discourages home slaughtering particularly in developing

countries like Ethiopia where home slaughtering is a common practice both in the rural and urban areas including major cities such as Addis Ababa.

- Due attention should be given to awareness creation programs in traditional chicken producers and consumers concerning the predisposing factors of avian tuberculosis both in chickens and humans; especially in areas where the concept of hygiene and modes of disease transmission are poor.
- Further studies should be conducted on the epidemiology, economic importance and public health significance of; avian tuberculosis; in countries like Ethiopia where information on the disease is very scant.

8. REFERENCES

Ada'a District Office of Livestock Development and Fisheries. (2017). Agro-ecological information, livestock and poultry population of Ada Chukala district, East Shewa, Oromia; Ethiopia. Unpublished official information.

Alders R., Spradbrow P. (2001) Controlling Newcastle Disease in Village Chickens: A Field Manual. Australian Centre for International, Canberra, Australia. Agricultural Research Monograph **82**: 112.

Amador E., Lloret L., Castillo A., Lopez Y. (2010). Identification of immunogenic proteins of *Mycobacterium avium* with diagnostic potential.*Inter. J. Infect. Disease*.**14(1):**129.

Aranaz A., Liibana E., Mateos A., and Domínguez L. (1997).Laboratory Diagnosis of Avian tuberculosis.*Seminars in Avian and Exotic Pet Med*. **6(1)**:9–17.

Bancroft J. and Cook H. (1994). Manual of histological techniques and their diagnostic application. London: Churchill Livingston & Longman. 244–247.

Biet F., Boschiroli M., Thorel M., Guilloteau L. (2005). Zoonotic aspects of *Mycobacterium bovis* and *Mycobacterium avium-intracellulare* complex.*Vet. Research*.**36(3):**411–436.

Bodmer T., Miltner E., Bermudez L. (2000). *Mycobacterium avium* resists exposure to the acidic conditions of the stomach. *FEMS Microbiol.Letters*.**182**(**1**):45–49.

Boset District Office of Livestock Development and Fisheries. (2017). Agro-ecological information, livestock and poultry population of Boset district, East Shewa, Oromia; Ethiopia. Unpublished official information.

Bradley A. (1992). A historical review of women's contributions to poultry production and the implicationsfor poultry development process. Proceedings 19th World's Poultry Congress, Amsterdam. The Netherlands.693-696.

Coetsier C., Vannuffel P., Blondeel N., *et al.* (2000). Duplex PCR for differential identification of Mycobacterium bovis, Mycobacterium avium, and Mycobacterium avium subsp. paratuberculosis in formalin-fixed paraffin embedded tissues from cattle. *J Clin Microbiol.* **38**:3048–4054.

Cromie R., Brown M. and Stanford L. (1992). The epidemiology of avian tuberculosis in white-winged wood ducks *Cairina scutulata* at The Wildfowl and Wetlands Trust, Slimbridge Centre. *Wild fowl*.**43**:211-214.

CSA. (2015) Agricultural sample survey 2012/13.Volume II. Report on livestock and livestock characteristics. Federal Democratic Republic of Ethiopia CSA.

CSA. (2009). Agricultural sample survey 2009/10. Report on livestock and livestock characteristics. Statistical Bulletin No. 468. Addis Ababa, Ethiopia.

Dhama K., Mahendran M., Tomar S. (2007). Avian tuberculosis: an overview. Poultry Punch. **24(3):**38–52.

Dhama K., Mahendran, M. and Tomar S. (2008). Pathogens transmitted by migratory birds: threat perceptions topoultry health and production. *Int. J. Poult. Sci,* **7 (6):** 516-525.

Dhama K., Mahendran M., Tiwari R., Dayal S., Kumar D., Singh S. and Sawant P. (2011). Tuberculosis in Birds: Insights into the *Mycobacterium avium* infections. Review article 712369, doi:10.4061/2011/712369.*Int. J. vet. Med.***: 14.**

Dvorska L., Matlova L., Ayele W., *et al.* (2007). Avian tuberculosis in naturally infected captive water birds of the Ardeideae and Threskiornithidae families studied by serotyping, IS901 RFLP typing, and virulence for poultry. *J. Vet. Microbiol*.**119(2–4)**:366–374.

Fukuoka Y., Nakano A., Nakajima S., Kimura H. (2003). Endobronchial lesions involved in Mycobacterium avium infection," *Respirator. Med*. **97(12):**1261–1264.

