



**EPIDEMIOLOGICAL STUDY ON THE ROLE OF CATTLE IN THE
OCCURRENCE OF PULMONARY TUBERCULOSIS IN HUMANS IN RURAL
COMMUNITIES OF AMHARA REGION, ETHIOPIA**

Araya Mengistu Kassa



A Dissertation for the Degree of Doctor of Philosophy (PhD) in Public Health

Addis Ababa University, Ethiopia.

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ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

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ABBREVIATIONS AND ACRONYMS

A:	Adenine
AFB:	Acid Fast Bacilli
AHRI:	Armauer Hansen Research Institute
AIDS:	Acquired Immunodeficiency Syndrome
ALERT:	All African Leprosy Education, Rehabilitation and Training Center
ANRS:	Amhara National Regional State
AOR:	Adjusted Odds Ratio
APPD:	Avian Purified Protein Derivative
BCG:	Bacillus Calmette-Guérin
BCS:	Body Condition Score
BD:	Becton, Dickinson and Company
Bp:	Base Pair
BRICS:	Brazil, Russian Federation, India, China, and South Africa
BTB:	Bovine Tuberculosis
C.I:	Confidence Interval
C:	Cytosine
CAS:	Central-Asia
CC:	Case-Control
CCIT:	Comparative Cervical Intra Dermal Tuberculin Test
CCS:	Comparative Cross-sectional
CDC:	Center for Disease Control
CM:	Centimeters
CMI:	Cell Mediated Immunity
CSS:	Cross-Sectional Survey
DF:	Degree of Freedom
DNA:	Deoxyribonucleic Acid
dNTP:	Dinucleotide Triphosphates
DOTS:	Directly Observed Treatments, Short Course
DR:	Direct Repeat

DST:	Drug Susceptibility Test
e.g.:	Example
EAA:	European-American-African
EAI:	East African- Indian
ECL:	Enhanced Chemiluminescence
EDTA:	Ethylene Diamine Tetra Acetic Acid
EHNRI:	the then Ethiopian Health and Nutrition Research Institute
EPTB:	Extra Pulmonary Tuberculosis
Ff:	Flank Forward
FIND:	Foundation for Innovation New Drugs
FMoH:	Federal Ministry of Health
Fr:	Flank Reverse
FW:	Flank Forward
G:	Guanine
GAP:	Global AIDS Program
GDP:	Gross Domestic Product
H:	Haarlem
HH:	House Hold
HIV:	Human Immunodeficiency Virus
ID ₅₀ :	Infective Dose 50
IFN:	Interferon
IL:	Interleukin
ILB:	International Laboratory Branch
Inj:	Injectable
Int:	Internal
IRB:	Institutional Review Board
IS:	Insertion Sequence
IU:	International Unit
Kbp:	Kilo Base Pair
LAM:	Latin- American- Mediterranean
LJ:	Löwenstein–Jensen

M.tb/MTB:	<i>Mycobacterium tuberculosis</i>
M:	Meter
MDR:	Multi-Drug Resistant
MDR-TB:	Multi-Drug Resistant Tuberculosis
MGIT:	Mycobacterium Growth Indicator Tube
MIN:	Minute
ML:	Milliliter
mm:	Millimeter
MTBC:	<i>Mycobacterium tuberculosis Complex</i>
NO:	Number
PCR:	Polymerase Chain Reaction
pH:	Expressing the acidity or alkalinity of a solution
PI:	Principal Investigator
PME:	Post Mortem Examination
PPD:	Purified Protein Derivative
PTB:	Pulmonary Tuberculosis
RD:	Region of Difference
Rev:	Reverse
RFLP:	Restriction Fragment Length Polymorphism
Rpm:	Revolution Per Minute
SDS:	Sodium Dodecyle Sulphate
SIT:	Shared International Types
SNPs:	Single Nucleotide Polymorphisms
SOP:	Shared Operating Procedure
SPoIDB4:	International Spoligotype Database 4
SPSS:	Statistical Packages for Social Sciences
St:	Saint
T:	Thymine
TAE:	Tris base, acetic acid and Ethylene Diamine Tetra Acetic Acid.
TB:	Tuberculosis
TBLN:	Tuberculosis Lymphadenitis

TE:	Tris base and EDTA
UK:	United Kingdom
USA:	United States of America
USD:	United States Dollar
UV:	Ultra Violet
VLA:	Veterinary Laboratory Agency
WHO:	World Health Organization
WV:	Weight-Volume
XDR:	Extensively Drug Resistant
XDR-TB:	Extensively Drug Resistant Tuberculosis

GLOSSARY

The definitions were collected from different sources by using Google. com engine, <https://www.google.com/> (1).

Chronic Cough: Any cough ≥ 2 weeks duration used as an indicator to index of suspicion for TB. Or cough was defined as acute if less than 2 weeks duration and as chronic otherwise or a productive cough for more than 2 weeks, which may be accompanied by other respiratory symptoms.

Cough: Is usually with **expectoration**, with or without blood stained sputum and may be accompanied by one or more of the following symptoms: weight loss; chest pain; shortness of breath; intermittent fever; night sweats; loss of appetite; fatigue and malaise.

Rural community: A community having smaller populations and dealing in lots of agricultural activities.

Purified Protein Derivative (PPD): Purified protein derivative tuberculin a sterile solution of a purified protein fraction precipitated from a filtrate of tubercle bacillus grown on a special medium; used in tuberculin tests. Or the Purified Protein Derivative (PPD) is an antigen used in the diagnosis of Tuberculosis infection.

Avian PPD: Tuberculin purified protein derivative, avian (Tuberculin PPD, avian) is a preparation obtained from *Mycobacterium avium*, strain D4 ER capable of revealing a delayed hypersensitivity in an animal sensitized to micro-organisms of the *Mycobacterium avium complex*.

Bovine PPD: Tuberculin purified protein derivative, bovine (Tuberculin PPD, bovine) is a preparation obtained from the heat-treated products of growth and lysis of *Mycobacterium bovis* strain AN-5 capable of revealing a delayed hypersensitivity in an animal sensitized to *Mycobacterium tuberculosis complex species*.

Comparative Cervical Intradermal Tuberculin Test (CCIT): The intradermal injection of biologically balanced bovine PPD tuberculin and avian PPD tuberculin at separate sites in the

mid-cervical area to determine delayed type hypersensitivity (Immune response) by comparing the response of the two tuberculin's 72 hours (plus or minus 4 hours) following injection.

Bovine: Of, relating to, or resembling a ruminant mammal of the genus *Bos*, such as an ox, cow, or buffalo. Or relating to, or belonging to the *Bovini* (cattle), a bovid tribe including domestic cattle.

Cattle: Cattle (colloquially cows) are the most common type of large domesticated ungulates. They are a prominent modern member of the subfamily Bovinae, are the most widespread species of the genus *Bos*. Or large ruminants with horns and cloven hoofs, domesticated for meat or milk, or as beasts of burden.

H37Rv: A Laboratory Virulent Strain of *Mycobacterium tuberculosis*

Pretest measurement: The measurement of skin fold of the injection site before administering PPD.

Posttest measurement: the measurement of skin folds of the injection site after 72hrs \pm 4hrs after PPD administration.

Negative reaction/cattle: If the increase in skin thickness at the bovine site of injection is less than or equal to the increase in the skin reaction at the avian site of injection.

Inconclusive/dubious result: If the increase in skin thickness at the bovine site of injection is from 1 to 2 or 1 to 4 mms greater than the avian reaction. There is some uncertainty as to the disease status of inclusive results and therefore they must be re-tested and occasionally, the presence of other diseases, or exposure to bacteria similar to *M. bovis*, can cause a positive test reaction, even if there is no TB infection.

Positive reaction/cattle: If the increase in skin thickness at the bovine site of injection is more than 2 or 4 mm greater than the reaction shown at the site of the avian injection.

Sputum: A mixture of saliva and mucus coughed up from the respiratory tract. Or is mucus-like material found in the lungs.

Body condition scoring (BCS): Body condition scoring is a management tool that can be used to evaluate the body status of cattle by using three major scoring grades, namely; lean, medium and fat for this study.

Household: Which consists of one or more people who live in the same dwelling and also share at meals or living accommodation, and may consist of a single family or some other grouping of people.

Bovine tuberculosis: A disease of bovine caused by *Mycobacterium bovis* and tested positive for intradermal tuberculin skin tests.

AFB positives: This bacterium is completely acid-fast (stain with carbol fuchsin and not decolorized by acid alcohol). A positive test result from the acid-fast stain confirms the patient has TB.

Presumptive TB case: Any person who presents with symptoms or signs suggestive of TB. The most common symptom of pulmonary TB is a productive cough for more than 2 weeks, which may be accompanied by other respiratory symptoms (shortness of breath, chest pains, haemoptysis) and/or constitutional symptoms (loss of appetite, weight loss, fever, night sweats, and fatigue).

Case of tuberculosis: A person showed at least one or more sputum positive for AFB and diagnosed as TB patient and decided to treat him/her with a full course of TB treatment.

Tuberculosis negative households: Households did not have TB history and did not have chronic cough which lasts for more than two weeks before commencement of the tuberculin test.

BASES FOR THE DISSERTATION

This dissertation is based on the following manuscripts (indicated the status in parenthesis), generated from the primary data, which are referred to text by their Roman numerals.

- I. Mengistu A, Enquasselasie F., Mulatu F., Hailu E., and Beyene D. 2013. Tuberculosis Infection in Cattle and Cattle Owners in North Western parts of Ethiopia. (Status: Accepted for publication in Journal of biology and Medical sciences).**
- II. Mengistu A, Enquasselasie F., Aseffa A. and Beyene D. 2014. Bovine Tuberculosis in rural Ethiopia: A comparative Cross-sectional Study on Cattle Owned by Households with and without Tuberculosis. (Status: Submitted to African Health Sciences-African Journals-online. Recommended to publish with minor comments/revisions and the revised version is resubmitted).**
- III. Araya Mengistu, Fikre Enquasselasie, Abraham Aseffa and Demissew Beyene. 2014. Bovine Tuberculosis (BTB) as a risk factor for developing Tuberculosis in humans in the rural community of Ethiopia: A Case-control study. (Status: Published on Ethiopian Medical Journal: *Ethiop Med J*, 2015, Vol. 53, No. 1).**
- IV. Mengistu A, Enquasselasie F., Hailu E., Aseffa A. and Beyene D. 2014. Identification and Characterization of *Mycobacterium Tuberculosis* Isolated from Cattle Owners in North Western and North Eastern Parts of Rural Ethiopia. (Published on International Journal of Innovation and Applied Studies, ISSN 2028-9324 Vol. 10 No. 1 Jan. 2015, pp. 85-94).**

ABSTRACT

Introduction: Tuberculosis is a major global public health problem resulting in considerable morbidity and mortality. According to 2012 World Health Organization report, an estimated 8.6 million people developed tuberculosis and 1.3 million died from the disease (including 320 000 deaths among Human Immunodeficiency Virus (HIV) positive people). Ethiopia is one of the highest tuberculosis burden countries in the world, which ranks 8th amongst the 22 high tuberculosis burden countries. In Ethiopia, it was also estimated that about 41% of tuberculosis cases were HIV positive. The transmission of *Mycobacterium tuberculosis* from humans to humans is well known globally; however, little is known about the transmission of *Mycobacterium tuberculosis* complex species between humans and animals, in particular cattle. Bovine tuberculosis is endemic in Ethiopia. Intimacy of cattle and humans in rural farming communities may transmit *Mycobacterium bovis* to humans. However, there is little information about the possible transmission of tuberculosis between humans and cattle in Ethiopia. The contribution of *Mycobacterium tuberculosis* to bovine reactivity is also unknown. Although bovine tuberculosis is a known zoonosis, it is mainly a disease of cattle. Humans could be infected by consuming raw milk and, to some extent, by inhaling droplet nuclei. Cattle owned by tuberculosis patients revealed higher bovine tuberculosis tuberculin skin test result and this might be due to the possible infection of cattle with *Mycobacterium tuberculosis*. The causative agent responsible for the infection in both populations could be identified using molecular techniques. This research work aimed to investigate the role of cattle in the occurrence of pulmonary tuberculosis in humans at a rural community in Ethiopia.

Methods: The study used both cross-sectional and case-control designs in Northeast and Northwest parts of the Amhara Region. A cross-sectional study was employed to determine the presence of bovine tuberculosis among cattle owned by presumptive pulmonary tuberculosis cases. A comparative cross-sectional study was conducted on 124 cattle owned by households with confirmed pulmonary tuberculosis and 381 cattle owned by households without tuberculosis to determine the likelihood of bovine tuberculosis among livestock's in the two groups. A case-control study was conducted on 35 households with at least one pulmonary tuberculosis case and 105 households without tuberculosis to determine the risk of bovine tuberculosis in humans.

Besides, laboratory based deletion typing and spoligotyping that was carried out for *Mycobacterium tuberculosis* isolated from acid fast bacilli confirmed pulmonary tuberculosis patients. A comparative cervical intradermal tuberculin skin test was conducted on all the study subjects (cattle) using bovine and avian purified protein derivatives as an antigen. Based on the skin test reaction measurement, the cattle were categorized as negative, doubtful and positive, if the measured difference between the bovine and avian injection site is below 1, 1- 4 and >4, or <1, 1-2 and >2, respectively. All sputum samples collected from presumptive tuberculosis cases as well as tuberculosis confirmed human patients were cultured on Lowenstein-Jensen medium (tubes containing glycerol and sodium pyruvate) and polymerase chain reaction, deletion typing, spoligotyping and single nucleotide polymorphism were performed for positive culture findings to identify the *Mycobacterium tuberculosis strains* involved for human tuberculosis infection. Closed questionnaires and forms were used to collect the required data.

Results: Of the 124 sputum samples collected from presumptive tuberculosis cases 4/124 (3.2%) were culture positive. Polymerase Chain Reaction using region of difference-9 as a marker has confirmed that 2/4 (50%) were found to be *Mycobacterium tuberculosis* while the rest were atypical *Mycobacterial species*.

Of the 381 cattle tested 5/381 (1.31%) were found to be tuberculin positive (prevalence of 1.31% with 95% CI: 0.2, 2.5) and 10/381 (2.63%) (Prevalence of 2.63% with 95% CI: 1.0, 4.2) were positive for the test according to a cutoff value ≥ 4 mm and ≥ 2 mm, respectively. About 67% (6/9) of the individuals who owned bovine tuberculosis positive cattle had the habit of drinking raw milk. Of the 10 positive cattle, five of them resided in lowland (Kola) areas (1300-1500 meters above sea level). However, none of the owners of tuberculin positive cattle were found to be tuberculosis positive.

Using >2mm as a cutoff value for the diagnosis of bovine tuberculosis, the study revealed that an overall apparent prevalence of 23.6% and individual cattle apparent prevalence of 8.7% for bovine tuberculosis in the study area with an odds of nearly three times (AOR= 2.90, 95% CI: 1.50, 5.54) higher bovine tuberculosis among cattle owned by households with tuberculosis. The findings indicated that 49.3% and 61.4% were having the habit of drinking raw milk and eating

uncooked meat, respectively. More than three fourth of the study subjects did not know the benefit of boiling milk. About 70.7% knew there is chance of disease transmission from animals to humans. Of the total respondents participated in a case - control study, 78.6% did not isolate their sick cattle and 87.1% kept the sick cattle with them.

Among the TB cases, 31.4% reported sharing the living room with their cattle as compared to only 9% of controls. It was also disclosed that 42.9%, 37.1% and 14.3% of the cases shared utensils, gave their urine to cattle and urinate on a cattle feed, respectively, as compared to 36.2%, 27.6% and 4.8% of the controls. Based on >2mms as a cutoff value bovine tuberculosis was recorded in 48.6% of the cases and 15.2% of controls, more than 8 times higher for cases (AOR=8.32, 95% CI; 2.82, 24.60).

In total, 70 acid fast bacilli positive sputum samples were collected in the study areas. The age of subjects ranged from 18 to 63 years with a mean age of 35.7 ± 13.24 years. Of the total 70 acid fast bacilli positive sputum samples, 50/70 (71.4%) were culture positive, from which 37.1 and 31.5% had the habit of drinking unboiled milk and eating uncooked meat, respectively.

Using deletion typing all the isolates were identified as *Mycobacterium tuberculosis*. Spoligotyping revealed 94% (47/50) interpretable patterns and three lineages namely; East-Africa-Indian (57.4%), Euro-American-African (38.3%) and Ethiopian (lineage-7) 2/50 (4.3%). Lineage 7 was registered only in North *Wollo Zone*. In this study 8 clusters (with cluster size ranging from 2 to 8), 8 unique and 10 new patterns were recorded. Shared International Types (SIT) (21, 25, 26, 35, 53, 109, 149 and 289) were found as clusters among which SIT 25 (7) and SIT 289 (8) were the predominant ones.

Conclusion: About 3.2% (4/124) of sputum samples collected from individuals with chronic cough were culture positive, of whom 50% (2/4) were found to be *Mycobacterium tuberculosis* by polymerase chain reaction. The findings indicate that bovine tuberculosis is a threat in Ethiopia and implicated possible transmission of tuberculosis between humans and cattle where human pulmonary tuberculosis cases could serve as a possible source of *Mycobacterium tuberculosis* infection to cattle. House sharing was found as a contributing factor in bovine

tuberculosis test reaction. Therefore, mechanisms should be developed to create awareness. Separate houses for cattle should be constructed to minimize the risk of cross infections and further study regarding the possible infection of cattle with *Mycobacterium tuberculosis* thus recommended. Similarly, households with bovine tuberculosis had a higher chance of getting pulmonary tuberculosis. It is, therefore, necessary to investigate whether the pathogen responsible is *Mycobacterium tuberculosis* or *Mycobacterium bovis* and appropriate measures should be taken to prevent spread of tuberculosis in both humans and livestock. This study also revealed that 3 *Mycobacterium tuberculosis* lineages, namely; the ancient, intermediate and modern were identified. Besides, considerable clustering, which indicates current tuberculosis transmission was also reported in the study areas.

Key words: *bovine/cattle, bovine tuberculosis, cough, households, human TB, lineage, North Gondar /Wollo, risk, skin test, Spoligotyp, tuberculosis, typing, Ethiopia.*

1. INTRODUCTION

1.1. Background

Human tuberculosis (TB), caused by *M. tuberculosis* (*M.tb*), and occasionally by *M. bovis* and *M. africanum* (2), is among the oldest diseases and the seventh leading cause of death worldwide (3). Human TB is a serious challenge, particularly for many of the developing countries in the world and the World Health Organization (WHO) (4) reported that 98% of TB deaths occurred in the developing world affecting mostly young adults in their productive ages. In 2013, WHO (5) reported a decline in the impact of tuberculosis by the year 2012, with 8.6 million people infected and 1.3 million died from tuberculosis. Shukla (6) mentioned that TB is still killing nearly 5000 people every day. If TB is left unchecked within 20 years, it will kill 35 million people (7). The emergence of Human Immunodeficiency Virus (HIV) has paved the way for the resurgence of *Mycobacterium tuberculosis* infection and resulting in an increased morbidity and mortality worldwide (8-10).

TB in Ethiopia is well known since years back and still remains being a major health problem, contributing to the morbidity and mortality of a considerable number of people. Ethiopia ranks 3rd in Africa and 8th amongst the 22 highest TB burden countries in the world (5). In Ethiopia, TB was the second leading cause of hospital admitted deaths in 2003/04 (11), and it is endemic in Amhara Regional State as well, where in 2008 the Regional Health Bureau reported 3.5% TB death rate (12). Besides, sputum samples collected from different health institutes in the Region were cultured and among 240 culture positive samples 237 were *Mycobacterium tuberculosis* strains based on RD9 analysis (13). Before years back tuberculosis in Ethiopia was markedly increasing (14), however, nationally, in 2013, the TB incidence rate has fallen to 224 per 100,000 of the population compared 369 in 1990 and TB prevalence rate has fallen by 50.5%. Besides, in the same year excluding HIV related deaths; TB mortality was estimated to be 32 per 100,000 of the population and about 13% of all new TB cases are also HIV co-infected (15).