Fulton R. and Thoen, C. (2003).Tuberculosis. In: Diseases of Poultry, Saif Y.M., Barnes H.J., Glisson J., Fadly F., Mc Dougald L. and Swayne, D. (Eds), Iowa State University Press, *Ames, IA*, USA. 836-844.

Gallagher J. (1998). Role of infected, non-diseased badgers in the pathogenesis of tuberculosis in the badger.Veterinary Record.**142:** 710-714.

Gerar Jarso District Office of Livestock Development and Fisheries. (2016). Agro-ecological information, livestock and poultry population of Gerar Jarso district, North Shewa, Oromia; Ethiopia Unpublished official information.

Hailu A. (2014). Agro-Ecological Conditions Effect on the Expression of the spatial Chickens Distribution. *J. Agri. Sci*. **4(9):** 476-480.

Heatley J., Mitchell M., Roy A., Cho D., Williams D. and Tully T. (2007).Disseminated avian tuberculosis in a bald eagle (Haliaeetus leucocephalus). J. Avian Med. and Surg. **21(3):**201–209.

Ikonomopoulos E., Fragkiadaki E., Liandris K., Sotirakoglou E., Xylouri and Gazouli M. (2009). Estimation of the spread of pathogenic mycobacteria in organic broiler farms by the polymerase chain reaction. *J. Vet. Microbiol*. **133(3):**278–282.

Inagaki T., Nishimori K., Yagi T., Ichikawa K., Moriyama M. Nakagawa T. (2009). Comparison of a variable-number tandem-repeat method for typing

Mycobacteriumavium with mycobacterial interspersed repetitive-unit-VNTR and IS*1245* restriction fragment length polymorphism typing. *J.Clin. Microbiol.***47**: 2156–2164.

Kaevska M., Slana I., Kralik P. and Pavlik I. (2010). Examination *of Mycobacterium avium* subsp. *avium* distribution in naturally infected hens by culture and triplex quantitative real time PCR. *Vet. Med.***55**: 325–330.

Kazda J., Pavlik I., Falkinham J. and Hruska K. (2009). The Ecology of *Mycobacteria*: Impact on Animal's and Human's Health. 1st ed. Springer Science and Business Media.**520:** 978-1420.

Kindu A. and Getaneh G. (2016).Prevalence of Avian Tuberculosis in Domestic Chickens in Selected Sites of Ethiopia.*J. Vet Sci. Technol.***7**: 377.

Kitalyi J. (1998). Village chicken production systems in rural Africa, Household food security and genderissue. FAO Animal Production and Health Paper No. 142. Food and Agricultural Organization of theUnited Nations, Rome, Italy. 81.

Koek N., Koek A., Wambua J., Kamau J. and Mohan K. (1999). - *Mycobacterium* avium-related epizootic in free-ranging lesser flamingos in Kenya. J. *Wild. Dis.* **35 (2):** 297-300.

Kríz M., Slaný J., Shitaye J. and Pavlík I. (2010). Avian mycobacteriosis in humans remains a threat in the Czech Republic. *Klin Mikrobiol Infekc Lek.* **16(1**):10–17.

Manarolla G., Liandris E., Pisoni G., *et al.* (2009). Avian tuberculosis in companion birds: 20-year survey. Vet. Microbiol.**133(4):**323–327.

Martin G. and Schimmel D. (2000).*Mycobacterium avium* infections in poultry—a risk for human health or not?*DutchTierarztl Wochenschr*.**107**:53–58.

Millan J., Negre N., Castellanos E., *et al.* (2010). Avian tuberculosis in free-living raptors in majorca Island, Spain.*Avian Patho.***39(1)**:1–6.

Mobius P., Lentzsch I., Moser L., Naumann G., Martin and Kohler H. (2006). Comparative macrorestriction and RFLP analysis of Mycobacterium avium subsp. avium and Mycobacterium avium subsp. hominissuis isolates from man, pig, and cattle. Vet. Microbiol.**117(2–4):**284–291.

Nazmul H. (2014). Investigation of Avian Tuberculosis in Layer Chicken. MSc. thesis, Bangladesh Agricultural University, Mymensingh.

Neonakis K., Gitti Z., Krambovitis E. and Spandidos D. (2008).Molecular diagnostic tools in mycobacteriology.*J Microbiol Methods*.**75(1)**:1–11.