The sources of infection for *M. tb* are mainly those humans who are developing active TB (16). However, *M. tb* has been isolated from different animal species, including bovines/cattle (17, 18), which should be seen as a challenge to further move in the prevention and controlling efforts made to tackle the disease. Although *M. tuberculosis* is the main cause of human TB, the main causative agent of bovine TB, *Mycobacterium bovis* (*M. bovis*) is well described to infect humans, primarily through close contact with infected cattle or consumption of contaminated animal products such as unpasteurized milk. Globally, most cases of zoonotic TB are caused by *M. bovis*, and cattle are the major reservoirs (19, 20).

Little is known about the transmission of *M. tb complex* from cattle to humans. Nowadays molecular techniques are becoming powerful tool that are widely used to type *Mycobacterium tuberculosis* complex species to know TB transmission dynamics (21-25). Understanding TB epidemiology, determining either TB relapse or exogenous re-infection, outbreak investigation and assessing laboratory cross contaminations as well as identification of the spread of clones is possible using genotyping (26-31).

Human TB of animal origin is an important public health concern particularly in developing countries. More than 94% of the world populations live in countries where the control of bovine tuberculosis (BTB) in cattle is limited or absent. In Africa, approximately 85% of cattle and 82% of the human population live in areas where BTB is prevalent (32). Research works undertaken in Ethiopia revealed the presence of bovine tuberculosis (33, 34). Knowing the possible sources of *Mycobacterium tuberculosis* could help the disease prevention and control. Isolation of *Mycobacterium tuberculosis* in cattle and the presence of BTB in Ethiopia were the bases to carry out the research work with the aim of investigating the role of cattle in the occurrence of pulmonary tuberculosis in humans in a rural community in Ethiopia.

1.2. Statement of the problem

Tuberculosis is a serious health problem to the world population and remains a major health constraint to humans, particularly for those who live in most developing countries, including Ethiopia. The problem is more serious in humans than in animals because of the potential to be infected by a group of *Mycobacterial species*, such as *M. tuberculosis* (the primary), *M.*

Africanum, *M. bovis* (primary animal's pathogen), *M. microti* and *M. canetti*. Poor living standards in the country, common tradition of house sharing with animals and the complication of the disease with HIV/AIDS magnify importance of the disease in Ethiopia. The attempts made so far to tackle TB have not been yet satisfactory. Shukla (6) mentioned that TB is still killing nearly 5000 people every day and 1.5 million deaths in 2013 alone. According to the report made by the World Health Organization (WHO), in 2005 two billion people were infected with *M. tuberculosis* and 5-10% of carriers will become sick in their lifetime (4).

The disease is still responsible for considerable human morbidity and mortality as well as economic losses by affecting young people at their productive ages, particularly in developing countries of the world. In general, if tuberculosis is left unchecked within 20 years, almost one billion people will become newly infected, 200 million will develop the disease and 35 million people will die of it (35). The problem could be worsened by prevailing HIV/AIDS, occurrence of multi-drug resistant (MDR) and extensively drug resistant (XDR) strains (36). Multidrug resistant TB and XDR-TB had been reported in a number of countries in the world and about 3.6% of new TB patients in the world were multi-drug resistant (MDR), where the levels are higher, about 20% in those previously treated TB patients. About 10% of MDR-TB cases are also resistant to the two most important second-lines anti TB drug classes, or called extensively drug-resistant TB (XDR-TB). In 2013, 92 countries reported at least one XDR-TB case (37).

Ethiopia is one of the highest TB burden countries in the world, and according to the current estimate, ranks 8th amongst the 22 global TB high-burden countries (5). The World Health Organization report in 2012 showed that there were about 1.6% of new MDR-TB cases and 12% of retreatment cases with MDR-TB in Ethiopia (38). In the country, it was also estimated that about 41% of TB cases were HIV positive. Drug sensitivity test survey conducted in the country between 2003 and 2006 showed that levels of MDR-TB were 1.6% and 11.8% in new cases and re-treatment cases of TB patients, respectively (39). These figures indicate how the disease is a threat in the country from different perspectives. The existence of the problem in the Amhara Region could be well described by the annual report made by the Regional Health Bureau in 2008. The report indicated 21.6% TB case detection and 3.5% TB death rates for the entire Region.

Tuberculosis is not only a deadly disease but also requires high cost for treatment, diagnosis, management, prevention, control as well as elimination efforts. According to reports, 80% of the victims are aged between 15 and 49 and are in the most economically productive years of their lives. TB carries a direct cost to the health service (diagnosis, treatment and control), patients and their family (drugs, transportation) as well as an indirect cost to society, the family and the community. The estimated costs to poor households are 12 billion USD per year. A systematic review conducted on costs to health services and the patient of treating TB showed that from the provider perspective, mean drug sensitive TB treatment costs per patient were US\$14,659 in high-income countries, US\$840 in upper middle-income countries, US\$273 in lower middle-income, and US\$258 in low-income countries while the respective costs for treating MDR-TB were US\$83,365, US\$5284, US\$6313 and US\$1218(40).

For instance, over the next 10 years TB will take 1-3 trillion USD the world's poorest countries, in particular developing nations where 94 and 98% of TB cases and deaths occur, respectively. TB and poverty show a vicious cycle (bad news for the 2.7 billion people who live on 2 USD per day or less); whereby TB exacerbates poverty, which in turn increases the likelihood of contracting TB. The World Bank estimated that loss of productivity attributed to TB is 4-7% of some countries gross domestic product (GDP) (41). Others, such as monitoring and evaluations of TB programs, capacity buildings for human resources and institutions as well as establishing new diagnostic laboratories are additional costs which can inflate the amount of money invested for TB. For instance, in 2007, Ethiopia requested a support from the Foundation for Innovative New Diagnosis (FIND) for the establishment of the *Mycobacteria* Growth Indicator Tube (MGIT) liquid culture system, the Capilia rapid identification system and the GenoType MTBDRplus line probe assay-based molecular system for rapid detection of MDR-TB. In collaboration with the then EHNRI (Now named as Ethiopian Public Health Institute), CDC-Ethiopia, the International Laboratory Branch (ILB) at GAP-CDC in Atlanta and other partners (Johns Hopkins University), FIND developed a comprehensive work plan to strengthen TB laboratory services at two central (EHNRI and St. Peter's Hospital) and four regional level laboratories. Besides, FIND posted one full-time international consultant for giving trainings and supplied those laboratories with MGIT instruments and reagents, GenoType MTBDR plus tests and instruments, and Capilia rapid identification tests (42). This huge estimate of money lost

would have been used for other developmental issues, particularly for countries like Ethiopia, if TB was controlled or prevented at the lowest minimum level.

The transmission of *M. tuberculosis* from humans to humans is well known and still researches are continuing. However, the transmission of *M. tuberculosis* from humans to animals, or vice-versa is not well studied. Isolation and characterization the causative agents of TB from animals and human patients could show the transmission pattern of the etiology between human and animals. Conditions like sharing common living rooms may facilitate the inhalation of infectious droplets by animals, while human patients sneeze or cough or drop/spit out their discharges and sputum on the floor and feed of animals. In most of the rural places in the region, there is a tradition, which is practiced to urinate on the grasses where animals are grazing or hays ready to feed. If a person is infected with tuberculosis, there is a chance to excrete the bacteria through urine and there will be a probability of picking the agent by animals (43). In this respect, those cattle contracting the infection may serve as a host for *M. tuberculosis* and used as a source of infection.

In Ethiopia, little is known about the transmission of *Mycobacterium tuberculosis* complex from cattle to humans. The reason why TB still remains challenging might be due to the possibility of *Mycobacterium tuberculosis* to adapt different host ranges like *Mycobacterium bovis*, which is closely related to it. The combination of the enormous burden of TB as well as the inconsistent availability of cost-effective interventions, makes TB one of the highest priorities for action in international health (6). Thus, this study aims to answer the possible role of cattle on the occurrence of human pulmonary tuberculosis.

1.3. Rationale of the Study

Ethiopian economy is currently led by agriculture. Most of its population lives in rural areas, which is estimated to be about more than 83% (44). The life of the rural community is dependent on agriculture and the contribution of livestock to their livelihood is so enormous, particularly for pastoralists and agropastoralists whose lives are mainly supported by livestock. Livestock is also used as a source of meat and milk as well as foreign currency earning. Therefore, the connection between animals, particularly cattle and farmers, pastoralists and agropastoralists is so close. Globally there are a number of neglected zoonoses and their effect could be immense in most of the developing world. In our country there is intimacy of cattle with humans is so vivid in the country (even to the level of common house sharing), this creates a very conducive environment for the transmission of diseases, particularly zoonotic ones, from animals to humans or vice-versa (*reverse-zoonoses*).

Despite the isolation of the agent from cattle, the role of animals in the maintenance and transmission of *M. tuberculosis* has not been studied in depth in Ethiopia in general and the Amhara Region in particular. Therefore, establishing the possibility of infection of *M. tuberculosis* from animals to humans and *vice versa*, as well as understanding the possible mode of transmission can help the prevention, control and eventual elimination of *M. tuberculosis*, which is maintained in cattle can result in the development of new infections or re-infection in the household and hence the finding of the same lineages from both populations will contribute a lot to the local community, the region, the nation as well as the world community. The finding also enables to bring attitudinal changes by the livestock owners, health workers as well as researchers on the issue and hence its contribution in the farming community, academic as well as research institutions will be so great. The research experience and the findings obtained here will be extrapolated and used to other areas in a wider scale to see the importance of cattle as a source of *M. tuberculosis* to human beings. Overall, the research targets on improving human health, supporting the efforts made to prevent and control TB in the country and playing a great role in the process of building a productive community. Thus, the study aims to assess the role of cattle on the transmission of pulmonary TB in humans in attempting to answer the following research questions.

1. Do *Mycobacterium tuberculosis complex species* detected from chronic coughers and their cattle react to bovine tuberculosis skin test?
2. Do cattle owned by pulmonary tuberculosis patients react differently to bovine TB skin test compared to those owned by non-tuberculosis patients?
3. Does bovine tuberculosis serve as a risk factor for the occurrence of human TB?
4. Are isolates from bovine skin test positive cattle the same as isolates of *M .tuberculosis complex* from humans?

1.4. LITERATURE REVIEW

1.4.1. The biology of *Mycobacterium tuberculosis*

1.4.1.1. *Mycobacterium tuberculosis* complex species

Tuberculosis, is caused by a genus called *Mycobacterium*, which belongs to the genera *Mycobacterium tuberculosis complex* that includes species such as *M. tuberculosis*, *M. africanum*, *M. canetti* and *M. bovis*, *M. microti* and *M. pinnipedii* (45, 46). *Mycobacterium tuberculosis* was thought to have been derived from *Mycobacterium bovis* (22), but more recent evidence suggests that both have a common ancestor (47).

The genus *Mycobacteria* are aerobic, non-spore forming, non-motile, slightly curved or straight rods (0.2 to 0.6 μm by 1.0 to 10 μm) or pleomorphic bacilli or coccobacilli which may branch and are acid fast. The distinguishing features of pathogenic *Mycobacteria* are the formation of characteristic cords (48). *Mycobacteria* are more resistant to disinfectants than vegetative bacteria (49, 50), and are easily inactivated by heat (> 65 °C for at least 30 min) and by ultra-violet (UV) light, but not by freezing or desiccation (51). *Mycobacteria* are able to survive from weeks to months on inanimate objects, if protected from sunlight (51), where direct sun light kills the bacilli in 5 minutes, but they can survive in the dark for long periods and therefore, indoor transmission could occur at a higher degree.

The genus, *Mycobacterium* comprises of some pathogenic to man and animals, some opportunistically pathogenic, while others essentially saprophytic living in water and soil (52, 53). The classic species of *Mycobacterium* that cause disease in man and animals include: *M. bovis*, *M. tuberculosis*, *M. paratuberculosis*, *M. avium*, *M. leprae* and *M. lepraemurium*. Tuberculosis in mammals is caused by *M. tuberculosis* complex (*M. bovis*, *M. tuberculosis*, *M. microti*, *M. africanum*) and by *M. avium* in birds. *Mycobacterium species* other than the *M. tuberculosis* complex that cause TB like diseases in man and animals are commonly called 'atypical *Mycobacteria*' (45). Atypical *Mycobacteria* are not pathogenic to man and animals, except in certain situations such as direct inoculation into wounds or introduction into immunocompromised hosts due to immunosuppression like in the case of HIV infections (46); however, they are very important during diagnosis as they sensitize man/animals to tuberculin test (54).

Mycobacterium species grow on media like Löwenstein-Jensen (LJ) and Stonebrink's medium. Tubercle bacilli are aerobes, but growth of *M. tuberculosis* and *M. bovis* can be enhanced at 5-10% CO₂ (carbon dioxide) and the optimal growth temperature is 37°C except for *M. avium*, which needs a temperature of 40-42°C (45).

Members of the *Mycobacterium tuberculosis* complex (MTBC) are slow growing and are causative agents of human and animal TB. Species in this complex include: *M. tuberculosis*, (the major cause of human tuberculosis), *M. bovis*, (the major cause of animal tuberculosis), *M. bovis* BCG, *M. africanum*, *M. canetti*, *M. caprae*, *M. microti* and *M. pinnipedii*. In older systems, *Mycobacteria* were grouped based upon their appearance and rate of growth, however, more recently classification is based upon common ancestors systems (28).

1.4.1.2. *Mycobacterium tuberculosis* lineages

In “the fourth international spoligotyping database” (27), the population genetics of 39,295 strains from 122 countries were identified; some main TB families/lineages were described. Those identified in eight regions of the world are described as follows: (I) the Central-Asia (CAS) family was essentially localized in the middle-East and Central Asia (21.2%), and preferentially in India (75%), (II) Haarlem (H) family was distributed 25% of the isolates globally while it dominated in South America by constituting around 50% of the isolates there, (III) the Latin-American-Mediterranean (LAM) constituted 50% of the other half of the strains from South America, (IV) the “T” family is less well defined and includes more than 600 unclassified strains and this lineage is currently stratified into 5 sub-clades (T1-T5), (V) the East-African-Indian (EAI) (also called CAS) family was more abundant in south-east Asia, particular in Vietnam and Thai Land (32%), (VI) the X family was highly prevalent in North America (21.5%) and Central American (11.9%) regions, (VII) the Beijing and the Beijing related strains represented about 50% of the strains in Far East- Asia and 13% of isolates globally. In some areas of the world the same lineages of *M. tuberculosis* were isolated from human TB patients and animals owned by them. In Ethiopia despite close intimacy between cattle and humans, the possibility of cross infection with this agent is not done in detail.

1.4.1.3. General Characteristics of *Mycobacterium tuberculosis*

Mycobacterium tuberculosis (MTB), also known as *tubercle bacillus* or *Koch's bacillus* is a pathogenic bacilli species in the genus *Mycobacterium* affecting mainly human beings. Primarily it is a pathogen of the mammalian respiratory system and in most of the cases it infects the lungs and causing pulmonary tuberculosis (55). The bacterium, *M. tuberculosis* divides every 15–20 hours, which is extremely slow compared to other bacteria, which tend to have division times measured in minutes, example *Escherichia coli* (*E. coli*) can divide roughly every 20 minutes) (56-58). It is a small bacillus that can withstand weak disinfectants and can survive in a dry state for weeks. Its cell wall, which is rich in lipids (e.g., mycolic acid), is likely responsible for this resistance and is a key virulence factor (58). The ability to construct *M. tuberculosis* mutants and test individual gene products for specific functions has significantly advanced the understanding of the pathogenesis and virulence factors of *M. tuberculosis*. Many secreted and exported proteins are known to be important in its pathogenesis (59).

Mycobacterium tuberculosis is a fairly large nonmotile rod-shaped, measuring 2-4 micrometers in length and 0.2-0.5 micrometers in width. *Mycobacterium tuberculosis* is an obligate aerobe and for this reason, in the classic case of tuberculosis, it is always found in the well-aerated upper lobes of the lungs. The bacterium is a facultative intracellular parasite, usually of macrophages. It takes 4-6 weeks to get visual colonies on media (48). *M. tuberculosis* can survive for months on dry inanimate surfaces (60). *M. tuberculosis* can survive in cockroach feces for 8 weeks, sputum on carpet (19 days) and wood (over 88 days), moist and dry soil (4 weeks), and in the environment for more than (74 days) if protected from light (possibly longer if in feces) (61-64).

1.4.1.4. Source of infection

The most common source of infection for TB is the human case whose sputum is positive for the tubercle bacilli (active TB patient). Moreover, those who are totally not treated or treated inadequately can also be used as a source of infection. People who have just started on TB treatment and who have a poor response to treatment can also be used as a source of infection and milk containing *Mycobacterium bovis* could be a source of infection to humans (65-68).

Besides, under WHO (69) recommendations individuals who suffered from a chronic cough for a duration of two and more than two weeks are considered to be presumptive TB cases and hence they are advised to be examined for tuberculosis. Because of the contagious nature of TB and its potential for devastating morbidity and mortality for individual patients and society, TB should be considered early in the evaluation of patients with chronic cough. In areas where TB prevalence is high or in populations which are at high risk of TB (e.g., HIV-seropositive persons), the tuberculosis diagnosis should be considered in all patients with chronic cough, sputum production, hemoptysis, fever, or weight loss. Some high-risk persons may have TB even with normal physical examination and chest radiograph findings, especially when they are immunocompromised (70). A patient with lung TB, usually sputum positive can disseminate while coughing, talking, singing, spitting and sneezing by releasing tiny infectious droplet nuclei and the nuclei can spread into the air and can then remain suspended in the air for long periods depending on the condition (65, 71).

1.4.1.5. *M. tuberculosis* in animals

Although transmission of *M. tuberculosis* between animals and humans has not been clearly reported, the bacterium *M. tuberculosis* has also been isolated from animal species (e.g., birds, elephants, and other mammals) and some are described hereunder.

Elephant

Michalak and his colleagues (72) reported short, fat, relatively scant number of acid-fast bacilli presumptively diagnosed as *M. tuberculosis* from Elephant lung caseous necrosis and mediastinal lymph node lesions. Of the 12 elephant handlers tested, 6 (50%) were PPD-positive. Three sputum samples were smear-negative for acid-fast bacilli, although one yielded *M. tuberculosis* upon culture. The sputum isolate from the handler with active TB was compared with the isolates from the Elephant. The isolates had identical IS6110 RFLP pattern, differing by ≤ 2 bands. Additionally, all isolates had the identical TBN12 RFLP pattern, except the isolate from the elephant that died from the disease, which demonstrated a shift of one band. Similarly, in the review made by Shimshony (73), there were 34 confirmed cases of TB in elephants in the United States between 1994 and 2005. Among the total 34 cases 31 of them were in Asian elephants and 3 in African elephants. In 33 of the cases, the etiologic agent was found to be *M. tuberculosis*.

Cattle

Among domestic animals, infection with *M. tuberculosis* has been most frequently identified in cattle (17, 74-76). According to published data, the prevalence of *M. tuberculosis* infection in cattle herds did not exceed 1% in the majority of studies (76-79). However, a few exceptions, like Algeria and Sudan with 6.2 and 7.4% prevalence, respectively, were reported (80, 81).

In a case report, in Slovenia 78 cattle were subjected to bovine TB skin test using Bovine and Avian PPD (Bioveta) and 16 of them showed positive reaction. Among the total 16 PPD positive cattle, 3 showed strongest reactivity to tuberculin test were slaughtered for further bacteriological and then molecular tests. The mandibular, mediastinal and portal lymph nodes were collected and sent for bacteriological examination. No acid-fast bacilli were detected on Ziehl-Neelsen-stained impression smears. However, after 28-days incubation of LJ media inoculated with the mixed specimen of mediastinal and portal lymph nodes, a few colonies appeared, which were acid fast positive on Ziehl-Neelsen staining. The bacteria were positive for Niacin, nitrate reduction and pyrazinamide reactivity and negative for thiopen-2-carboxylic acid hydrazide susceptibility test and it was aerobic.

Additionally, both the AccuProbe *Mycobacterium tuberculosis* Complex Assay (GenProbe, San Diego, Calif.) and an in-house PCR, targeting the 123-bp portion of IS6110 (17), identified the isolate as a member of *M. tuberculosis* complex. A banding pattern is seen, which is specific for *M. tuberculosis*. In the meantime *M. tuberculosis* was isolated from sputum of one of the cattle farm workers. Both isolates were analyzed by restriction fragment length polymorphism (RFLP) typing using IS6110-based, following the standardized protocol (17). The *M. tuberculosis* isolates from the cow and farm worker, together with a number of epidemiologically unrelated strains, were analyzed by IS6110-based. The *M. tuberculosis* isolates from the cow and farm worker showed an identical RFLP pattern. A 100% homology of the two isolates was also confirmed by computer-assisted analysis of RFLP patterns and this might suggest possibility of reverse zoonoses (17). Even though Zebu breeds are resistant to TB infections compared to exotic and their crosses *M. tuberculosis* has also been isolated in Ethiopia from TB lesions of cattle (82).

Although *M. tuberculosis* in cattle most frequently produces a quickly vanishing infection rather than a progressive disease, the infected animals do react positively when challenged with tuberculin despite having short tuberculin sensitization duration (83, 84). Thus, when a tuberculin-positive animal is recognized during a routine tuberculin testing for the first time in the previously tuberculin-negative herd, and particularly when the tuberculin-positive animal is young, the possibility of human TB infection among farm workers or animal attendants should be considered (85). As reported by several authors (79, 85-87), animal attendants with active pulmonary TB represent an important source of *M. tuberculosis* for animals, spreading the mycobacterium via sputum, urine, or feces (88) and as a result of such spreading, the classical form of TB may occasionally develop in animals living in close contact with humans with active TB (84).

A study of TB infection conducted in cattle in three districts of Western Gojjam, Ethiopia, indicated a three-fold higher prevalence in bovines owned by tuberculosis patients than those owned by non-tuberculosis patients. *M. tuberculosis* (15.4%), *Mycobacterium bovis* (44.1%) and atypical *Mycobacteria* (38.5%) were identified from milk of tuberculin-positive cows using biochemical tests in the study. Similarly, *M. tuberculosis* (74.5%), *M. bovis* 4.9%) and atypical *Mycobacteria* (8.5%) were identified from sputum and fine needle aspiration specimens of tuberculosis patient cattle owners. Mutual transmission of mycobacterium from animals to humans and vice versa has been signified (18).

Sheep and goats

Sheep and goats are quite resistant to *Mycobacterium* infections, however, a study conducted in pastoral community of Ethiopia showed that *Mycobacterium tuberculosis* (SIT-149) was isolated from goats tuberculous lesions (89).

Dogs

Bonoyska and colleagues (90), conducted experimental studies to assess the susceptibility of dogs to *M. tuberculosis* infection in Bulgaria (Sofia). The study showed that in most cases, the disease runs subclinically with lesions localized mainly in the lungs, lymph nodes, small intestine, liver, kidney and spleen. Histologically, granulomatous inflammation, caseation and predominant

epitheloide macrophages with single lymphocytes were demonstrated. According to that study, 83 (86.5%) of PCR results coincided with bacteriological findings, 82 (85.4%) with the pathological and 71 (74.0%) with bacteriological and pathological results. From the results obtained, it is possible to say that *M. tuberculosis* infected dogs can also serve as source of infection to humans and other animals.

Green-Winged Macaw (*Ara chloroptera*)

Infection with *M. tuberculosis* in birds is rare, however, the Psittacine birds, parot family are known to be infected with *M. tb* mainly due to close contact with tuberculous owners. Previously there were four reports of Amazon parrot infections with *M. tb* (91-94). Besides a report in Neyork City revealed isolation of *Mycobacterium tuberculosis* from the eyelid, skin, tongue, and lungs of a green-winged macaw (*Ara chloroptera*) bird (95). The authors confirmed that two persons living in the same household were culture positive for pulmonary tuberculosis 3 to 4 years before tuberculosis was diagnosed in the bird. They noted that an infected bird may be a sentinel for human infection (95). All of the parrots presented with cutaneous growths on the head and neck and had localized granulomas in the eye and retrobulbar tissues, the sinuses and nares, and the oral cavity. Two parrots were found to have systemic infections at necropsy (91, 93).

1.4.1.6. Bovine tuberculosis (BTB)

BTB is a disease of mainly cattle caused by *Mycobacterium bovis*. The causative agent belongs to the *Mycobacterium tuberculosis* complex (MTBC) (96-98), which can infect most mammalian species (99). The MTBC is a group of very closely related pathogens, which can cause tuberculosis disease with similar pathology in a variety of mammalian hosts (96). BTB is generally a respiratory disease and is chronic, which is difficult to diagnose clinically in cattle, although emaciation, loss of appetite, chronic cough and other signs of pneumonia could be symptoms developing at relatively late stages of the infection (20).

BTB can have a direct effect on milk and meat production as well as animal reproduction and indirect effect like international trades (100). BTB can also infect wildlife, for instance, in Kruger National park in South Africa, 38% of the buffaloes were infected with *M.bovis* strains,

which originally introduced from domesticated cattle (101), and this cycle could hamper disease control/eradication schemes (102, 103). Animals like lions and cheetah are spillover hosts for the disease causing agent (101). BTB also has a zoonotic potential and it is a great public health concern (104, 105), and zoonotic transmission of *M. bovis* may occur in countries where the disease remains enzootic. In San Diego (California), USA, *M.bovis* infection accounted for 45% of the TB cases in children of Hispanic origin where BTB is prevalent (105).

It is reported that persons infected with *M. bovis* were 2.6 times more likely to die during treatment than those with *M. tuberculosis* infections (105). The poor, especially in developing countries, are thought to be at highest risk to contract zoonotic tuberculosis (106). The disease is more important in countries like Africa, particularly the arid and semi-arid regions, where >50% of African cattle, sheep and goats are raised (107) and millions livelihood depends on livestock farming (106). Consumption of contaminated milk represents the most important route of infection (19, 20). BTB is also endemic to Ethiopia, though the prevalence varies from place to place. The disease causing agent also isolated from different animal species, including human TB patients. The disease severity in cattle could vary according to breed, age, sex, management systems and body conditions (108, 109).

1.4.1.7. Modes of transmissions

Tuberculosis, which is less contagious compared to others, is spread through water/air droplets which are expelled when TB diseased individuals cough, sneeze, speak, spit, or sing and among these coughing generates the largest number of droplets of all sizes. To transmit infection, the particles must be fresh enough to carry a viable organism. The frequency and vigor of cough and the ventilation of the environment influence transmission of the infection (110). As the exhaled particles remain airborne, they become dehydrated and decrease in size until they reach to 1-5 μ , named as droplet nuclei (contain about 3 bacilli) (48). The tubercle bacilli can enter the body through the airway that is nasal passages and lungs; the droplet nuclei can avoid the defense system of the upper airway and bronchi, and finally reach the alveoli where infection can occur (48). As a rule, close, frequent, or prolonged contact is needed to spread the disease and persons with prolonged, frequent or intense contact are at higher risk of acquiring infection having a chance of 22% (111). Of course, if a severely infected patient emits huge numbers of bacilli, the

chance of transmitting the infectious agent is much greater. Pertaining to proximity, only about one in three close contacts and fewer than 15% of more remote contacts are likely to become infected with TB (112).

The probability of transmission depends upon: infectiousness of the person with TB (quantity expelled), environment of exposure, duration of exposure, and virulence of the organism and the chain of transmission can be stopped by isolating patients with active disease and starting effective anti-tuberculous therapy (113). Drug-resistant TB is transmitted in the same way as drug-susceptible TB. Primary resistance develops in persons initially infected with resistant organisms. Secondary resistance (acquired resistance) develops during TB therapy due to inadequate treatment regimen, not taking the prescribed regimen appropriately or using low quality medication (113). The fetus of an infected mother may contract TB by swallowing the bacilli in the amniotic fluid during pregnancy if the mother is a TB patient (112). Tuberculosis transmission is unlikely to occur from individuals with extra-pulmonary TB because infectious particles are usually not aerosolized (113).

1.4.1.8. Airborne transmission

The risk of infection is exogenous that the agent is transmissible through air. For this type of transmission, the agent must remain buoyant in the air. The velocity of a droplet falling to the ground depends on the surface and diameter. For instance, in moisture saturated air droplets would fall to the ground from a height of 2meters in less than 10 seconds. The liquid droplets tend to evaporate and thereby diminishing their size. The duration of time droplets remain in unsaturated air is proportional to its size where very small droplets evaporate immediately and large droplets settle rapidly and reach the ground without evaporation. The droplet nuclei are about 1 to 5 microns in diameter. If inhaled, droplets less than 0.1micrometer are more likely to reach the alveoli and then produce infection. Contrary to this, droplets higher than 5 micrometers will not reach the alveoli and hence will not produce infection. Besides the distance, air circulation and ventilation also determine the probability of airborne transmission and susceptible host to acquire infection. The volume into which the bacilli are expelled determines the probability that a susceptible individual becomes infected. Ventilation dilutes the

concentration of infectious droplet nuclei. Since surgical masks do not filter particles less than 5mm and do not seal the mouth as well as the nose they are low efficient in the prevention (68).

The risk of developing tuberculosis after getting infection is largely endogenous, which determined by the integrity of the cellular immune system (most likely elders). The importance of any risk factor in public health is determined by both the strength of the association and the prevalence of the risk factor in the population. One cough can generate 3000 droplet nuclei. Talking for 5 minutes can generate 3000 droplet nuclei and singing can generate 3000 droplet nuclei in one minute. Sneezing generates the most droplet nuclei by far (tens of thousands, about 40,000 droplets), which can spread to individuals up to 10 feet away. Each one of these droplets may transmit the disease since the infectious dose is very low for this bacterium that is inhaling less than 10 bacteria may cause an infection (48). The probability that TB will be transmitted depends on three factors: the infectiousness of the person with TB, the environment in which exposure occurred, and the duration of exposure (113).

1.4.1.9. Tuberculosis

The reaction to *M. tuberculosis* infection is not similar in all humans and animals. Instead, they display a whole spectrum of symptoms. The extreme ends of this spectrum are referred to as the "polar manifestations" of mycobacterioses. At one extreme, some infected people display only a cell mediated immunity (CMI) reaction to the infecting *Mycobacterium*. This end of the spectrum is the contained form of mycobacterioses. At the other end of the spectrum, infected people display only a humoral reaction and do not display a CMI reaction and this end of the spectrum is the aggressive form of mycobacterioses (114). Most people infected with *Mycobacteria* do not display either of the extreme forms mentioned above, but display symptoms that are somewhere in between the two extremes. The position in the spectrum is determined by the strength of their CMI reaction to the infecting *Mycobacteria*.

Some *Mycobacteria* are capable of interfering with the immune system of the infected host, thus causing a spectrum of reactions in different hosts. For example, they may produce a toxin that interferes with the action of certain T-cells, thus suppressing a correct T-cell reaction to their presence (114). Some *Mycobacteria*, can evade (the exact evading mechanism is unknown) the defenses of the immune system by hiding inside macrophages that normally ingest and destroy

bacteria, although they are not always successful at destroying them. When macrophages fail to destroy the *Mycobacteria*, the human immune systems form granulomas around the infected macrophages where layers of two different types of T cells surround the infected macrophage, sealing it inside a barrier called granuloma from which it cannot escape. Since the infecting *Mycobacteria* is "contained" inside the granuloma, this form of *Mycobacterial* disease is known as the contained form, which is typified by low numbers of infecting *Mycobacteria* and high levels of inflammation (114).

1.4.1.10. The burden of tuberculosis

In 2012, an estimated 8.6 million people developed TB and 1.3 million died from the disease (including 320 000 deaths among HIV-positive people). The rate of new TB cases has been falling worldwide for about a decade, achieving the Millennium Development Goal to reverse the spread of TB by 2015. Globally, TB mortality rate has fallen by 45% since 1990. Although most TB cases and deaths occur among men, the burden of disease is high among women. In 2012, an estimated 410, 000 women died from TB (250, 000 among HIV-negative women and 160, 000 among HIV-positive women). There were also an estimated 74,000 TB deaths among HIV-negative children (115). Tuberculosis is a global pandemic with higher rates in Africa (a quarter of all TB cases), while half of all new cases are in Asia (Bangladesh, China, India, Indonesia, Pakistan and The Philippines) and almost 9 million new TB cases occurred in 2004 in the globe (116). However, due to rapid increase in Africa, global TB incidence is still growing at 1% per year while intense control efforts help to decline in other regions (117). Africa alone is estimated to have approximately 170 million TB patients and various sources estimated that between 2000 and 2020 nearly one billion people will be newly infected with TB, 200 million will become sick and 35 million will die (118).

The World Health Organization (WHO) estimated that among the TB infected people, 5 to 10 percent will become sick in their lifetime. Of the 8.7 million annual TB cases in 2011, about 0.5 million occurred in children (under 15 years of age) (38). Of 90-95% persons infected with *M. tuberculosis*, the immunological defense either kills the inhaled or ingested bacilli or keeps them suppressed (silent focus) causing latent Tuberculosis infection. If untreated, TB leads to deaths

within 5 years in at least half of the patients, about 20 to 25% would have natural healing and 25 to 30% would remain chronically ill, thus continuing to spread the disease in the community (119).

The disease is responsible for millions of deaths annually with a global case fatality of 27%, although it has reached up to 81% in African countries; with a lowest fatality rate in America, at 6% (120, 121) and it accounts for 2.5 % of the global burden of disease and is the commonest cause of death in young women, killing more women than all causes of maternal mortality combined (122). It is the second cause of death worldwide after HIV/AIDS (122, 123), and therefore unless intensive efforts are made, it is estimated that between 2002 and 2020, millions of people will be newly infected, over 150 million people will get sick, and 36 million will die of TB - if control is not further strengthened (124). Tuberculosis hinders economic development because 75% of the people with TB are within the economically productive age groups of 15-54years (125).

Over 95% of TB deaths occur in low and middle-income countries, and it is among the top three causes of death for women aged 15 to 44. In 2012, an estimated 530 000 children became ill with TB and 74 000 HIV-negative children died of TB (5). From a TB patient perspective the mean total costs for TB treatment ranged from 55 to 8198 USD dollars, with an un-weighted average of USD 847. On average, 20% (with maximum of 62%) of the total cost was due to direct medical costs, 20% (with maximum of 84%) to direct non-medical costs, and 60% (16–94%) to income loss (126).

Each person with active TB infects on average 10-15 people every year (124). Globally, six million people die every year due to HIV, tuberculosis and malaria, of those, nearly one-third of the deaths are due to TB. TB accounts for about 25% of all avoidable adult deaths in developing countries (127). According to WHO 2008 estimate, the largest number of new TB cases occurred in the South-East Asia Region, which accounted for 34% of incident cases to the globe. However, the estimated incidence rate in sub-Saharan Africa is nearly twice that of the South-East Asia Region with over 350 cases per 100 000 population. The number of new cases arising each year is still increasing globally in the WHO regions of Africa, the Eastern Mediterranean and South-East Asia (128).

As literatures indicate, infection with HIV increases the incidence of TB. Since HIV weakens the immune system, someone who is HIV positive and infected with TB bacilli is many times more likely to become sick with TB than someone who is infected with TB bacilli, but not HIV positive. TB and HIV form a lethal combination, each speeding the other's progress. People living with HIV and infected with TB are 21 to 34 times more likely to develop active tuberculosis than people without HIV (129). Therefore, the increasing global burden of TB is linked to human immunodeficiency virus (HIV) infections (130) since as many HIV-infected individuals are co-infected with TB, that could contribute to the rise in the incidence and mortality (131, 132). According to the WHO Global TB report, in 2011, the proportion of TB cases co-infected with HIV was highest in the African Region where the Region accounted for 79% of TB cases among people living with HIV (38, 119). In the same year (2011), an estimated 990,000 deaths (14/100,000 populations) occurred among HIV negative cases of TB including 0.30 million deaths among women.

In Africa, the dual epidemics of TB and HIV is imperative, where HIV has been the most important contributing factor in increasing the incidence of TB over the last 10 years. In regions, like sub-Saharan Africa, up to 80% of individuals with active TB disease are also HIV-positive. The dual epidemics are also of growing concern in Asia, where two-thirds of TB-infected people live and where TB now accounts for 40 percent of AIDS deaths. The overlap of TB-HIV co-infection with MDR-TB and extensively drug-resistant TB present a tremendous challenge and threatens progress in controlling TB and HIV/AIDS and in eliminating the mortality associated with these diseases. Persons who are infected with TB and HIV are 30 times more likely to develop active TB disease; similarly infection with TB enhances replication of HIV and may accelerate the progression of HIV infection to AIDS (133).

In Ethiopia, a national population based TB prevalence survey conducted in 2010/11 revealed that the prevalence of smear positive TB among adults and all age groups was found to be 108 and 63 per 100,000 populations, respectively. The study also indicated the prevalence of bacteriologically confirmed TB to be 156/100,000 populations and by extrapolations of the result, the prevalence of all forms of TB in Ethiopia is estimated to be 240/100,000 populations (119). According to the WHO global TB report 2012, there were an estimated 220,000 (258 per 100,000 populations) incident cases and 200,000 (237 per 100,000 populations) prevalence cases

of TB, with an estimated 15,000 deaths (18/100,000 populations due to TB, excluding HIV related deaths in Ethiopia in 2011(38). Based on 2011 health and health related indicators survey of FMoH, TB was the third leading cause of death in Ethiopia (119). In the country during the year 2010/11 there were a total of 159,017 TB cases and among those, 151,866 (95.5%) were all forms of new cases of TB. The proportion of new smear-positive, smear negative and EPTB among all new cases was 32.7%, 34.8%, and 32.5% respectively. Re-treatment cases represented about 2.9% of all notified TB cases (119). By the year 2012, TB/HIV surveillance, 91.2% of HIV infected individuals which were newly enrolled in HIV care were screened for TB; of those 8.6% were found to have active TB. It was also reported that of 87% of TB cases undergone HIV test 19.7% tested HIV positive. Anti-TB drug resistance survey conducted in 2005 by EHNRI/FMoH among 804 newly diagnosed TB cases, 1.6% were found to be infected with multidrug resistant TB (MDR-TB). The rate of MDR-TB among specimens from 76 previously treated TB cases was 11.8% (119). According to the WHO 2012 report, there were an estimated 1700 and 550 MDR-TB cases among notified new and re-treatment pulmonary TB cases in 2011, respectively in Ethiopia (38).

1.4.1.11. Public health impacts of Tuberculosis

The public health impacts of human tuberculosis are immense. The existence of latent TB where one-third of the world population infected with TB (134), the disease direct morbidity and mortality, the costs incurred to curbe the disease problem and subsequent managements (economical concern), loss of production as well as the emergence of drug resistant problems could be stated as major public health impacts of TB. Besides, susceptibility to TB in HIV-infected populations is another serious health problem throughout the world. As it is known, TB is in the top three diseases which is a highly contagious and life-threatening infection. Among the issues, Multidrug-resistant TB (MDR-TB), which poses a grave and growing threat to global public health, has been increasing in incidence in many areas during the past decade. These situations, particularly the global resurgence of TB and the rapid emergence of MDR-TB, underscore the importance of the development of new antituberculous drugs. However, there are a number of constraints (the research is expensive, slow and difficult, and requires specialized facilities for handling MTB) that have deterred companies from investing of new anti-TB drugs. There are few animal models that closely mimic the human TB disease and hence the

development time of any anti-TB drug will be long (135). Once a drug resistant strain has developed, it can be transmitted directly to other individuals just like drug susceptible TB and its spread is undermining efforts to control the global TB epidemic (136). WHO estimates that 450,000 people developed MDR-TB in 2012, leading to 170,000 deaths; but less than one in four cases were identified and treated appropriately. An estimated one in ten cases of MDR-TB have XDR-TB, which had been reported in 92 countries by the end of 2012 (137).