OIE. (2004).Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees). Volume1. Paris, France. 347-359.

OIE. (2001). Mycobacteriosis in birds.*Rev. Sci. tech. Off. int. Epiz.***20 (1):**180-203.

OIE. (2010). Manual of diagnostic tests and vaccines for terrestrial animals; Avian tuberculosis. Office International des Epizooties, Paris, France Chapter 2.3.6:497-508.

OIE. (2014). Avian tuberculosis.Version adopted by the World Assembly of Delegates of the OIE.Chapter 2.3.6.

Pattison M. (2008). Poultry diseases.*Elsevier Health Sciences.*

Pavlas M., Michalska A. and Hunady M. (1993).Diagnosis of avian tuberculosis-mycobacteriosis by rapid agglutination.*Acta vet.* **62**: 63-69.

Quaranta V., Santagada G., Latorre L. and Raimondi P. (1996). Serious outbreak of tuberculosis by *Mycobacterium avium* in a pheasant-breeding in Italian. *Acta*

Quinn J., Carter E., Marker B. and Carter P. (1994). Mycobacterium species. In: Clinical Veterinary Microbiology, Wolfe Publishing, London.156-170.

Radkowski M., Uradzinski J. and Szteyn J. (1996).The occurrence of infectious and parasitic diseases in poultry slaughtered in the district of Olsztyn, Poland.*Avian Dis.* **40:** 285-289.

Radomski V., Thibault C., Karoui C., *et al*. (2010). Determination of genotypic diversity of Mycobacterium avium subspecies from human and animal origins by mycobacterial interspersed repetitive-unit-variable-number tandemrepeat and IS1311 restriction fragment length polymorphism typing methods. *J. Clin. Microbiol*.**48(4):**1026–1034.

Shin S., Lee J., Koh W., *et al.* (2010). Efficient differentiation of Mycobacterium avium complex species and subspecies by use of five-target multiplex PCR.*J. Clin. Microbiol*.**48(11):**4057–4062.

Shitaye J., Beran V., Svobodová J., Morávková M., Babák V. and Pavlík I. (2009).Comparison of the conventional culture, the manual fluorescent MGIT system and the automated fluorescent MGIT 960 culture system for the detection of Mycobacterium avium subsp. avium in tissues of naturally infected hen.*Folia Microbiol*.**54(2):**137–141.

Shitaye J., Halouzka R., Svobodova J., Grymova V., Grym M., Skoric M., Fictum P., Beran V., Slany M. and Pavlik I. (2010). First isolation of *Mycobacterium genavense* in blue headed parrot (*Pionus menstruus*) imported from Surinam, South America, to the Czech Republic: a case report. *Vet. Med*.**55**:339–347.

Shitaye J., Matlova L., Horvathova A., Moravkova M., Dvorska L., Treml F., Lamka J. and Pavlik I. (2008). *Mycobacterium avium subsp. avium* distribution studied in a naturally infected hen flock and in the environment by culture, serotyping and IS901 RFLP methods. *Vet. Microbiol*.**127**:155–164.

Slana I., Kaevska M., Kralik P., Horvathova A. and Pavlik, I. (2010). Distribution of *Mycobacterium avium* subsp. *avium* and *M. a. hominissuis* in artificially infected pigs studied by culture and IS901 and IS1245 quantitative real time PCR. *Vet. Microbiol*.**144**: 437–443.

Soini E. and Viljanen M. (1996).Genetic diversity among Mycobacterium avium complex AccuProbe-positive isolates," *J. Clin.Microbiol*.**34(1):**55–57.

Sultan A., Gezahegne M., Adane W., Gobena A. (2015).Preliminary Study on Avian Tuberculosis and associated risks in domestic chickens at Shashemene District, Ethiopia.*Inter. Peer-reviewed J*. **3**: ISSN 2409-5117.

Tadelle D., Kijora C. and Peters K. (2003). Indigenous chicken ecotypes in Ethiopia: Growth and feedutilization potentials. *Int. J. Poult. Sci,* **2:** 144-152.

Tadelle K. (1998). Epidemiology and zoonotic importance of bovine tuberculosis in selected sites of Eastern Shoa, Ethiopia. MSc Thesis, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia and Freie University, Berlin, Germany.