As of 2013, 3.7% of new tuberculosis cases have MDR-TB, where levels are much higher in those previously treated for tuberculosis reaching about 20%. About 9% of MDR-TB cases also had resistance to two other classes of drugs, or extensively drug-resistant TB (XDR-TB). By March 2013, 84 countries had reported at least one XDR-TB case. WHO estimated that there were about 0.5 million new MDR-TB cases in the world in 2011 and about 60% of these cases occurred in Brazil, China, India, the Russian Federation and South Africa alone (“BRICS” countries). Forty eight percent of patients with MDR-TB enrolled on treatment in 2009 were reported to have been successfully treated (138). The drug resistant issue now days also overburdened by the occurrence of totally drug resistant *Mycobacterial tuberculosis* strains (139).

Apart from susceptible tuberculosis, Ethiopia is also among the high MDR-TB burden countries (5), where in 2013, the estimated annual number of MDR-TB cases was 2,000-2,500. The prevalence of MDR-TB is increasing at an alarming rate from a baseline rate of 1.6% among new TB cases in 2005 to current level of 2.3% in 2014; similarly, the rate has increased from 11.8 to 17.8% among previously treated cases (140). Extensively drug resistant -TB burden in Ethiopia is not known, but two XDR TB cases were reported from research done in the National Referral Laboratory, Ethiopia(141). The available evidences also indicates clear geographic variations with the highest prevalence rates in major urban settings, for example Addis Ababa and largely pastoralist regions like Afar and Somali (142). Besides the high TB/HIV co-infection is also a major concern to the country.

1.4.1.12. Risks for human tuberculosis

Anyone can get TB, but risk factors could contribute for the disease occurrence. Factors like, age of the individual, presence of chronic diseases, weakened immune system by abnormalities like HIV/AIDS, diabetes, end stage kidney disease, certain cancers, anticancer drugs, malnutrition etc., traveling or living in certain areas (like Sub-Saharan Africa, India, China, Pakistan), poverty and substance abuse as well as occupations/living place would contribute to TB occurrence (143). The risk factors associated with the development of TB include human immunodeficiency virus infections, fibrotic lesions, silicosis (approximately 30 folds), carcinoma, hemophilia, immunosuppressive treatment/s, hemodialysis, underweight, diabetes (3 times higher), gastrectomy, bacilli infective dose, age (adolescent and > 60years) and renal failure (10-15 times higher).

Genetic factors like sex, body build (example, low body mass index), blood groups (higher in blood groups AB or B than O or A), and virgin populations can also be considered as a risk factors. Besides, environmental factors smoking (20 cigarettes per day will increase the risk by 2-4 times), alcohol abuse, nutrition (malnutrition, vegetarian and vitamin D deficiency) play a significant role for the development of tuberculosis. Furthermore, though there is no solid evidence concerning the role of pregnancy, there is an indication that the post-partum period might double the risk of progression to TB (144-147). Levels of education as well as hygienic conditions also serve as a risk factor for the occurrence of tuberculosis (148, 149) .

For the development of *Mycobacterial* infections, the agent infective dose, strain virulence (cell wall and protein), drug resistance and infection with *M. bovis* as well as HIV/AIDS are among reported risk factors (150). Tubercle bacilli could present in sputum, gastric lavage fluids, cerebrospinal fluid, urine, and in a variety of tissues. The tubercle bacilli may survive in heat-fixed smears and may be aerosolized in the preparation of frozen sections and during manipulation of liquid cultures. Because of the low infective dose of *M. tuberculosis* (i.e., ID₅₀ <10 bacilli), sputa and other clinical specimen from presumptive cases or known cases of tuberculosis must be considered potentially infectious and handled with appropriate precautions. Accidental needle-sticks are also a recognized hazard (151). The principal means of transmission occurs by infective particles. Active TB patients will usually cough, as a result of typical chronic pulmonary inflammation, which constitutes the main dissemination mechanism for the pathogen

to new hosts (152). Infection usually occurs from person to person through the inhalation of the infective particles (153). Experiments with animal models demonstrated that particles in suspension containing 1 to 10 bacilli are enough to cause infection. The main determinants of risk of infection are the concentration of bacilli in an exhaled particle from a source, its aerodynamic features, the ventilation rate, and the exposure period (150, 154).

For centuries, TB has been linked anecdotally with environmental risk factors that go hand-in-hand with poverty: indoor air pollution, tobacco smoke, malnutrition, overcrowded living conditions, and excessive alcohol use. Now scientists are presenting convincing evidence to back these associations, leading some TB experts to argue that control programs must confront underlying risk factors to limit the spread of the disease. Confronting that risk factors could offer broad population-level benefits (155). The environment determines the path that TB droplet nuclei take after they have become aerosolized. This determines whether or not a susceptible individual can inhale these particles and possibly become infected with TB. Characteristics of the environment include the size of the space and the ventilation, where, the smaller the space and the poorer the ventilation, the higher the risk (156).

1.4.1.13. Diagnosis of tuberculosis

The diagnosis of TB is made on the basis of laboratory test results, which is the standard test for tuberculosis. Tuberculin skin test (which detects the presence of infection, not of active TB) by using pure protein derivative (PPD) antigen prepared from cultures of *M. tuberculosis* is also used as tool (157). Because the symptoms of TB cover a wide range of severity and affected body parts, diagnosis on the basis of symptoms is not always possible. Often, the first indication of TB is an abnormal chest x-ray or other test results rather than physical discomfort. To verify the test results of chest x-ray, a sample of sputum or a tissue sample (biopsy) or body fluids like spinal fluid or urine or liver as well as bone marrow can be taken for culture. Three to five sputum samples should be taken early in the morning. If necessary, sputum for culture can be produced by spraying a salt solution into the windpipe(157).

One important new advance in the diagnosis of TB is the use of molecular techniques to speed the diagnostic process as well as improve its accuracy. As of late 2005, four molecular

techniques are increasingly used in laboratories around the world. They include polymerase chain reaction to detect *Mycobacterial* DNA in patient specimens; nucleic acid probes to identify *Mycobacteria* in culture; restriction fragment length polymorphism analysis to compare different strains of TB for epidemiological studies; and genetic-based susceptibility testing to identify drug resistant strains of *Mycobacteria* (157). Advances in molecular biology and molecular epidemiology enhance better understanding of the molecular basis of drug resistance in TB.

Non-conventional diagnostic approaches, including the search for biochemical markers and detection of immunological response facilitates for early detection of *M. tuberculosis* and these methods have provided new tools for rapid and effective diagnosis of TB. However, the high cost of most of the techniques, and the need for sophisticated equipment and skilled personnel have precluded their implementation on a routine basis, especially in developing countries (158-160). In general clinical symptoms, chest radiography, culturing on LJ or other media following decontamination and liquefaction procedures, smear based identifications (AFB) which is still considered as the major epidemiological tool in the diagnosis of tuberculosis, although less important biochemical tests, drug susceptibility test (DST), PPD skin test and Post-mortem examinations (PME particularly in cattle) and molecular techniques that used to study the distribution and determinants of disease occurrence in human populations are employed to diagnose TB or could be used as epidemiological tools (161-165).

The World Health Organization (WHO) aims to detect at least 70% of infectious TB cases and cure 85% of detected cases (166) to control TB, however, the lack of resources in TB high burden countries (121), smear microscopy constitutes a simple, inexpensive and fast recommended passive case detection method. According to WHO, two sputum specimens are collected from presumptive pulmonary TB cases, who are persons with persistent cough for at least 2 weeks (167) and examined for Acid Fast Bacilli (AFB) through Ziehl–Neelsen staining. This screening increases the probability of ascertaining a TB case and gives maximal detection of infectious cases (168).

Case detection is primarily passive by direct smear examination of persons who present to the health service with productive cough for more than 2 weeks. Health seeking behavior of those individuals with chronic cough for 2 weeks and more is not well practiced in the community and

studies regarding population or community based presumptive tuberculosis cases is scarce in the study *Zone*. Acute or chronic infections of the respiratory systems may lead to acute or chronic cough. Both latent and active TB could be often undiagnosed in many health institutes. Cough is among the cardinal signs of TB (169). Since tuberculosis is contagious, individuals with chronic cough should be investigated. In particular, chronic cough was found to be both highly sensitive and to have a reasonably high positive predictive value for smear-positive TB at the primary health-care level in studies in the pre-HIV era leading up to the development of the DOTS strategy (170-172). Cough was defined as acute if less than 2 weeks' duration and as chronic otherwise (173).

Upon progression, TB would lead to the formation of cavitation that could contain sputum with about 1 million TB bacilli per ml. Besides the destruction of healthy lung tissues they eventually reach to the airway and if the TB bacilli destroy the wall of the airway, there will be a connection to the outside, thus transported up into the trachea and coughed up. People with pulmonary TB cough a lot (seen in about 85% of people with pulmonary TB), initially; people with pulmonary TB have a dry, persistent cough, often worse at night and is accompanied by fever and excessive sweating. As the destruction of lung tissue worsens, the sputum could be stained with blood (174).

In a study done at six health care facilities in San Juan de Lurigancho district, Lima, Peru, the smear positivity was 7.3% (321/4376). Of the 4376 adults submitting sputa, 55.3% (2418) reported cough for <14 days. In this group, smear microscopy yielded 3.2% (78/2418) positive results vs. 12.4% (243/1958) in patients coughing for 14 or more days. Having cough for >2 weeks, being referred by health care staff, was among independent determinant of smear positivity (175). The sputum of an infectious patient requires some 5,000 bacilli in 1ml of sputum to yield a positive smear and 10,000 to identify a smear as positive with a 95% probability. It should be important to note that patients with a positive smear are by far more infectious than those having a negative one. Despite the presence of droplet nuclei in the air, probability of becoming infected varies depending on the distance between the source and the recipient. Although sputum smear positive patients are the major source of infection for the community smear negative, patients are also responsible for 15-20% of transmissions (68).

1.5. Conceptual Framework

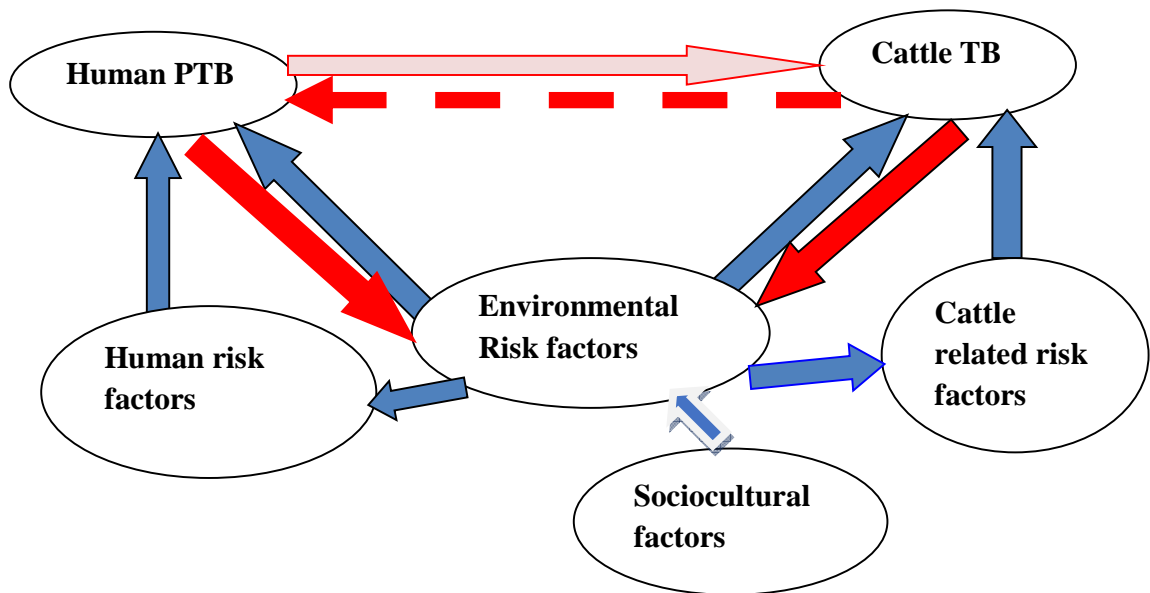


Figure 1: Conceptual Framework of the Study

Description of the conceptual model: TB patients/presumptive TB cases live in the same house with their cattle. Patients discharge the causative organism to the environment with possible contamination of cattle feed and water. The sociocultural characteristics will also play a role in the transmission dynamics. In fact, there will be aerosol transmission of the agent to cattle or susceptible house members and ingestion by cattle. When cattle are infected, they will discharge the agent and either humans or animals will pick the infectious organism. Humans will get either new infection or re-infection from cattle and humans again will release the causative agent and cattle become infected with the agent. By doing so, a sort of vicious circle will be created and the disease will reveal itself for a long period of time in one house. This framework clearly shows the possible epidemiological role of cattle in the occurrence of human pulmonary TB (PTB) by harboring the causative agent, *M. tuberculosis*.

2. OBJECTIVES

2.1. General Objective

Epidemiological study on the role of cattle in the occurrence of pulmonary tuberculosis in humans in rural communities of Amhara Region, Ethiopia.

2.2. Specific Objectives

1. To determine tuberculosis infections in cattle and cattle owners in north western parts of rural Ethiopia.
2. To compare BTB status among cattle owned by TB positive and TB negative households in north western and north eastern parts of rural Ethiopia.
3. To assess Bovine Tuberculosis (BTB) as a risk factor for developing pulmonary tuberculosis in humans in north western and north eastern parts of rural Ethiopia.
4. To Identify and Characterize *Mycobacterium tuberculosis* Isolated from Cattle Owners in north western and north eastern parts of rural Ethiopia.

2. MATERIALS AND METHODS

2.1. Study Area

The Amhara National Regional State (ANRS) is found in the North-western part of Ethiopia. Geographically the Region is located between 9°20' and 14°20' latitude North and 30°20' and 40°20' longitude east. The climate of the area is characterized by long rainy season (June to September) and short rainy season (February to March) and extended dry season (October to February). The average annual rainfall is 750 mm, while the average monthly ambient temperature ranges from 18⁰c to 40⁰c. The case detection and TB death rate for the entire Region was 21.6% and 3.5%, respectively (176). In the Region livestock and livestock products account for some 80% of mean farm cash income (including the value of subsistence consumption). The farming community of the area follows traditional system of livestock production. The main sources of variability in output lie in livestock density rather than individual animal productivity

(177). With other risk factors, increased livestock density in the household will increase diseases transmission, particularly zoonotic ones. This in fact leads to congestion during housing that favors disease transmission, particularly of zoonotic diseases like TB. According to the Ministry of Agriculture (177), the average number of cattle per household is around three.

The study was particularly conducted in *Meket, Gidan, Gubalafto/Woldeya, Habru* and *RayaKobo Districts* of North Wollo Zone, and *Dembia, Chilga, Dabat, Debark, Adarkay* and *Wegera Districts* of North Gondar Zone, Amhara National Regional State (ANRS), Northwest and Northeast parts of Ethiopia, where the livelihood mainly is mixed farming (**Figure-2**). Based on the 2007 Census (178), the study place covered an estimated area of 58,117.13 square kilometers with a total population of 4,429,931, of whom majorities are rural dwellers accounting for about 90% and 85% in North Wollo and North Gondar Zones, respectively (178). The study areas in North Wollo Zone included, *Meket, Habru, Gidan, Gubalafto, Weldeya* and *Raya-Kobo Districts*, where the Ethiopia lineage (Lineage-7) *M. tuberculosis* has been reported were selected purposefully. In addition *Dembia, Chilga* and *Debark Districts* in North Gondar Zone were included for this study.

These study sites were selected purposefully due to reasons described below. The Armauer Hansen Research Institute has been conducting research on bovine tuberculosis in these areas, including in *Weldeya* and *Gondar* Hospitals. Those hospitals were used to collect fine needle aspirates from extra-pulmonary TB patients whereas abattoirs were used to collect tuberculous lesions from cattle and the practice supported the current study. In addition to this, in *Weldeya* area the presence of Lineage-7 has recently been reported by AHRI and this gives an extra interest to include the area in this study. Furthermore, *Gondar* Hospital is under University of *Gondar* which is the host institute of the PhD candidate and hence using the available resources for the research is much easier. Hospitals and health centers with better facilities which practiced TB diagnosis in the two selected *Zones* of the Amhara National Regional State were used for this study. *Gondar* University and *Weldeya* Referral Hospitals were used as main sites to collect acid fast bacilli positive sputum samples from health institutes at their respective *Zones*. Besides in North Wollo Zone a community based household study was conducted to assess tuberculosis infections in humans and their cattle.

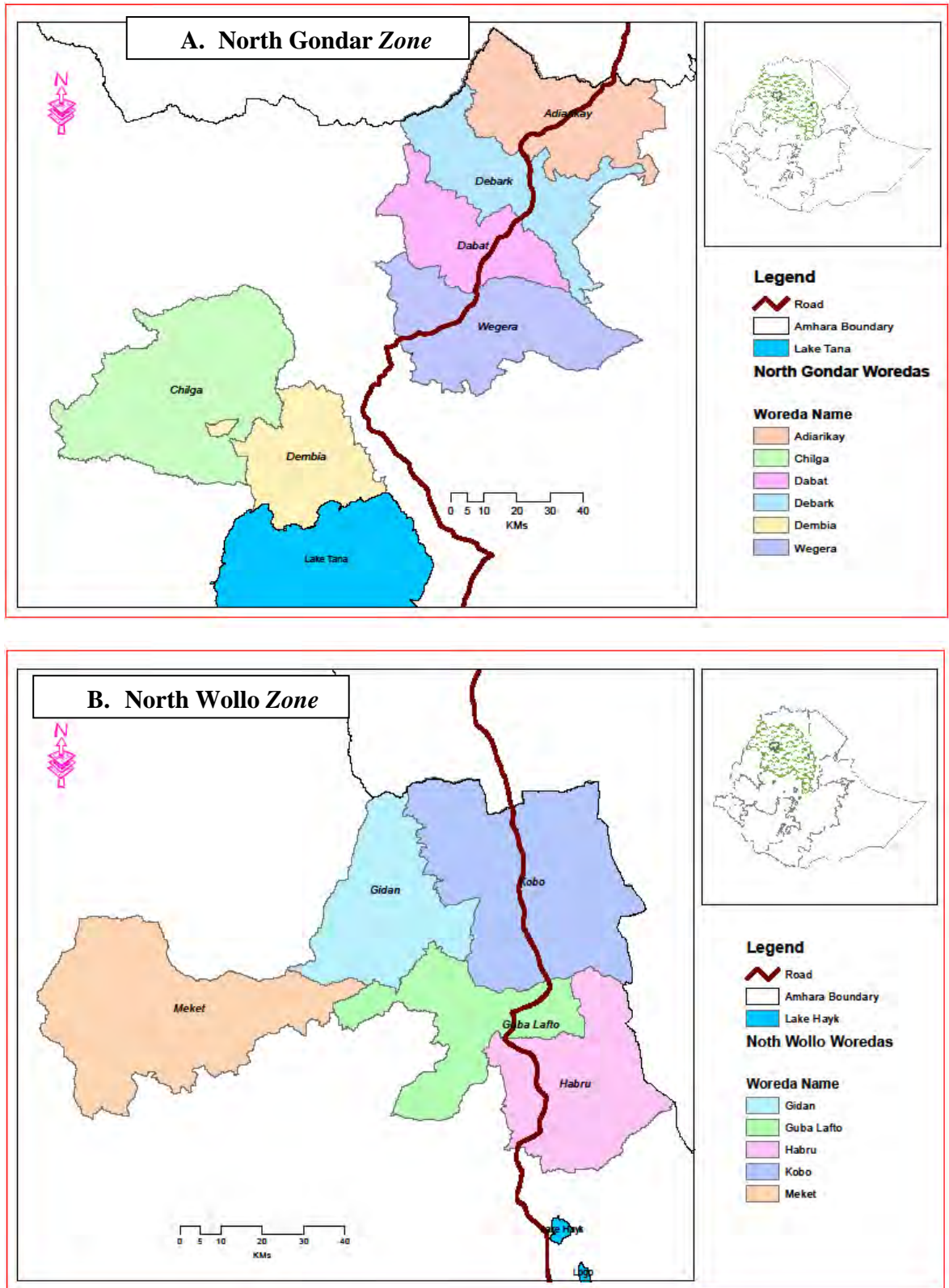


Figure 2: Map of the study sites

2.2. Study Design and Duration

The study included three epidemiological designs; namely, a cross-sectional survey (CSS), a comparative cross-sectional (CCS) and a case- control type (CC), which were undertaken 5-24 December, 2012 (CSS), September to November, 2013 (CCS and CC), and August 2012 to December 2014 (laboratory works).