Tadelle, D. & Ogle, B. (2001).Poultry production systems in the central highlands of Ethiopia.*Trop. Anim.Health and Prod,* **33(6):** 521-537.

Tadesse S., Woldemeskel M., Molla B., Tibbo M., Kidane D., Medhin G. and Britton S. (2004). Avian mycobacteriosis in domestic chickens from selected agro-climatic regions in Ethiopia. *Int. Appl. Res.Vet. Med.* **2(1):**17-25.

Tell L., Woods L. and Cromie R. (2001). Avian tuberculosis in birds.*Rev. Sci. Tech. Off. Int. Epiz,* **20**: 180-203.

Tell L., Woods L. and Cromie R. (2002). Mycobacteriosis in birds. Rev. *Sci. Tech. Int. Epiz.***20(1):**180-203.

Thibault V., Grayon M., Boschiroli M., Hubbans C., Overduin P. and Stevenson K. (2007). New variable-number tandem-repeat markers for typing *Mycobacteriumavium* subsp. *paratuberculosis* and *M.avium* strains: comparison with IS*900* and IS*1245* restriction fragment length polymorphism typing. *Journal of Clinical Microbiology,* **45**:2404–2410.

Thoen C. (1978). Comparison of Four Culture Media for Isolation of *Mycobacterium avium* Complex from Porcine Tissues. Diagnostic Bacteriology Laboratory, and Pathology, Toxicology, and Parasitology Laboratory, National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Ames, Iowa 50010.

Thoen C. (1997). Tuberculosis. In: Diseases of Poultry. 10th ed. Iowa: Iowa State University Press. 67–178.

Thoen C. (1998). Tuberculosis. In: Calnek BW, editor. *Disease of Poultry*, London, UK.167–178.

Thoen C. (2013). Last full review/revision by Charles O. Thoen, DVM, PhD.

Thomas N. (2007). Infectious diseases of wild birds.*John Wiley and Sons.*

Thursfield M. (1995). Veterinary Epidemiology. 3rd edn.University of Edinburgh. Blackwell Sciences: 225-228.

Tirkkonen J., Pakarinen A., Moisander M., Makinen J., Soini H. and Ali-Vehmas T. (2007).High genetic relatedness among *Mycobacterium avium* strains isolated from pigs and humans revealed by comparative IS1245 RFLP analysis. Vet. Microbiol.**125(1-2):**175–181.

Une Y. and Mori T. (2007).Tuberculosis as a zoonosis from a veterinary perspective; Comparative Immunology.Microbiol and Infec. Dis. **30 (5-6)**:415–425.

WHO. (1998). Laboratory Services in tuberculosis control.World Health Organization.48-49.

Wikipedia. (2014).Socio-economic profile of East Shewa by Government of Oromia Region last accessed 1 August 2006.

Wilton S., Cousins D. (1992). Detection and identification of multiplemycobacterial pathogens by DNA amplification in a single tube PCR.*MethApp*. **1**: 269–273.

Yamamoto T., Shibagaki T., Yamori S., *et al.* (2008). Strategy for the detection and differentiation of *Mycobacterium avium* species in isolates and heavily infected tissues; Research in Vet. Sci. **85(2):** 257–264.

Zander D. and Mallison E. (1991).Principles of disease prevention and control. In: Calnex BW, *et al.* (eds.) Diseases of Poultry.4 ed. Ames, IO: Iowa State University Press.

9. ANNEXIES

Annex 1: Questionnaire prepared to assess the perception of back yard chicken producers in the study areas on avian tuberculosis

Addis Ababa University

College of Veterinary medicine and Agriculture

Questionnaire prepared to appraise the perception of back yard chicken raisers and chicken consumers on the risk of acquiring avian tuberculosis

Part -1: General Information

Date_____

Name of the respondent _____ Sex_____

Age_____

Educational level_____ Family size _____

Woreda_____

Kebele_____

Part-2: Specific Questions

1. Do you have a chicken?
   A. Yes B. No
2. If yes, how many chickens do you have?
   A. 1-5 B. 6-10 C. 10-15 D. >15
3. Which breed are they?
   A. Local B. Cross C. Exotic D. A and C
4. Is there a separate house for your chickens?
   A. Yes B. No

5. If no, how do you keep them?
   A. On a perch in my own home
   C. On a perch in a shoats pen
   B. On a perch in a cattle barn
   D. On a perch in equine shade
6. In how many days interval do you clean a chicken house or its perch?
   A. Every day
   C. Every week
   E. Every Month
   B. Every 2-3 days
   D. Every 2-3 weeks
   F. Rarely
7. Is it common to consume chicken and its products in your locality?
   A. Yes
   B. No
8. If yes, do you know any disease of chicken which can be transmitted from chicken to humans through the consumption of chicken or chicken products like eggs?
   A. Yes
   B. No
9. If you know them, please specify?

   _____

   _____.
10. Do you think that chickens can be affected by avian tuberculosis?
   A. Yes
   B. No
11. If yes, what are the clinical signs of avian tuberculosis in chicken?
   A. Poor body condition
   C. Decreased appetite
   E. Regression of comb and wattle
   B. Coughing
   D. Ruffled hair coat
   F. All
12. Is there any risk of transmission of avian tuberculosis from chicken to humans?
   A. Yes
   B. No
13. If yes, what are the predisposing factors?
   A. Poor hygiene of the chicken house or perch
   B. Consumption of raw and /or under cooked chicken products
   C. Keeping chickens in the same house with humans
   D. Unlimited intimacy of children with chickens
   E. Poor personal and environmental hygiene
   F. All
14. Have you or your family ever been affected by tuberculosis?
   A. Yes
   B. No

15. If yes, what was the source of infection for that particular case?
    A. TB patient in our neighbor
    B. TB patient in the transportation vehicle
    C. TB patient in the market place
    D. Unknown
16. Did the patient treated properly with effective drug of TB?
    A. Yes B. No
17. If yes, how was the response of the disease to the therapy?
    A. It was good
    B. It was poor and hence, recovery was obtained through repeated therapy
    C. The patient was died
18. Finally, if you have any comment on chicken production in your area and the risk of TB in humans?

_____

_____

_____.

Thank you very much!!!

Annex 2: Questionnaire translated to the local language (afan oromo) of the study areas

Yunivarsittii finfinnee koollajjii yaala beeyladaa fi qonnaatti gaafilee horsiistonnii lukkuu fi fayyadamtoonni lukkuuf buu'aalee lukkkuu hubannoo dhibee lukkuu ''avian tuberculosis'' jedhamurratti qaban baruuf qopha'an

I. Gaafilee waliigalaa

Guyyaa_____

Maqaa nama gaafatamee_____ Saala_____ Umurii_____

Sadarkaa barnootaa_____ Lakk. Maatii_____

Aanaa_____ Ganda_____

II. Gafilee Hubannoo Horsistonni Lukkuu Dhibee Lukkuu 'Avian Tuberculosis' Jedhamee Beekamu Irratti Qaban Ilaalchisee Qopha'an.

1. Lukkuu qabduu?
   A. Eeyyee B. Hinqabu
2. Eeyyen yoo jettan, lukkuu meeqa qabdu?
   B. 1-5 B. 6-10 C. 10-15 D. >15
3. Lukkuun keessan sanyummaan isaanii sanyii kami?
   B. Sanyii biyya keessa B. sanyii makaa C. sanyii biyya alaa D. A fi C
4. Lukkuuwwan keessan mana addaa qabuu?
   A. Eeyyee B. Hinqaban
5. Hinqaban yoo jettan, eessa buluree?

C. Mana jirreenyaakoo keessa, Qooxiirraa C. Mana hoolaf ra'ee keessa, Qooxiirra

D. Mana loonii keessa, Qooxiirra D Mana fardaaf harree keessa, Qooxiirra

6. Guyyaa meeqa meeqatti mana lukkuu ykn qooxii isaanii qulquleessituuf?

A. Guyyuma guyyaadhaan C. Torbanitti al tokko E. Ji'atti al tokko

B. Guyyaa 2-3tti al tokko D. Torban 2-3tti al tokko F. Darbee-darbee

7. Lukkuu fi hanqaaquu nyaachuun isin biratti baramaa dha?

A. Eeyyee B. Hinqabu

8. Baramaa taanaan, dhibeewwan lukkuu lukkuu ykn hanqaaquu hin waaddamne ykn hinaffeellamne nyaachuudhaan lukkuurraa namatti daddarban beektuu?