2.3. Source and Study Population

Individuals who owned cattle and came to hospitals for diagnosis of tuberculosis and were willing to participate constitute the source population. Besides, willing individuals in the rural community who owned cattle were also used as a source population particularly by allowing tuberculin skin test to be conducted on their cattle as a comparative or control groups. Cattle owned by both individuals also served as source population.

Individuals with chronic cough who are presumptive pulmonary TB cases, acid fast bacilli confirmed pulmonary TB patients and their cattle were used as the study population. Households without TB who owned cattle were used as controls in both comparative cross sectional and case control studies.

2.4. Exclusion criteria

The following were excluded from the study.

- ☞ Individuals having unproductive cough and those with less than 2weeks duration of cough,
- ☞ Households who have no cattle,
- ☞ Patients under TB treatment and children aged below 15 years,
- ☞ Individuals with sputum not produced within 15 minutes,
- ☞ Individuals having a history of tuberculosis as well as a history of cough for more than two weeks,
- ☞ Cows delivered few days before skin test and cattle younger than 6 months old as well as cattle added to the herd with less than 6 months of durations,
- ☞ Cows at last trimester and sick or poor body conditioned cattle during the time of visit,
- ☞ Cattle under treatment and oxen used for plough during the study time,
- ☞ Aggressive cattle.

2.5. Sample Size and Sampling Methods

2.5.1. Sample size determination

For the cross sectional tuberculosis infection study, single population proportion sample size determination formula, which is indicated below (179) was used. For this study since the prevalence of lineage 7 in cattle was not known, 50% prevalence, 5% precision level and 95% confidence interval was taken as an assumption and therefore, 314 bovines were required to be tested. *Gidan, Gubalafto, Rayakobo and Meket Districts* were selected purposefully and the sample size was distributed proportionally to each District. Thus, 39, 79, 79 and 117 cattle were planned to be tested in *Gidan (Eyela Kebele and Tikur Wuha Kebeles), Gubalafto (Woynye, Goshu Ber, Ahuntegegn and Sibilkay Kebeles), RayaKobo (Addis-alem and Robit area Kebeles) and Mekete (033 and 044 Kebeles) Districts*, respectively. Assuming on average of 3 cattle are owned by a single household in Amhara Region, approximately 105 households were required to be included in the study. For comparison purpose the unmatched case-control with double population proportion sample size determination formula was employed using EpiInfo (180) epidemiological software. With the assumption of 15% (18) bovine TB among non-TB-households and 41.4% among TB positive-households and a confidence interval of 95% with a power of 80%, a ratio of cases to comparison group of 1:3 and adding 10% non-response rate the required sample size was 35 households with TB cases and 105 households without TB. All culture positive isolates were used for molecular typing.

$$n = \frac{(Z_{\alpha/2})^2 \times \text{expected prevalence (pe)} (1-\text{expected prevalence})}{d^2}$$

Where n = number of sample size

d^2 = absolute precision (5%)

Pe = expected prevalence of *M. tuberculosis* in cattle (50%)

$Z_{\alpha/2}$ = is 1.96 (the z-score at 95% Confidence interval)

2.5.2. Sampling methods

Previous experience of bovine tuberculosis work, isolation of 4 *Mycobacterium tuberculosis* from 19 cattle tissue samples in *Woldeya*, availability of trained veterinary personnel regarding identifications of bovine TB lesions, and the PI's (principal investigator) awareness of the study areas as well as the detection of lineage 7 in North *Wollo Zone* were some of the reasons that guided the selection of the sites purposefully. Individuals suffering from chronic cough with duration of two or more weeks were considered as presumptive tuberculosis cases (181, 182). Aiming to have *Mycobacterium species* isolate, chronic coughers were identified in selected *Districts* of North *Wollo Zone* by health extension workers. The *Kebeles* were selected based on the report of having a high TB occurrence or being suspected to have TB by the local authorized personnel. Pulmonary TB confirmed cases were recruited from health institutes, namely; *Hara*, *Rayakobo*, *Meket*, *Aykel* and *Dembia* health center as well as *Woldeyia* and *Debark* hospitals. Control or comparative households were selected during the field work.

2.6. Survey Instruments

2.6.1. Instruments for sputum sample collection, culturing and skin testing

Materials used for sputum sample collection were, universal bottles, ice box, ice packs, refrigerator/freezer and gloves. Ropes, automatic digital caliper, tuberculin syringes, cotton, blades, gloves, purified protein derivatives, ice box, ice packs, alcohol, adrenalin, albendazole and battery/cell were used for cattle intradermal skin testing. Sputum, LJ media, phosphate buffer saline solution, tubes, racks, pen/marker, logo, microscope, biosafety cabinet, vortex machine, centrifuge, sodium hydroxide, hydrochloric acid, phenol red, M-Torck papers, plastic bags, aluminum foil, loop, slides, acid fast staining reagents and materials and incubator were the materials used for culturing.

2.6.2. Instruments for the PCR

Mycobacteria positive control strains: *M. tuberculosis* H37RV and *M. bovis* AF2122/97. PCR Thermocycler, laminar flow cabinet, cleaning agent DNA away/equivalent, pipettes, filter tips, nuclease free water (Qiagen), HotStar Tagmaster Mix kit (Qiagen; Product No. 203445, which includes DNA polymerase, buffer, MgCl₂, and dNTPS), stock solutions of oligonucleotide primers, namely: 100µM RD4-FlankFW (5'-CTC GTC GAA GGC CAC TAA AG-3'), 100µM RD4-FlankRev (5'-AAG GCG AAC AGA TTC AGC AT-3'), 100µM RD4-internalFW (5'-ACA CGC TGG CGA AGT ATA GC-3'), 100µM RD9-FlankF (5'-AACACGGTCACGTTGTCGTG-3'), 100µM RD9-FlankRev (5'-CAAACCAGCAGCTGTCGTTG-3'), 100µM RD9-InternalRev (5'-TTGCTTCCCCGGTTCGTCTG-3'), 100µM RD10- FlankFW (5'-TTG CTT CCC CGG TTC GTC TG-3'), 100µM RD10- FlankRev (5'-GAA GCC CTA CAT CGC CAA G-3'), 100µM RD10- InternalRev (5'-GAA GTC GTA ACT CAC CGG GA-3') and heat killed cells that need to be detected. Besides, Agarose gel electrophoresis equipment's: DNA ladder and loading dye, agarose gel, 1XTAE running buffer and ethidium bromide were also used (183).

2.6.3. Instruments for Spoligotyping

Spoligomembrane of good quality (can be used many times) and all reagents needed for PCR amplifications. Equipment's like water baths, glass miniblottedter, measuring cylinders, scales, magnetic stirrers, pH meter, thermocyclers, autorad and hybridization oven were used. Among the consumables intended to be used were Stock solutions, Buffer solutions (10% SDS, Sodium Dodecyl Sulphate), 0.5M EDTA 2X SSPE (Saline sodium Phosphate-EDTA) as well as other chemicals/salts needed for hybridization and detection also utilized. In addition positive and negative controls and samples to be typed were used (183).

2.7. Data Collection Methods

2.7.1. Questionnaire

A questionnaire was used to identify the possible factors that could be associated with the infection and transmission of BTB, zoonotic risk, in TB households. Most of the questions in the questionnaire were closed and organized to collect data on socio-demographic, livestock related, human knowledge of zoonoses diseases as well as tuberculosis, consumption habits and human-livestock interface. The questionnaire was pretested before the actual study. Moreover forms were used to record relevant information pertaining to activities related to the research work. Skin test and different laboratory techniques were also used to collect results regarding bovine tuberculosis and the disease causative agents.

2.7.2. Intradermal skin test on Cattle

Comparative cervical intradermal tuberculin test was conducted as per the manufacturer's recommendation (184). The operation was performed on either side of the animal middle neck region. Two circular areas of about 2.5cm diameter, about 12–15 cm apart, on the cervical area of the skin, were shaved using a scalpel blade. The initial skin thickness was measured with a digital caliper (185). Measurements were read to the nearest millimeter. For the study, Lelystad (Netherlands) products and AVITUBAL-25,000inj., *Mycobacterii avium* (strain D 4 ER, 25,000IU/ml) and BOVITUBAL (*Mycobacterium bovis*, strain AN-5 25,000 IU/ml) PPD antigens were used (186). From the products 0.1 ml (2000IU/ml) of bovine tuberculin PPD at one site and 0.1ml (2000IU/ml) of avian tuberculin PPD at the other site were injected into the dermis using BD1/2 tuberculin syringe (187). Correct injection was confirmed by palpating a small pea-like swelling at each site of injection. Skin thickness was measured again at both injection sites after 72 hrs \pm 4hrs. The reaction at each site was derived by calculating the difference of the two tests. The results were interpreted according to the manufacturer recommendations (184) using the cutoff point for positivity of the test, that is >4mms difference, calculated as the difference between skin thicknesses of bovine tuberculin (B) and avian tuberculin (A) injection sites (B – A). But, Ameni *et al.*, (188) reported >2mm difference as

positive in Ethiopia and his cut off value also used considered for this study to calculate the difference.

Based on the manufacturer's recommendation and appraisal by Ameni *et al.*, (188) the skin test results were interpreted as positive, if the reaction number to bovine exceeds the reaction to avian tuberculin by more than 2 mm and 4mm; or clinical symptoms such as diffuse or extensive edema, exudation, necrosis soreness or inflammatory reaction of relevant lymphatic vessels or lymphatic nodes were discovered in the application place of bovine tuberculin. Results were considered dubious if the reaction measurement for bovine TB is from 1-2 mm and 1-4mm greater than the reaction to avian tuberculin or no clinical symptoms were discovered. If the reaction number is the same or lower than for avian tuberculin with no clinical symptoms were observed in either site; the result was considered as negative.

2.7.3. Cattle body condition scoring

For the purpose of this study, the method mentioned by Nicholson and Butterworth, (189) with modifications was used to determine the body condition of the zebu cattle. All cattle older than six months of age in herds owned by the selected households were subjected to skin test. Most of the cattle included in the study were local zebu breeds and some crosses kept under extensive management systems. For each tested cattle, sex, breed, age, source and body condition score (BCS) were collected and recorded at the time of the test. The body condition of each of the study cattle was scored using guidelines established by Nicholson and Butterworth, mainly based on observations of anatomical parts such as the vertebral column, ribs, and spines and were scored as lean (score 1 to 2), medium (score 3) or fat (score 4 to 5) (189).

2.7.4. Sputum sample collection and Mycobacteria isolation

M. tuberculosis isolation sputum samples were collected from individuals having chronic cough, according to Cheesbrough (190). Presumptive TB cases were asked to produce an "on spot" sputum specimen in a 20 ml screw plastic container under the supervision of a trained laboratory technician in the open air. Those cases who produce sputum were asked to give additional sputum samples on the second day and then on the spot. The sputum samples obtained from each patient was put in an icebox with ice packs (4°C), and immediately after collection, it was

transferred to the hospital laboratory to be stored at -20°C. The samples collected from each site transported to Armauer Hansen Research Institute (AHRI) TB laboratory to be processed.

Briefly, an equal amount of Phosphate-buffered saline solution was added to the sputum and then decontaminated, digested with equal volume of 4% sodium hydroxide for 15 minutes. Then vortexing was done in a closed tube for 1-3 (5) minutes till the mixture becomes homogeneous. The homogenate was centrifuged at 3000 rpm for 15 minutes. Neutralization done by using concentrated hydrochloric acid. In order to monitor neutralization 1 or 2 drops of phenol red was added. The supernatant was decanted and the sediment was inoculated onto Lowenstein-Jensen medium (tubes containing glycerol and sodium pyruvate) using the drop method. The tubes were incubated at 37°C and examined for growth weekly for 12 weeks (191, 192). Cultures were considered negative if there was no *Mycobacteria* growth after 12 weeks of incubation. Growth of *Mycobacteria* was confirmed by detection of a typical colonial morphology and by microscopy for AFB after Ziehl-Neelsen staining. Positive cultures were sub-cultured onto another set of media and were incubated for another 3–4 weeks for further identification. Polymerase chain reaction using RD4 and RD9 as a marker was conducted. Armauer Hansen Research Institute (AHRI) *M. tuberculosis* (H37Rv) and *M. bovis* (AHRI No.1632) isolates were used as a positive control.

2.7.5. DNA extraction

To run molecular techniques the genetic material of isolated mycobacterium should be extracted. To extract a loop full of colonies was taken from culture positive Lowenstein-Jensen media and was transferred to the 1.5ml tube containing 200 µl of 1% Tris-EDTA (TE) buffer. The re-suspended bacteria was heated in a dry bath at 90°C for 40 min and centrifuged at 10,000x g for 10 min. The supernatant was processed for target DNA isolation in the laboratory using the method described by Chakravorty and Tyagi (193).

2.7.6. PCR using direct repeat deletion technique

Differentiation of *Mycobacterium tuberculosis* complex species is made by PCR amplification of species -specific DNA fragments. There is little evidence for recombination among these species and therefore they are suggested to be strictly clonal (194). The evolutionary relationship among

the complex is based on specific “Region of Difference” (RD) genetic elements that are deleted along the phylogeny. The assumption of clonality among these species suggests that a lost RD can not to be restored; these RD regions are either present or deleted in a species of the complex, which helped to characterize unknown isolates. Deletion typing is a multiplex PCR using three different primers. A strain with the RD4 deleted (*M.bovis*) and RD9 present typed as *M. tuberculosis*. An isolate that has the RD9 deleted (*M.bovis* or *M. africanum*), but the RD10 present can be classified as *M. africanum*.

Polymerase Chain Reaction (PCR) was performed for all culture positive samples to identify the species of *M.tuberculosis* complex causing tuberculosis both in cattle and in human using both RD4, RD9 and RD10 regions as a marker. The amplification products were analyzed by electrophoresis on 1.5% (W/V) agarose gel containing 1x ethidium bromide to be visualized with Ultraviolet rays (195). If the PCR results confirm the presence of both RD4 and RD9 regions, then the disease causing organism will be either *M .tuberculosis* or *M. microttii* but not the rest of *M. tuberculosis* species. If RD9 is present: RD9-Ff and RD9Int will amplify a product size of 1.5Kbp; thus it is *M. tuberculosis*. In order to differentiate strains in MTBC Spoligotyping was performed. In General to run the PCR-deletion typing and spoligotyping procedure the standard operation procedure used at AHRI, which is adopted from Veterinary Laboratory agency (VLA), UK, was followed.

2.7.7. Spoligotyping

Spoligotyping (spacer oligonucleotide typing) was carried out using the protocols used for AHRI spoligotyping. Spoligotyping includes three main methods namely; PCR amplification of specific spacer sequence of the strain, hybridization to a spoligomembarne and detection. This method used to detect and type bacteria of the genus *Mycobacterium tuberculosis* complex (MTC). The genome of MTC carry a single region on the chromosome called the direct repeat locus and DNA polymorphism in this locus allow for strain typing. It comprises of multiple copies of a 36- base pair (bp) direct repeat (DR) that is reiterated usually between 10 and 50 times. Each repeat is separated from the next by a sequence of non-repetitive DNA, called a spacer. Each spacer sequence contains between 37-41 bp. In the MTC more than 100 different spacer sequences have been identified. Of these 43 (which are derived from DNA sequences of

the DR regions in the strains of *M. tuberculosis* H₃₇Rv and *M.bovis* BCG) have been selected for use in spoligotyping. The presence or absence of specific spacers allows for typing of MTC isolates. A copy of the insertion sequence IS6110 is commonly inserted into a specific site in one of the repeats in the DR locus. The 43 spacer nucleotides used in spoligotyping are synthesized and modified with a “5’-amino-modifier C6” and they can covalently bound to a biodyne C membrane (183).

The PCR was performed on heat killed cells and only small amount of DNA template was required. The PCR amplification of the spacers is accomplished by using the primers RDa (5’-GGT TTT GGG TCT GAC GAC-3’) and RDb (5’-CCG AGA GGG GAC GGA AAC-3’), which can anneal to all repeat sequences and thereby enables for amplification of all spacers that occur in the DR region of the specific strain. The PCR products will become labeled with biotin since the DRa primer is biotinylated. The PCR products amplified from each sample were loaded on to the membrane at right angle to the 43 parallel spacer lines by using a miniblottedter and left for hybridization. After hybridization the membrane was washed with buffer to remove the non-hybridized and non-specific bound PCR products. The step was followed by incubation with conjugated streptavidin-peroxidase, of which streptavidin binds to the biotin labeled PCR products (183).

The presence or absence of spacers in specific strains was then detected by an enhanced chemiluminescence (ECL) detection system onto an autorad (photographic film). When the membrane was exposed to ECL a substrate for peroxidase, the autorad will detect light signals where hybridization has occurred and thereby produces a pattern that allows for spoligotyping of an isolate. The details of the procedures could be traced from AHRI SOP. Acceptable results include clearly defined squares on a clear background. Ensure that the controls have worked, especially the negatives (water) control (183). Spoligo pattern was analyzed by comparing it with online spoligotype data base website, SPOLDB4.0 (<http://www.pasteur-guadeloupe.fr:8081/SITVITDemo>).

2.7.8. Single Nucleotide Polymorphisms (SNPs) typing

The lineages of the isolates were predicted by Spoligotyping, but final confirmation for Lineage 3 and Lineage 4 were done by SNPs typing using real-time PCR. We performed the TaqMan Real-time PCR (Rotor Gene) SNP assay for lineage 4 (Euro-American-African, EAA- lineage) and lineage 3. The lineage 4 was defined by katG 463 SNP and the assay was performed as described (196) the lineage 3 was defined by Rv3804c_0012s SNP and assay was performed using standard procedures (197).

2.7.9. Tissue samples

Six highly reactive animals for tuberculin skin test were bought and sacrificed. Specimens of suspected lesions obtained from the sacrificed animal/s used for *Mycobacterial* isolation and about 8-10g of suspected tissue were collected for culturing (198, 199). Identified tissue samples were put in a universal bottle containing phosphate buffer saline solution. The tissue samples obtained from each slaughtered animal were put in a cooler with ice packs (4°C) and immediately after inspection transferred to the hospital laboratory to be stored at -20°C or refrigerator. The samples collected from each site were put in a cooler with ice packs (4°C) to transport to Armauer Hansen Research Institute (AHRI) tuberculosis laboratory within two weeks post collection. The tissue samples transferred to deep freezer in AHRI and then processed and cultured in AHRI TB laboratory.