A. Eeyyee B. Hinbeeku

9. Beektu taanaan kanneen keessaa muraasa isaanii natty himaamee?

A. Influeenzaa lukkuu C. TB lukkuu E. Dhibee Gumbro

B. Fangilii D. Koleeraa Lukkuu F. Taayphoyidii lukkuu

10. Lukkuun dhibee sombaa ykn TB dhaan ni qabamti jettanii yaadduu?

A. Eeyyee B. Hinqabmtu

11. Ni qabamtti yoojettan, mallaattoon dhibee sombaa ykn TB lukkuurratti maalfaadha?

A. Huuqachuub B. Nyaata hir'isuu

C. Guttiyyee fi amartiinshee xixxiqaachuu fi daddalachaa'uu D. Bifa sokorreessuu

E. Qufaasisuu F. Hunduu deebiidha

12. Dhibeen sombaa ykn TBn lukkuu kun lukkuurraa namatti daddarbuu ni danda'aa?

A. Eeyyee B. Hindaddarbu

13. Nidaddarba yoo jettan, wantoonni namni dhibee kanaan akka qabamu sababa ta'uu danda'an maalfaadha?

A. Mana ykn Qooxii lukkuu qulqulina hinqabne

B. Lukkuu ykn hanqaaquu dheedhii nyaachuu

C. Lukkuuwwan mana jireenyaa namaa keessa bulchuu

D. Da'imman xixxiqqoon bakka lukkuun ooltu ykn bultu fi qulqullina hinqabne yemmuu taphatan

E. Qulqulina dhuunfaa fi naannoo dhabuurraa kan ka'e

F. Hunduu deebiidha

14. Isin ykn maatiin keessan dhibee sombaatiin qabamtanii beektuu?

A. Eeyyee B. Hinbeeku

15. Qabamtanii beektu taanaan, ka'umsisaa maal ture ykn eessaa isin qabe?

A. Nama dhibee sombaatiin qabameetu olla keenya jira ture

B. Nama dhibee sombaatin qabame waliin konkolaataa tokko yaabbanneet isarraa naqabe

C. Iddoo gabaatti nama dhibee sombaatiin qabamerraa naqabe

D. Waan irraa naqabe hinbarre

16. Isin ykn maatiin keessan dhibee sombaa kanaan erga qabamtanii booda yaala barbaachisaa dhibee kanaaf ta'u argattanii turtanii?

A. EEyyee B. Hinarganne

17. Yaalamtaanii turtan taanaan, bu'aan yaalichaa akkam ture ykn qoricha isiiniif ajajamee ture erga fixxanii booda daftanii fayyitanii?

A. Eeyyee, bu'aan yaalichaa baay'ee gaarii ture

B. Fayyuuf baay'een rakkadhe; baay'ee narra ture, deddeebi'ee yaalameen nadhiise

C. Namichi dhukkubsatee ture ni du'e

18. Waluumaagalatti, akka naannoo keessanitti ,horsiisa lukkuutiin walqabatee balaa dhibeen sombaa lukkuu kun lukkuurraa namatti daddarbuu ilaalchisee yaada yooqabaattan?

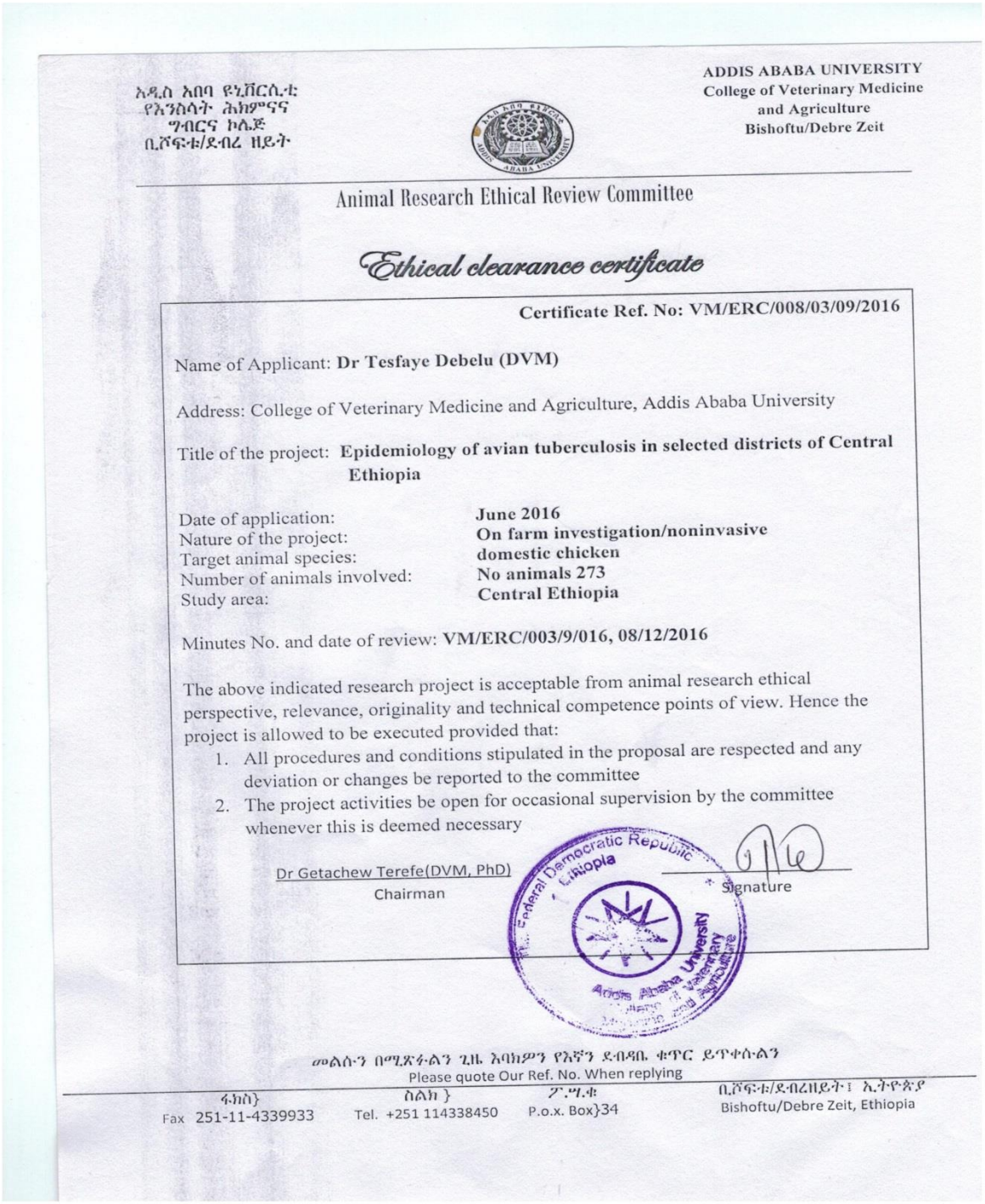
_____

_____

_____.

Galatoomaa!!!

Annex 3: Ethical clearance certificate



አዲስ አበባ ዩኒቨርሲቲ
የእንስሳት ሕክምናና
ግብርና ኮሌጅ
ቢሾፍቱ/ደብረ ዘይት

ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu/Debre Zeit

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/008/03/09/2016

Name of Applicant: **Dr Tesfaye Debelu (DVM)**

Address: College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: **Epidemiology of avian tuberculosis in selected districts of Central Ethiopia**

Date of application:	**June 2016**
Nature of the project:	**On farm investigation/noninvasive**
Target animal species:	**domestic chicken**
Number of animals involved:	**No animals 273**
Study area:	**Central Ethiopia**

Minutes No. and date of review: **VM/ERC/003/9/016, 08/12/2016**

The above indicated research project is acceptable from animal research ethical perspective, relevance, originality and technical competence points of view. Hence the project is allowed to be executed provided that:

1. All procedures and conditions stipulated in the proposal are respected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee whenever this is deemed necessary

Dr Getachew Terefe(DVM, PhD)
Chairman

Signature

Federal Democratic Republic Ethiopia
Addis Ababa University College of Veterinary Medicine and Agriculture

መልሱን በሚጽፉልን ጊዜ አባክዎን የእኛን ደብዳቤ ቁጥር ይጥቀሱልን
Please quote Our Ref. No. When replying

ፋክስ} Fax 251-11-4339933
ስልክ } Tel. +251 114338450
ፖ.ሣ.ቁ P.o.x. Box}34
ቢሾፍቱ/ደብረዘይት፣ ኢትዮጵያ Bishoftu/Debre Zeit, Ethiopia

Annex 4: Flow chart showing the activities that were undertaken during the research work and their chronological order.