In short, tissues were minced in a tissue grinder by adding phosphate buffer saline solution and then decontaminated by adding 4% sodium hydroxide. Then vortexing was done in a closed tube for 1-3 (5) minutes till the mixture becomes homogeneous. The homogenate was centrifuged at 3000 rpm for 15 minutes. Neutralization was done by using concentrated (1%. 0.1N) hydrochloric acid. In order to monitor neutralization 1 or 2 drops of phenol red was added. The supernatant was decanted and the sediment was inoculated onto Lowenstein-Jensen medium (tubes containing glycerol and sodium pyruvate) using the drop method. The tubes were incubated at 37°C and examined for growth weekly for 8-12 weeks (200). Cultures were considered negative if there is no *Mycobacteria* growth after 12 weeks of incubation and growth of *Mycobacteria* was confirmed by detection of a typical colonial morphology and by

microscopy for acid fast bacilli (AFB) after Ziehl-Neelsen staining. Positive cultures were sub-cultured onto another set of media and incubated for another 3–4 weeks for further identification.

2.7.10. Milk samples

It is believed that some of tuberculin reactor cows will excrete tubercul bacilli in their milk. It is impossible to buy and sacrifice all reactor animals under the situation that we have in Ethiopia. The teats of skin test positive cow/s were washed and swabbed with 70% alcohol to minimize possible contaminations. Milkers were asked to wash their hands using detergents before milking their cows. After complete dry of the hands of the milker and the teats, the cow was milked and a total of 30ml of the last streak milk was collected from each cow using sterile 100ml universal bottles(201).

Seven milk samples obtained from test positive lactating cows was put in a cooler with ice packs (4°C) and immediately after collection transferred to the hospital laboratory to be stored at -20°C or refrigerator. The samples collected from each site, were put in a cooler with ice packs (4°C) to transport to AHRI tuberculosis laboratory to process and culture. The subsequent procedures are the same as tissue sample processing.

2.8. Data Management and Analysis

During the study period good data management practices such as study monitoring, timely data archiving and transferring, sorting and filing were done. The collected data were coded and entered into EpiInfo version 3.2.1(180) by double data entry. Data cleaning and analysis were done using SPSS software version 20.0 (202). Descriptive statistics like frequency distributions, percentages, means and standard deviations were used to describe the findings. Tables, charts and graphs were used to present the results. Logistic regression was employed to assess the risk factors. Significance was considered at a p-value <0.05. The manufacturer >4mms and Ameni *et al.* (188) >2mms cutoff values of the skin test measurements were used to determine the skin test results. All data were collected in collaboration with experts and checked regularly while collecting the data by the principal investigator. All tuberculin tests were conducted by the same

person and sputum sample collection and culturing were done by laboratory personnel. Analysis employed for each specific objective is presented in **Table-1**.

2.8.1. Variables

The variables for this research could be divided into two, namely dependent and independent variables.

Dependent variables

Human TB

Mycobacterium tuberculosis isolated from pulmonary TB confirmed and presumptive TB cases and skin test positivity (Bovine TB)

Independent variables

Cattle age, sex, breed, body condition and source (origin of cattle)

House sharing, common water source, disease knowledge, utensil share, house ventilation, feeding urine, spitting on animal feed.

2.9. Data Quality Control

Training was given to data collectors on how to collect data from owners through the prepared questionnaires and supervised while administering the questionnaire. To ensure the performance of the questionnaire, pre-test was conducted. All standard operations procedures were maintained in all types of activities, such as intradermal skin test, post-mortem examinations, sample collections as well as transportations of specimen and laboratory works. The validity of the laboratory results were checked by determination of growth by appreciating their morphology, AFB staining of the culture and looking acid fast bacilli and sub cultured for further identification. The data were entered by EPiINFO and double entry was applied. Data were checked many times for its accuracy before commencing data analysis.

2.10. Ethical Considerations

Ethical clearance was obtained from the College of Health Sciences, Addis Ababa University Institutional Review Board (IRB) and AHRI/ALERT (Armauer Hansen Research Institute) ethics committee. Permission was obtained from the Amhara Regional Health Bureau and from North *Gondar* and North *Wollo* as well as respective District health departments. In addition, permission was also obtained from Zonal department of agriculture and District agricultural offices in each *Zone*. The aim of the study was explained to all participants of the study and willingness was asked to participate in the study. If he/she was willing to participate, written consents were taken and sputum samples were collected and then their cattle were tested for bovine tuberculosis. The participants were told their right to withdraw from the study at any time. Confidentialities were maintained and to maintain confidentiality codes were used rather than participants' names. Based on the results, individuals who owned positive as well as doubtful cattle were advised and those chronic coughers whose sputum samples were cultured positives communicated through health institutes to receive appropriate anti-TB treatments and conduct family screening. Individuals still had cough and other TB related signs after treatment were consulted to go to health centers for possible checkups. All participants were given health education on safe food practices. All tested cattle received antiparasitic drugs as per the recommended dosage. Besides, all oxen were slaughtered in humane and their carcass buried. Possible slight swelling on the site of tuberculin injection could be seen as harm to the cattle. Summary of objectives, designs, study subjects and data analysis is presented in **Table-1**.

2.11. Summary of the Study

Table 1: Summary of objectives, designs, study subjects and data analysis

Objective	Design	Study subjects	Data analysis
To determine Tuberculosis infection in cattle and cattle owners in north western parts of rural Ethiopia.	Cross-sectional	124 TB presumptive PTB cases 381 cattle owned by them	Descriptive X ² test
To compare BTB status among cattle owned by TB positive and TB negative households in north western and north eastern parts of rural Ethiopia.	Cross-sectional comparative	481 cattle owned by 35 HH with TB and 105 HH without TB	Descriptive Logistic regression
To assess Bovine Tuberculosis (BTB) as a risk factor for developing pulmonary tuberculosis in humans in north western and north eastern parts of rural Ethiopia.	A Cross-sectional Case-control	-Bovine TB positive cattle -35 HH with TB and 105 HH without TB	Descriptive Logistic regression
To Identify and Characterize <i>Mycobacterium tuberculosis</i> Isolated from Cattle Owners in north western and north eastern parts of rural Ethiopia.	Laboratory based*	70 AFB positive sputum samples	Descriptive

*Laboratory based: Not a design, but we used it for the collected samples

3. DISSMINATION OF RESULTS

The results will be disseminated to users by means of arranging seminars and workshops. Leaflets could also be used to address a number of communities. Posters could be prepared to post at different places and during international and national meetings. Mass Medias could be attempted to disseminate in a wider scale and reach to most of the communities. Above all four publications will be done to address the scientific community and those interested institutes as well as individuals.

4. RESULTS

4.1. Tuberculosis Infection in Cattle and Cattle Owners in North Wollo Zone, Ethiopia

The study covered *Dega* (highland) 3 (30%), *Woina Dega* (midland) 6 (60%) and *Kolla* (lowland) 1 (10%) agroecological Zones. One hundred twenty four individuals were reported to have a chronic cough in the four districts of North Wollo Zone. Nearly three fourth of the participants were males. The age of participants ranged from 18-80, with a mean age of 50 (\pm 15), and nearly one third of the participants aged 40-50 years. About 60% of the individuals were having family size ranging from 1 to 5. About 92% of the participants reported to be followers of Orthodox Christianity. Of the participants, about 71.8% were illiterate/traditional (**Table-2**).

Table 2: Characteristics of presumptive TB cases in North Wollo Zone, Eastern Amhar region, Ethiopia, 2013.

Characteristics	Frequency (n=124)	%
EcoZones		
Dega	3	30
Woina Dega	6	60
Kolla	1	10
Sex		
Male	91	73.4
Female	33	26.6
Age		
<29	10	8.1
29-39	19	15.3
40-49	37	29.8
50-59	27	21.8
>60	31	25.0
Family size		
1-5	74	59.7
6-10	50	40.3
Religion		
Orthodox	114	91.9
Muslim	10	8.1
Education		
Illiterate /traditional	89	71.8
Primary	30	24.2
Secondary and above	5	4.0

As it is presented in **Table-3**, 17.7% (22/124) of the participants had tuberculosis history in their household. Of the individuals with TB history 86.4% (19/22) started treatment and all of them have completed their treatment. Nearly 60% of the participants were suffering from chronic cough for more than 2 months. Chronic cough is not seen as a serious problem in the study area and hence nearly 77% of them were not taking any action, and of those who took actions only one person was seeking modern treatment and the others seek traditional. Nearly three fourth (73.4%) and more than half (52%) of the participants were having the habit of taking unboiled milk and raw meat, respectively. Of the participants only 12% were having contact with tuberculosis diagnosed patients. Although there is no association, all culture positive individuals were having the habit of taking unboiled milk as well as raw meat. From the collected 124 presumptive TB cases sputum samples 4 *Mycobacterial species*, which accounted about 3.2%, were grown in LJ media. Polymerase chain reaction using deletion method revealed that 2/4 (50%) of the isolates were *M. tuberculosis*.

Table 3: TB history, feeding habits and sputum culture result of presumptive TB cases in North Wollo zone, Eastern Amhara region, Ethiopia, 2013.

Characteristics	Frequency (n=124)	%
Household TB history		
No	102	82.3
Yes	22	17.7
Cough duration		
2weeks-4weeks	37	29.8
>4weeks -8 weeks	13	10.5
>8 weeks	74	59.7
Action taken to combat cough		
No	95	76.6
Yes	29	23.4
Household contact with TB patient		
No	109	88.0
Yes	15	12.0
Drinking unboiled milk		
Yes	91	73.4
No	33	26.6
Eating raw meat		
Yes	64	51.6
No	60	48.4
Sputum culture result		
Negative	120	96.8
Positive	4	3.2

As indicated in **Table-4** below, all positive cultures (100%) were found in males and out of which 2 of them were living in the mid-altitude. Equal positive cultures were recorded in those who were illiterate and attending formal and informal education. Using the mean age 50 years as a cutoff point 3 of the 4 culture positives were in those who were above 50 years and 3 of 4 culture positives in those who had cough duration of more than 4 weeks. Three of the positives took traditional treatments to cure cough situation. All culture positive individuals reported not having a TB history in their house and none of them had contact history. Of the total participants who had their cattle tested, 73.4 and 51.6% had habit of taking unboiled milk and uncooked meat, respectively. Polymerase Chain Reaction (PCR) using RD9 as a marker indicated that 2 among 4 sputum culture positives were *M. tuberculosis*. The PCR indicated that two of them were atypical *Mycobacterial species*.

Table 4: Distribution of sputum culture positive results with some predictors in North *Wollo* zone, Eastern Amhara region, Ethiopia, 2013.

Category	Sputum culture result (n=124)	
	Total	Positive (%)
Sex		
Male	93	4 (3.2)
Female	31	0 (0)
EcoZone		
Highland	55	1 (0.8)
Midland	55	2 (1.6)
Lowland	14	1 (0.8)
Education		
Illiterate	86	2 (1.6)
Formal & informal education	38	2 (1.6)
Age (mean=50 ± 15)		
Below the mean	68	1 (0.8)
Above the mean	58	3 (2.4)
Cough duration		
2- 4 weeks	37	1 (0.8)
>4 weeks	87	3 (3.2)
TB history in the household		
No	102	4 (3.2)
Yes	22	0
Taking appropriate Rx		
No	105	4 (3.2)
Yes	19	0
Completing Rx course		
No	105	4 (3.2)
Yes	19	0
Habit of drinking raw milk		
No	33	0
Yes	91	4 (3.2)
Habit of taking raw meat		
No	60	0
Yes	64	4 (3.2)
Contact with TB patient		
No	109	4 (3.2)
Yes	15	0

In this work, 381 cattle owned by these households were tested intra-dermal by using APPD (Avian purified protein derivative) and BPPD (Bovine purified protein derivative). Characteristics of cattle's are presented in **Table-5**. Of the total cattle tested males contribute 192 (50.4%). The mean age of cattle was 6.0341 ± 3.5246 and 63.5% (almost two-third) were having medium body conditions.

Table 5: Cattle characteristics and their skin test results in selected *Kebeles* of North *Wollo* zone, Eastern Amhara region, using >2mm as a cutoff value, Ethiopia, 2013.

Category	Cattle tested (No)	CIDT result (n=381)			X ² /P-value
		+ve No (%)	-ve No (%)	Inclusive No (%)	
Breed					
Local	365	10 (2.63)	345 (90.6)	10 (2.63)	0.5
Cross	16	0	14 (3.7)	2 (0.5)	
Source					
Home bred	286	10 (2.63)	268 (70.3)	8 (2.1)	0.07
Purchase	95	0	91 (23.9)	4 (1.1)	
Sex					
Male	192	5 (1.3)	183 (48.0)	5 (1.3)	0.97
Female	189	5(1.3)	176 (46.2)	7(1.8)	
Body condition					
Lean	65	0	66 (17.3)	0	0.1
Medium	242	6 (1.5)	227 (59.6)	8 (2.1)	
Fat	74	4 (1.1)	66 (17.30)	4 (1.1)	
EcoZone					
Dega	173	4 (1.1)	166 (43.6)	3 (0.8)	0.005
Woina Dega	151	1 (0.3)	148 (38.8)	2 (0.5)	
Kolla	57	5(1.3)	45 (11.8)	7 (1.8)	

Based on manufacturer recommendation, 5 (1.31%) cattle were found positive, however, according to previous cut off proved by Ameni *et al.*, in 2008 10 (10/381) (2.63%) were positive for the test. From the tested cattle nearly two-third (63.5%) of the cattle and among the positive once 60% of them were having medium body conditions. Lean cattle did not show positive reaction. Highest positivity (with a reading >4mms) was recorded in Raya-Kobo District, *Addis alem Kebele*, where 5/57 (8. 8%) cattle revealed bovine TB. The total households in the *Addisalem Kebele* were 312, of which 14 households (4.5%) were reported as having chronic cough and of these reactor cattle were recorded in four households, which is about 28.8%. According to the manufacturer recommendation 12 (3.2%) inconclusive test results were obtained. In general, according to the cutoff point by Ameni *et al.*, 2008 among the ten positive cattle, females contribute 50% and 4 (40%), 1 (10%) and 5 (50%) cattle were positive for the test in *Dega*, *Woinadega* and *Kolla Kebeles*, respectively (**Table-5**).

About 3.2% of the cattle showed inconclusive results. Excluding inconclusive test result cattle, Fisher's exact test showed a significance difference ($P < 0.05$, $\chi^2 = 12.13$ with 2 df) in skin test reaction among different agro-climatic *Zones*. When inconclusive cattle considered as positive and negative, the differences were significant having a P-value of 0.0001 and 0.005, respectively.

According to manufacturer recommendation, from 381 tested cattle 5 of them showed positive results (BTB), in particular in *Rayakobo District, Addis-alem Kebele*, which is characterized by *Kola* (equivalent to low land) agroclimatic *Zone* and this, contributes about 1.31%. Based on this the prevalence of bovine tuberculosis in this study was 1.31% (95% CI: 0.002, 0.025). When the cut off skin fold measurements was taken as >2mm (188), the number of positive cattle recorded lifted to 10, which in turn raised the prevalence to 2.63 (95% CI: 0.01, 0.042).

4.2. Distribution of Bovine Tuberculosis in North Gondar and North Wollo Zones, Amhara region, Ethiopia

A total of 481 cattle were tested, with 76% from North *Gondar*, with male to female ratio of 1:1.2 and a mean age of 5.2 ± 3.3 years. Among them, 76% (365/481) cattle were home-bred and 87.1% (419/481) were local; 61.3% (295/481) had medium size, 24.1% (116/481) were lean and 14.6% (70/481) fat (**Table-6**).

Using >2mms as a cutoff value, 4.6% (22/481) and 4.2% (20/481) had skin test positive results in households with TB and without TB, respectively. With the same cutoff value, the individual cattle prevalence of bovine TB was 15.3% (95% CI: 9.4, 21.2%) and 5.9% (95% CI: 3.4, 8.5) among TB and non-TB households, respectively. In this study an overall individual apparent prevalence of 8.7% (95% CI: 6.2, 11.3) was recorded.

On the other hand, using >4mms cutoff value, the individual cattle prevalence of bovine TB was 5.6% (95% CI: 1.8, 9.3) and 0.6 % (95% CI: 0.2, 1.4) among TB and non-TB households, respectively, making the overall individual cattle prevalence of 2.1% (95% CI: 0.8, 3.4). About 30% (42/140) of the households/herds and 13.5% (65/481) of the individual cattle had doubtful bovine TB results based on >4mms cutoff value, of which 31.4% (11/35) were from TB households and 29.5% (31/105) without TB households.

Table 6: Characteristics of tested cattle in North *Gondar* and North *Wollo* zones, Amhara Region, Ethiopia, 2014.

Category	Households (n=481)	
	With tuberculosis (n=144)	Without tuberculosis (n=337)
	No (%)	No (%)
Study Area		
North <i>Gondar</i>	117 (81.2)	247 (73.3)
North <i>Wollo</i>	27 (18.8)	90 (26.7)
Sex		
Male	75 (52.1)	145(43.0)
Female	69 (47.9)	192 (57.0)
Mean age of cattle	5.6 ± 3.0	5.2 ± 3.0
Breed		
Local	126 (87.5)	293 (87.0)
Cross	18 (12.5)	44 (13.0)
Source		
Homebred	103 (71.5)	262 (77.7)
Purchase	41 (28.5)	75 (22.3)
Body condition		
Lean	28 (19.5)	88 (26.1)
Medium	85 (59.0)	210 (62.3)
Fat	31 (21.5)	39 (11.6)
Bovine tuberculosis		
>4mms		
Dubious	20 (13.9)	45 (13.4)
Negative	116 (80.5)	290 (86.0)
Positive	8 (5.6)	2 (0.6)
>2mms		
Dubious	6 (4.2)	27 (8.0)
Negative	116 (80.6)	290 (86.0)
Positive	22 (15.2)	20 (6.0)

As it is shown in **Table- 7**, households with TB amounted for 20% (7/35) (95% CI: 6.7, 33.7) and 48.6% (17/35) (95% CI: 32.0, 65.1) BTB, while those without TB revealed 2% (2/105) (95% CI: 0.7, 4.5) and 15.2% (16/105) (95% CI: 8.4, 22.1) BTB using >4mms and >2mms cutoff values, respectively. An overall household/herd apparent prevalence of 6.4% (9/140) (95% CI: 2.4, 10.5) and 23.6% (33/140) (95% CI: 16.5, 30.6) BTB were registered using >4mms and >2mms cutoff values, respectively.

Table 7: Skin test results at household/herd level in North *Gondar* and North *Wollo* zones, Amhara Region, Ethiopia, 2014.

		n=140	
Measurements	Test Result	Household with TB (n=35)	Household without TB (n=105)
		No (%)	No (%)
BTB >4mms	Negative	28 (80)	103(98.1)
	Positive	7 (20)	2 (1.9)
BTB >2mms	Negative	18 (51.4)	89 (84.8)
	Positive	17 (48.6)	16 (15.2)

As presented in **Table- 8**, based on >2mms as a cutoff value, adjusted for age, body condition, source, sex and breed of the individual animal the odds of bovine TB that cattle owned by TB cases was about 3 times (AOR=2.90, 95% CI: 1.50, 5.54) higher compared to those cattle owned by households without TB and the difference was highly significant (p= 0.002). In this study, sex, age, breed, source and body condition of the cattle were not associated with the occurrence of bovine TB.

Table 8: Bovine tuberculosis in North *Gondar* and North *Wollo* zones, based on >2mm as a cutoff value, Amhara region, Ethiopia, 2014.

Category	Bovine tuberculosis (n=481)		AOR	95% CI	
	Positive No (%)	Negative No (%)		Lower	Upper
Sex					
Male	18 (3.7)	202 (42.0)	1.0		
Female	24 (5.0)	237 (49.3)	1.35	0.67	2.70
Age [§] (mean) in years	5.6 ± 3.0	5.2 ± 3.0	0.99	0.89	1.10
Breed					
Local	34 (7.1)	385 (80.0)	1.0		
Cross	8 (1.7)	54 (11.2)	1.42	0.40	3.41
Source					
Homebred	28 (5.8)	337 (70.1)	1.0		
Purchase	14 (2.9)	102 (21.2)	1.603	0.76	3.34
Body condition					
Lean	6 (1.2)	110 (22.9)	1.0		
Medium	29 (6.0)	266 (55.3)	1.90	0.60	4.80
Fat	7 (1.5)	63 (13.1)	1.60	0.50	5.10
Household TB status					
Negative	20 (4.1)	317 (66.0)	1.0		
Positive	22 (4.5)	122 (25.4)	2.90	1.50	5.54

[§]: age was not categorized

4.3. Bovine Tuberculosis (BTB) as a risk factor for developing Tuberculosis in humans in the rural community of Ethiopia

As shown in **Table 9**, on average the age of participants was 42.13 ± 13.70 , ranging from 15 to 82 years old. Males accounted for 88% (120/140) and among the cases more than half were females 51.4% (18/35). Majority of the participants (80.7%) were married, (43% were married among TB cases compared to 93.3% to that of non –TB). Illiterate or traditionally educated individuals accounted for 66% and 97.1% of the study participants were Orthodox Christianity followers. Of the TB cases 17.1% were attended high school and above levels of education compared to 7.6% of the controls. About 23.6% (33/140) of individual herds were positive for bovine tuberculosis.

Table 9: Socio-demographic characteristics of study participants in North Gondar and North Wollo zones, Amhara Region, Ethiopia, 2014.

Category	Households (n=140)		
	With tuberculosis (N=35)	Without tuberculosis (N=105)	Total
	No (%)	No (%)	No (%)
Socio-demographic			
Sex			
Male	17(48.6)	103(98.1)	120 (85.7)
Female	18 (51.4)	2 (1.9)	20 (14.3)
Age (Mean)**	42.13 \pm 13.70		
Marital status			
Married	15 (42.9)	98 (93.3)	113 (80.7)
Single	16 (45.7)	6 (5.7)	22 (15.7)
Divorced/widowed	4(11.4)	1 (1.0)	5 (3.6)
Educational status			
Illiterate /traditional	16 (45.7)	73 (69.5)	89 (65.6)
Primary	13 (37.2)	24 (22.9)	37 (26.4)
High school and above	6 (17.1)	8 (7.6)	14 (8.0)
Religion			
Orthodox	34 (97.1)	99 (94.3)	133 (95.0)
Others	1 (8.9)	6 (5.7)	7 (5.0)
Household (HH) BTB*			
HH with BTB	17 (48.6)	16 (15.2)	33 (23.6)
HH without BTB	18 (51.4)	89 (84.8)	107 (76.4)

*The mean age for both households

**BTB: bovine tuberculosis

In the study areas, all farmers were keeping cattle for the purpose of farming and milk use. Nearly half, 49.3% (69/140) and 61.4% (86/140) were having the habit of drinking raw milk and consumption of uncooked meat, respectively. About 67.1% (94/140) of the participants did not know the benefits of drinking boiled milk. Among the study participants, 70.7% (99/140) and 85.7% (120/140) knew the possible transmission of disease from animals/cattle to human and human tuberculosis, respectively. Twenty nine point three percent (41/140) and 21.4% (30/140) of cattle owners responded the presence of cattle having cough and showing losing weight, respectively. Of the participants, 78.6% (110/140) did not isolate their cattle when they get sick and 87.1% (122/140) keep sick cattle with themselves (**Table-10**).

Table 10: Feeding habits and disease knowledge among cattle owners in North *Gondar* and North *Wollo* zones, Amhara Region, Ethiopia, 2014.

Category	Households (n=140)		Total No (%)
	With TB (n=35) No (%)	Without TB (n=105) No (%)	
Consumption of raw milk			
No	10 (28.6)	61 (58.1)	71(50.7)
Yes	25(71.4)	44 (41.9)	69 (49.3)
Consumption of raw meat			
No	23 (65.7)	31(29.5)	54 (38.6)
Yes	12 (34.3)	74 (70.5)	86 (61.4)
Knowledge assessment			
Consumption of boiled milk benefit			
No	22 (62.9)	72 (68.6)	94 (67.1)
Yes	13 (37.1)	33 (31.4)	46 (32.9)
Knowledge on zoonoses diseases			
No	17 (48.6)	24 (22.9)	41 (29.3)
Yes	18 (51.4)	81(77.1)	99 (70.7)
Knowledge on PTB			
No	7 (20.0)	13 (12.4)	20 (14.3)
Yes	28 (80.0)	92 (87.6)	120 (85.7)
Cattle related knowledge			
Cattle that cough			
No	22 (62.9)	77 (73.3)	99 (70.7)
Yes	13 (37.1)	28 (26.7)	41 (29.3)
Cattle with wasting			
No	21 (60.0)	92 (87.6)	113 (80.7)
Yes	14 (40.0)	13 (12.4)	27 (19.3)
Sick cattle isolation			
No	28 (80.0)	82 (78.1)	110 (78.6)
Yes	7 (20.0)	23 (21.9)	30 (21.4)
Keeping sick cattle			
No	4 (11.4)	14 (13.3)	18 (12.9)
Yes	31 (88.6)	91 (86.7)	122 (87.1)

Among the participants, 22.1% (31/140) exercised sharing a house with their cattle/animals. Of individuals with tuberculosis 31.43% (11/35) were sharing their living rooms. Assessing some possible risk factors, 7.1% (10/140) individuals were urinating on cattle feed/hay, 30.0% (42/140) were giving their urine to cattle or cattle licking their urine, 25.7% (36/140) answered cattle lick their hands, about 38% (53/140) sharing utensils, 32.9% (46/140) spitted sputum in the house, 44.3% (62/140) of the houses were having ventilations (window or holes) and nearly 18% (25/140), were using common water sources (**Table-11**).

As it is presented in **Table- 11**, adjusted for taking unboiled milk, sharing living rooms, cattle licking human urine, urinating on cattle feed, human spitting sputum in the house, house ventilation, using the same water source and knowing zoonoses diseases. The odds of households with positive bovine tuberculosis to acquire tuberculosis was more than 8 (95% CI; 2.82, 24.60) times higher compared to bovine TB negative households. Raw milk taking (AOR=8.80, 95%CI; 2.60-29.81), human urinating on cattle's feed (AOR=12.10, 95% CI; 1.80, 81.20) and house ventilation (AOR= 5.50, 95%CI; 1.60, 18.9) were also the contributing factors for the possible circulation of *Mycobacterium tuberculosis complex* in the households.

Table 11: Risk factors associated with the occurrence of human TB in North *Gondar* and North *Wollo* zones, using >mm as a cutoff value, Amhara Region, Ethiopia, 2014.

Category	Households Human TB (n=140)		AOR	95% CI	
	Controls (N=105)	Cases (N=35)		Lower	Upper
	No (%)	No (%)			
Bovine TB					
Negative	89 (84.8)	18 (51.4)	1.0		
Positive	16 (15.2)	17 (48.6)	8.32	2.82	24.60
Sharing living rooms					
No	85 (81)	24 (68.6)	1.0		
Yes	20 (9)	11(31.4)	0.31	0.01	0.99
Urinating on hay/feed					
No	100 (95.2)	30 (85.7)	1.0		
Yes	5 (4.8)	5 (14.3)	12.10	1.8	81.22
Urine licking					
No	76(72.4)	22(62.9)	1.0		
Yes	29 (27.6)	13(37.1)	1.10	0.32	3.60
Cattle licking hand					
No	78 (74.3)	26 (74.3)	1.0		
Yes	27 (25.7)	9 (25.7)	0.14	0.03	0.60
Sharing utensils					
No	67 (63.8)	20 (57.1)	1.0		
Yes	38 (36.2)	15 (42.9)	1.97	0.70	5.90
Spitting sputum in the house					
No	75 (71.4)	19 (54.3)	1.0		
Yes	30 (28.6)	16 (45.7)	2.64	0.90	7.70
House ventilation					
No	61 (58.1)	17 (48.6)	1.0		
Yes	44 (41.9)	18 (51.4)	5.50	1.60	18.9
Using same water source					
No	89 (84.8)	26 (74.3)	1.0		
Yes	16 (15.2)	9 (25.7)	1.80	0.51	6.11
Habit of taking unboiled milk					
No	61 (58.1)	10 (28.6)	1.0		
Yes	44 (41.9)	25 (71.4)	8.80	2.6	29.81

4.4. Identification and Characterization of *Mycobacterium Tuberculosis* Isolated from Cattle Owners in North Western and North Eastern Parts of Rural Ethiopia.

The socio demographic characteristics of AFB patients showed that age of participants ranged from 18 to 63 years old with a mean age of 35.7 years \pm 13.24, of whom, 45 (64.3%) were between 18 and 40 years, 39 (55.7%) were from North *Gondar Zone* with 1:1 male to female ratio. Among the participants, 64.3%, 82.9%, 41.5%, 60.0%, 38.6%, 20.0%, 52.9%, 38.6% and 71.4% were illiterates, rural dwellers, farmers, married, were having contact with TB patients before, treated with anti-TB drugs before, took unboiled milk, took uncooked meat and revealed culture positive results, respectively (**Table-12**).

Seventy AFB positive sputum samples were collected in North East and North West Ethiopia from pulmonary tuberculosis patients. Sputum samples were collected from 8 different health institutes which are found in North *Gondar* and North *Wollo Zones*. Of the total AFB positive sputum samples 28/70 (40%) and 22/70 (31.4%) were culture positive from North *Gondar* and North *Wollo Zones*, respectively. Among culture positives 22/70 (31.4%), 28/70 (40%), 36/70 (51.4%), 42/70 (60%), 23/70 (32.8%), 33/70 (47.1%), 17/70 (24.3%), 10/70 (14.3%), 26/70 (37.1%) and 22/70 (31.5%) were males, females, illiterate, rural dwellers, farmers, married, those had previous contact with TB patients, those received anti-TB treatment before, those experienced drinking unboiled milk and those practiced consuming uncooked meat, respectively. Those leading school lives (primary, secondary and college) contributed about 22.9% (16/70) for culture positivity, which could serve as a good medium for TB transmission due to the possible chance of the congregation (**Table-12**).

Table 12: Socio-demographic characteristics of AFB positive patients and sputum culture results in North *Gondar* and North *Wollo* zones, Amhara Region, Ethiopia, 2014.

Category	Frequency No (%)	Culture result (n=70)	
		Negative No (%)	Positive No (%)
Zone			
North <i>Gondar</i>	39 (55.7)	11(15.7)	28(40.0)
North <i>Wollo</i>	31 (44.3)	9(12.9)	22(31.4)
Sex			
Male	35 (50)	13(18.6)	22(31.4)
Female	35 (50)	7(10.0)	28(40.0)
Educational status			
Illiterate/informal education	47 (67.1)	11(15.7)	36(51.4)
Primary	13 (18.6)	6(8.6)	7(10.0)
Secondary and above	10 (14.3)	3(4.3)	7(10.0)
Dwelling			
Urban	12 (17.1)	4(5.7)	8(11.4)
Rural	58 (82.9)	16 (22.9)	42 (60.0)
Occupation			
Merchant	5 (7.2)	2 (2.9)	3(4.3)
Student	14 (20.0)	7 (10.0)	7 (10.0)
Housewife	19 (27.1)	5 (7.1)	14(20.0)
Government employee	3 (4.3)	0 (0)	3(4.3)
Farmer	29 (41.5)	6(8.6)	23(32.8)
Marital status			
Single	19 (27.1)	7(10.0)	12(17.1)
Married	42 (60.0)	9 (12.9)	33 (47.1)
Divorced/widowed	9 (12.9)	4 (5.7)	5(7.2)
History of contact with TB patients			
No	43 (61.4)	10(14.3)	33(47.1)
Yes	27 (38.6)	10 (14.3)	17(24.3)
Anti-TB treatment before			
No	56 (80.0)	16(22.9)	40 (57.1)
Yes	14 (20.0)	4(5.7)	10 (14.3)
Taking unboiled milk habit			
No	33 (47.1)	9(12.9)	24(34.3)
Yes	37 (52.9)	11(15.7)	26(37.1)
Taking uncooked meat habit			
No	43 (61.4)	15(21.4)	28(40.0)
Yes	27 (38.6)	5(7.1)	22(31.5)
Sputum sample	70 (100)	20 (28.6)	50 (71.4)

After deletion typing the isolates were characterized using spoligotyping and were compared with the International SpoIDB4 database. Of these, 71.4% (50/70) were found culture positive and spoligotyping was conducted on these isolates. Ninety four percent (47/50) and 6 % (3/50) showed good interpretable patterns. Forty six point eight percent (22/47) and 53.2% (25/47) were from North *Wollo* and North *Gondar* Zones, respectively. As it is presented in **Figure-3**, three lineages namely; lineage 3 (East African-Indian) 57.4% (27/47), lineage 4 (Euro-American-African) 38.3% (18/47) and lineage 7 (Ethiopia) 4.3% (2/47) were recorded in the study areas.

Among lineage 3, 5 SITs and 6 new SITs, lineage 4, 9 SITs with 4 new SITs and lineage 7, 2 SITs (North *Wollo Zone*) were identified in both areas. In general 16 shared international types with 10 new ones were disclosed by the current study. Among the SITs, SIT 25, 26 and 289 were the predominant proportions. Among the 10 new SITs, 6 of them were from *Gondar Zone*. Based on spoligotyping 8 clusters (with a cluster size ranging from 2-8), 8 unique and 10 new patterns were identified in the study areas. SITs 21, 25, 26, 35, 53, 109, 149 and 289 were found as clusters in both *Zones*. From 3 cluster size/isolates of SIT 53, and SIT 26 were found in North *Wollo Zone*, each accounted for 66.7% (2/3). SIT 149 with cluster size 2 and SIT 109 with cluster size 2 were noticed from North *Wollo Zone* only. SIT 35 and SIT 21 each with cluster size 2 reported in North *Gondar Zone*, particularly the first one registered in *Chilga* District. Among SIT 25 having a cluster size 7, 57.1% (4/7) and SIT 289 with cluster size 8, 75% (6/8) occurred in *Debark District* areas and *Chilga* District of North *Gondar Zone*, respectively (**Table-13**). The rest was identified as unique.

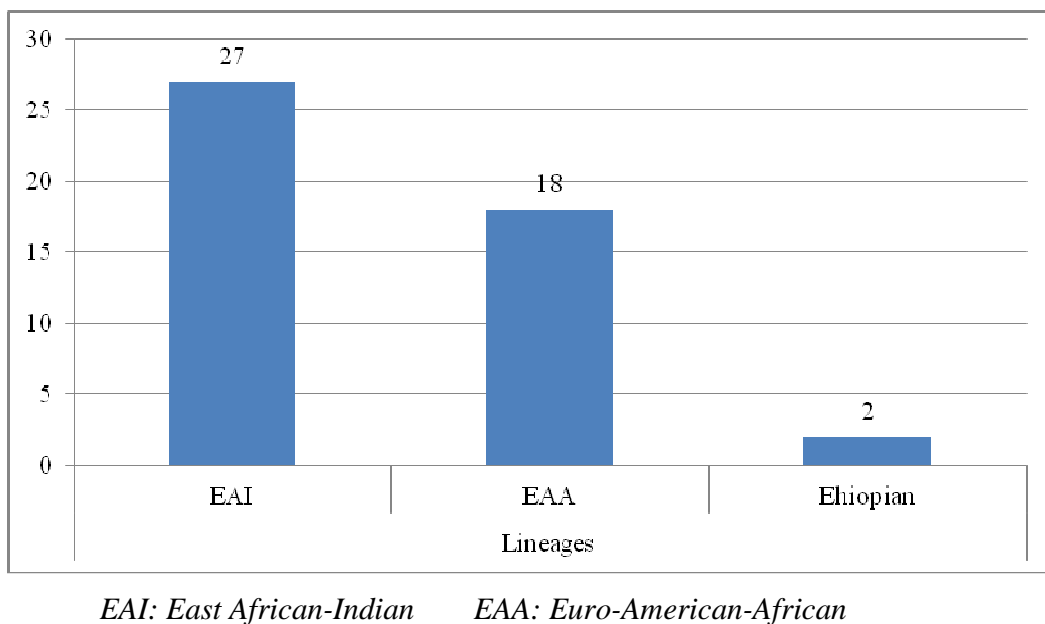


Figure 3: *Mycobacterium tuberculosis* lineages identified from cattle owners in North *Gondar* and North *Wollo Zones*, Amhara region, Ethiopia, 2014

Table 13: Description of Spoligotype International Types (SITs) representing *M. tuberculosis* isolated from cattle owners AFB positive sputum samples in North Gondar and North Wollo zones, Amhara Region, Ethiopia, 2014.

n= 47

SITS	Spoligotype patterns	L	No	Distribution
21		3	2	NG
25		3	7	3 NW , 4NG
New		3	1	NG
New		3	1	NG
New		3	1	NG
1199		3	1	NG
289		3	8	2NW, 6NG
New		3	1	NW
26		3	3	2NW, 1NG
New		3	1	NW
New		3	1	NG
910		7	1	NW
1729		7	1	NW
53		4	3	2NW , 1NG
52		4	1	NW
New		4	1	NW
41		4	1	NW
42		4	1	NW
149		4	2	NW
New		4	1	NW
New		4	1	NG
35		4	2	NG
New		4	1	NG
134		4	1	NG
4		4	1	NG
109		4	2	NW

SITs: Spoligotypes/Share International types L: Lineage NW: North Wollo NG: North Gondar No: Number of isolates

Black boxes: Interpretable patterns (represents the presence of the specific spacers at position), while the rest are not interpretable patterns.

4.5. Summary of the Main Findings

Tuberculosis infections were recorded in both humans (presumptive as well as AFB confirmed TB cases) and cattle population owned by them with 3.2% (4/124) isolation of *Mycobacterium species* and 2.6% (10/381) BTB based on >2mm measurement difference, respectively. Of the *Mycobacterium species*, half of them were belonging to *Mycobacterium tuberculosis* and the rest were atypical *Mycobacteria*. There were higher BTB positive cattle 8.8% (5/57) in low altitude areas compared to mid and high altitudes. About 67% (6/9) of the presumptive TB cases who owned positive cattle were having the habit of drinking raw milk. The comparative study revealed an overall herd BTB and individual apparent prevalence of 23.6% and 8.7%, respectively, with an odds of nearly three times (AOR, 2.90, 95% CI: 1.50, 5.54) higher BTB in cattle owned by TB confirmed households compared to those cattle owned by households without TB. Evaluation of BTB as a risk factor for the occurrence of human pulmonary TB showed that the odds of households with BTB is about 8 times (AOR= 95% CI; 8.32, 2.82-24.60) higher than those households without BTB to develop PTB. From the collected AFB sputum samples (who own cattle) 71.4% (50/70) were culture positive and based on molecular techniques 94% (47/50) were confirmed as *Mycobacterium tuberculosis* and three lineages namely, East-African-Indian (57.4%), Euro-American-African (38.3%) and Ethiopian (lineage-7-North wall area) (4.3%) with clusters ranging from 2-8 size SITs were recorded in the study areas. Unfortunately we failed to isolate *Mycobacterium tuberculosis complex* species either from milk or tissue samples collected and processed from CCIDT positive cattle.

5. DISCUSSION

Objective 1: Tuberculosis Infection in Cattle and Cattle Owners in North Western parts of Ethiopia

Human TB, which is primarily caused by *M. tuberculosis* (203), could also be caused with *M. bovis* (BTB) since it is a known zoonosis (204). The global contribution of *M. bovis* to human TB has been estimated to be about 3.1% of all human TB cases (19). Its role in human TB in Africa is not well studied, but the agent is isolated in some African countries including Ethiopia from human sputum samples (18, 201, 205-216). Reports from countries like Egypt, Nigeria, Zaire and Tanzania confirmed bovine type human tuberculosis from their patients (217-220).

Of 124 sputum samples collected from chronic coughers, 4 culture positives were isolated accounting for about 3.2%. A tuberculosis survey conducted in Ethiopia indicated 0.2% (110/51667) culture positivity from presumptive pulmonary TB cases (221). A study conducted in the rural community of South-west Ethiopia revealed a culture positivity of about 4% (222). The current result (3.2%) is almost similar to the last report. The differences between these results is expected to be due to differences in the quality of sputum sample, time of sputum sample collections, cold chain systems during collections and transportations as well as the time of processing after collection. Fifty percent of the isolates were confirmed as atypical *Mycobacterial species*. As it is indicated in literature people could be infected with atypical *Mycobacteria* or *Mycobacteria* other than tuberculosis (MOTT) and some of them could lead to disease development in different parts of the body including the lungs (208, 223).

In the current study 381 cattle owned by chronic coughers were tested for bovine TB and prevalence of about 2.63% was documented. The prevalence of BTB in *Meskanmareko*, *Woldeya*, *Bakogazer Districts* and *Bale* mountains were 7.9%, 1.2%, 4.3% and 2.0%, respectively (with an overall prevalence of 3.1%) (224). Studies reported that, prevalence of BTB in small scale dairy production systems ranged from 3.5% in *Asella* to 14.2% in *Wolaita Sodo* areas (225, 226), and prevalence in intensive dairy farms ranged from 4.5 to 73.6% in *Desie* area(227, 228), whereas in the traditional management system it ranged from 3.4% (West

Wolega) to 22% (*Kombolcha*) (227, 229). The present finding is higher than the findings from *Woldeya* area, but almost similar with the overall findings reported by Tschopp *et al.* (224).

This finding showed relatively lower values compared to the reports for small scale and intensive dairy production as well as traditional management systems. In general the low prevalence recorded in this research compared to different management systems might be explained by the differences in breed types/composition, the way of management of cattle, the environment and the body condition of the cattle during testing season, which is related to the availability of feed for the cattle. The difference between *Dega*, *Woinadega* and *Kolla* areas in BTB was significant ($P < 0.05$, $\chi^2 = 12.13$ with 2 df). A study in Ethiopia indicated that the prevalence is relatively high in the highlands, having an altitude of >2000 meters above sea level (230). Indeed the presence of difference in skin test positivity in geographical locations is described by Phillips *et al.*, (231). However, this needs further investigation, because reports in human TB showed a higher prevalence in low altitudes (232, 233).

All cattle that showed a positive result for the test were local zebu breed and 100% home-bred, having a longer contact period (with a mean age of 6.034 ± 3.53 years) with the household. Longer contact period might act as a source of infection to the family members as well as cattle including other animal species and at large to the community. As a result of longer duration of stay with the household, females, particularly lactating cows will serve as a permanent source of infection since it has been reported that a single cow can excrete sufficient viable *Mycobacteria* in milk to make even pooled milk infective (198) and infection of the udder has been found in varying proportions of tuberculous animals as high as 5.4% (198, 234). Studies in selected areas of Nigeria revealed that 12/35 (34.3%) of the positive milking cows excrete *Mycobacteria* confirmed by acid fast bacilli stains (235). A similar study conducted on 805 milk samples in pastoral areas of Tanzania revealed 31 (3.9%) acid fast positivity for *Mycobacteria* and among those two of the isolates were confirmed as *M. bovis* (201). Even apparently healthy cattle can excrete the bacilli (236, 237). The condition could be worsened if infants/children and individuals whose immunity is compromised consumed such type of milk and its products. Besides taking unboiled milk, these positive cattle could be sold for different reasons and play a role in the BTB transmission in the area where they are relocated.

In this study area 74% presumptive TB cases had the habit of taking raw/unboiled milk and from the 9 households having positive BTB on their cattle, 6 (nearly 67%) of them were taking raw/unboiled milk. According to reports made by Felleke and Geda (238), in Ethiopia, it is estimated that 68% of the total milk produced is used for human consumptions in the form of fresh milk, butter, cheese and yogurt. The percentage of taking raw milk among chronically coughing individuals is higher compared to their findings and this could be related to the study settings whereby all participants in this study were rural inhabitants having little awareness about the effect of consuming unboiled milk. Polymerase chain reaction conducted from Albanian milk samples in Tirana University revealed the presence of the MTC gene in 36/50 (72%) of the tested samples would support the possibility of ingesting the disease causing agent (239).

About 60% of the study individuals were suffering from chronic cough for more than two months and nearly 77% of the participants were not taking any action to cure their cough. Besides, three-fourth (75%) of the culture positive ones had cough duration of more than two months. These will increase the chance of disease transmission; if the individual is infected with tuberculosis since smear negative-culture positive cases are responsible for 20% of tuberculosis transmission(240, 241). A person with active tuberculosis can release droplet nuclei containing up to 3 bacilli while coughing, sneezing or talking. Coughing and a single sneezing can generate about 3000 and 40,000 droplet nuclei, respectively(65, 242). In the rural community most family members are living together in a one room house and therefore, the likelihood of TB transmission would be high since the number of infectious droplets released from diseased individuals is high. Besides, the infective dose of *Mycobacterium tuberculosis complex species* is very low (ID₅₀ 1-10 bacilli) (243, 244). Three-fourth of the culture positive individuals were aged above 50 years old and this might be related to the decline in host immunity as age increases and this is supported by Chan and his colleagues (245) referring that immunologic abnormalities in elders led to tuberculosis development. Different authors also indicated that active TB is characterized by a profound and prolonged suppression of *M. tuberculosis* (MTB) specific T-cell responses, as evidenced by decreased production of the cytokines interleukin (IL) -2 and interferon (IFN) - γ (246-250).

Objective 2: Distribution of Bovine Tuberculosis in North Gondar and North Wollo Zones, Amhara Region, Ethiopia

The current study, mainly conducted on local zebu cattle and some cross breeds, indicated the importance of bovine tuberculosis (BTB), particularly in cattle owned by TB diagnosed households in the study area. Based on >4mm measurement difference an overall household/herd of 6.4% and 2.1% of an individual cattle apparent prevalence of BTB were documented. Herd (household) and individual cattle apparent prevalence of 20% and 5.6% registered, respectively among TB households (cases). In the control groups 2% and 0.6% of herd and individual cattle apparent prevalence, respectively were recorded. On the other hand, using >2mms as a cutoff value, 23.6% and 8.7% of an overall household/herd and individual cattle apparent prevalence of bovine TB were observed. Using the same cutoff value 48.6% in households with TB and 15.2% in households without TB apparent prevalence of bovine tuberculosis were documented.

Adjusted for age, sex, body condition, breed and source, at an individual cattle level, the risk of a cattle to be positive for bovine TB, owned by TB affected individuals was about three times higher (using >2mms as cutoff) compared with those cattle owned by non-tuberculosis households. In Ethiopia, a herd prevalence of 19%, 44% and 51.4% of bovine tuberculosis (BTB) were reported in *Boji* district, *Afar* and *Jima* dairy herd, respectively (251-253). A study conducted in and around *Mekele* area, Tigray regional state, Ethiopia, revealed an overall farm BTB prevalence of 13.9% and 54% of herd prevalence (254).

Similarly, a cross sectional study of BTB conducted in Ambo and *Toko Kutaye* districts, Ethiopia, showed an overall individual and herd prevalence of 1% and 7.02%, respectively (255). Besides, in the districts BTB was more prevalent in cattle owned by tuberculosis households (1.36% at an individual and 12% at herd levels) compared to non-tuberculosis households (0.6% and 3.13% at individual and herd levels). The prevalence and the BTB status more or less agree and support the present result. However, contrary to the above mentioned study from cattle owned by the TB patient's higher prevalence of BTB at herd level was registered in this comparative cross-sectional study.

Studies done in different parts of Ethiopia indicated the presence of high prevalence of BTB in cattle owned by TB diagnosed owners than TB negative ones at herd and individual cattle levels. For instance, of 11 TB households, 36.4% (4/11) (251) and 62.5% (5/8) (256) in Northwest Ethiopia and Jimma dairy herd, respectively, had BTB reactor herds. Fetene *et al.*(18), reported a significantly higher prevalence of BTB in cattle owned by TB patients than in cattle owned by non-TB owners and at the same time ascertained by isolating *M. tuberculosis (M.tb)* and *M. bovis* from sputum and fine needle aspiration specimens of TB patient cattle owners. Besides *M. tb* was isolated in grazing cattle in Central Ethiopia. In Nigeria 11.8% prevalence was reported in cattle owned by TB positive owners (257) and Aishatu (258) denoted that high TB prevalence trend among human patients in Nigeria has similar trend among cattle populations.

The presence of higher BTB reactor cattle in cattle owned by TB positive households than TB negative ones could suggest that either of them could be a source of infection for the other creating a sort of vicious cycle (19). From the current study and other literature, human TB may be transmitted to cattle or that cattle TB may be acquired from humans (255, 259, 260) and, therefore, *M. tb* might be a possible cause of the skin test positivity in cattle. A study carried out in and around *Mekele* reported that those who owned cattle had 5% TB patients in their house compared to 3% TB patients in the house for those who did not own cattle (254). A report in Spain indicated *M.tb* infection of cattle and the source of infection was human TB patient (261). Apart from the possible contribution of *M.tb* for skin test positivity, the difference in skin test result might be related to the differences in the cattle husbandry system, nutritional status of the animal, breed differences(19) number of TB case households, number of cattle (herd size) (262) owned by the TB cases, coverage as well as cattle stay/relocation in the household, the use of proper cold chain systems and technical disparity as well as cattle immunity/circulating antibodies (263).

Based on the current comparative intradermal skin test referring to >4mms, the proportion of dubious/inclusive herds 42/140 (30%) as well as individual cattle 65/481 (13.5%) seems important because of the fact that such cattle could serve as a source of infection to other cattle or humans and this was evidenced by culturing of *Mycobacterium species* from suspected BTB lesion (264) and milk samples (258). Besides, a study conducted in India showed 26.7% doubtful skin test result and among the doubtful cattle 66.7% revealed positive results with interferon assay (265).

Objective 3: Bovine Tuberculosis (BTB) as a risk factor for developing Tuberculosis in humans in the rural community of Ethiopia

Bovine tuberculosis, one of the neglected zoonotic diseases, is a worldwide problem and control of the disease remains difficult for many developing countries, including Ethiopia. In Ethiopia, bovine tuberculosis was reported from different corners of the country reaching with a herd prevalence of up to 62.5% (256).

Based on case control study, among the participants, 70.7% knew disease transmission from cattle to humans and mentioned some of the diseases, particularly anthrax and rabies, while their knowledge regarding BTB was limited to few individuals with some form of education. Ameni and his colleagues reported that 38.3% of the respondents knew about BTB (266). This difference on the awareness of BTB might be attributed to lower participation of veterinary personnel's in the public health issues, little attention given by health extension workers or high participation of illiterates in the current study compared to *Wuchalie-Jida* (Ethiopia) districts.

Of the study households, higher proportion of participants (85.7%) knew human tuberculosis. The higher percentage of awareness about humans TB might be related to the existing health extension activities practiced and implemented in the country health programs, in mass media, particularly radio. Despite creating awareness as to the possible risk of food borne diseases, nearly half (49.3%) of the participants, were having a habit of consuming raw milk and 61.4% of them were eating uncooked meat. Besides, more than two-third (67.1%) of the respondents in the current study did not know the benefit of using boiled milk. In a study conducted in rural *Torodi* (Niger), more than 68% of the household families consumed unpasteurized milk (148) and a study carried out in *Wuchalie-Jida* (Ethiopia) districts showed 52.1% of the interviewed households used to drink raw milk and of the respondents from *Wuchale-Jida* 90% ate raw as well as cooked meat (266). Relatively a lower percentage of raw meat and milk consumption habit was recorded in the current study, compared to studies done in Niger, while a relatively closer figure is reported from *Wuchali-Jida* area and this might be related to having similar cultural settings between the study sites in Ethiopia, while the higher figure in Niger could be justified as having different socio-cultural settings. The habit of raw meat consumption in the current study was very low compared to

the findings from *Wuchali-Jida* district and this might be a reflection of the effects of increased community awareness about the use of boiled milk and cooked meat consumption.

Our study revealed that, 22.4% (31/140) households were sharing living rooms with cattle. Although sharing living rooms was not seen as a contributing factor in the occurrence of human TB in households with BTB, cattle which harbor *Mycobacterium tuberculosis complex species* could serve as a source of infection to humans by releasing droplet nuclei during coughing (267). Similarly Ayele and co-authors(20) reported that inhalation of dust particles or bacilli containing aerosols released from cattle could cause pulmonary TB in humans. The survival of the bacilli in the dark for hours will facilitate transmission of *M. tuberculosis complex species* (71). The more droplet nuclei, space, ventilation, air circulation as well as air pressure are the factors that could increase the probability of *M. tuberculosis complex* transmission (268).

Adjusted for different predictors, the odds of human TB in households with BTB was more than 8 (95% CI; 2.82-24.60) times higher compared to households without BTB. As close contact is a predisposing factor for TB transmission between humans (71, 268), prolonged close contact(269-273) between the cattle with TB and humans could result in longer duration of exposure that would lead to higher risk for the disease transmission between the two populations. Most of the houses in the current study were not having adequate ventilation or were completely closed. Having house ventilation was found as a contributing factor in the occurrence of human TB in households with bovine BTB. The odds of house ventilation for the occurrence of human TB in households with bovine TB was 5.50 (95% CI: 1.60, 8.90) times to households without BTB. It is known that inadequate ventilation could result in insufficient dilution or removal of infectious droplet nuclei which enhance the transmission of *M. tuberculosis complex* (71, 268). However, in this study the definition used for ventilation and the perception of ventilation by respondents might be inadequate, and can be a concern for information bias, in the association between ventilation and TB.

In this study, about 7.1% of the participants were urinating on animal feed and this was found to be as contributing factor (AOR: 12.10, 95%CI; 1.80-81.20) for the occurrence of human TB in households with BTB. As evidenced by different authors, urine could contain *Mycobacterium*

bacilli when a person is infected with either disseminated or localized TB. Mortier and his colleagues (274) isolated *M. tuberculosis* from human urine samples and Torrea and his colleagues (275) detected *Mycobacterium tuberculosis* complex in HIV-infected and uninfected pulmonary and extra pulmonary tuberculosis patients in Burkina Faso. In Italy (276), researchers also detected *Mycobacterium tuberculosis* complex using rapid gen-probe amplification test. The possible explanation for this contribution is that when infected individuals urinate on animal's feed, the feed will be contaminated by tubercle bacilli and then taken by cattle. In this case, infected cattle might serve as a continuing source of infection to the owners thereby re-infection or new infection could be possible despite the patients received adequate treatment and cured of the disease.

Apart from that unboiled milk drinking was associated with the manifestation of TB in households whose cattle was having BTB and the risk was 8.80 (95%CI: 2.6, 29.81) higher compared to households without BTB. In line with this the odds of house sharing and habit of hand licking for the occurrence of human PTB who own cattle were 0.31 (95% CI: 0.01, 0.99) and 0.14 (95% CI: 0.03, 0.60) lower compared to their counterparts, respectively. These factors might favor the circulation of the disease causing agent between human and cattle populations.

The risk of bovine TB to the occurrence of human TB in the current study could be seen in light with the detection of 6.2% and 7.4% *M. tuberculosis* prevalence in cattle in Algeria and Sudan, respectively (80, 81). Besides, *Mycobacterium species* could be excreted with the milk (277). This might suggest the possible role of cattle in the maintenance as well as transmission of tuberculosis to humans.

Objective 4: Identification and Characterization of *Mycobacterium Tuberculosis* Isolated from Cattle Owners in North Western and North Eastern Parts of Rural Ethiopia.

Genotyping is contributing a lot to understand the epidemiology of TB and provides information on its transmission dynamics. In this study, 78.7% of SITs were matched with standard spoligotype database, while 21.3% were new ones. The current study revealed the presence of four lineages in northwest and northeast Ethiopia. The majority (57.4%) were the East African-Indian lineages (EAI; L-3) followed by Euro-American-African (EAA; L-4) (38.3%) lineages, which were found in both *Zones*, while Ethiopian (L-7) (4.3%) were found in North *Wollo Zone* only. A study done in Amhara region revealed 15.6% of lineage 7(278). Since EAI strains reported as one of the major genotypes in many countries of Southeast Asia and Africa (279), the higher proportion of this lineage finding in the current study could be the main reason. In contrast to the current finding, lower value of EAI lineages were reported in a study conducted on farmers in mixed type multipurpose cattle raising region of Ethiopia, the majority 78.5% were Euro-American lineage followed by East-African Indian 17.7% (280) and Rebuma and his colleagues also reported the presence of those lineages in both *Zones* and reported 71% (442/662) and 25% (153/622) lineage 4 and 3 from PTB patients, respectively, and 70% (229/328) and 24% (79/328) lineage 4 and 3 from TB lymphadenitis (TBLN) patients, respectively (281). The Authors also reported relatively higher percentage of 13% (17/133) of a new lineage, named as lineage-7 (281), which placed between ancient lineage 1 and modern lineages of *Mycobacterium tuberculosis* phylogeny prominently in North *Wollo (Weldeya)* area compared to the current study. The difference in the proportion could be due to sample size in the case of North *Wollo Zone* and geographical situations in the case of central Ethiopia. In Uganda, researchers reported 22% (394/1746) and 11% (187/1746) L- 4 and 3, respectively (282). The current finding showed relatively higher values compared to the work in Uganda and this might be due to the presence of dominant and common types of prevailing isolate differences in both countries. Lineage East African-Indian showed higher cluster size by SIT 25 (7 cluster size) and 289 (8 cluster size). Clustering showed the possibility of a shared source of recent *Mycobacterium tuberculosis* infections in the study area.

6. VALIDITY AND GENERALIZABILITY

6.1. Validity

To maintain the validity of the study the following issues were addressed. The questioner was pretested and training was given ahead to data collectors prior to work proper. Tuberculin skin test was carried out by an expert following the recommendations and all laboratory works were done by laboratory technologists and molecular biologist. Tuberculosis suspected and active cases were identified by health professionals. Samples were collected by laboratory technologist and meat inspection was supported by meat inspectors. Cold chain systems were tried to meet standards despite power fluctuation. Data were sorted and double entered timely. Data cleaning, coding and recoding were done before analysis. Selection bias was controlled by clearly identifying or defining the study population and selection of the appropriate control or comparison groups. Information bias was prevented by well designed (closed as well as precise) pretested questionnaire and using standard test materials and procedures. Logistic regression was used to control potential confounding effects of some variables on the main outcome variable.

6.2. Reliability

Cattle skin testing by an expert following the recommendations, sputum samples collections and laboratory works by laboratory technologists and molecular biologists, screening of presumptive TB and confirmed cases by health professionals and collection of TB lesions from humanity-slaughtered oxen tissue by trained meat inspectors were employed to maintain consistency. Furthermore, standard operating procedures were followed at each activity.

6.3. Generalizability

Due to the nature of the disease, the study followed purposive sampling method and based on participants willingness and therefore, these could preclude from generalizing the findings. However, the findings of this study could be generalized to areas having similar settings in the country, in particular in the Northeastern and Northwestern parts of the Amhara Region.

6.3. Implications

The results could support TB program activities, which mainly focus on the disease prevention and control. Furthermore, it could draw the attention of policy makers to look at cattle as a possible source to human PTB, and this led them to decide to deal in studies like this one in a more detailed and well organized manner at different corners of the country.

7. STRENGTH, LIMITATIONS AND CHALLENGES

7.1. Strength

Although failed to isolate the etiological agent from BTB positive cattle, the study indicated cattle as a possible source of human pulmonary tuberculosis. BTB situation in households with TB using 1 case: 3 controls/comparative groups should be seen as strength of the study. Testing almost all eligible cattle should also be considered as strong side of the study. With the limited resources that we had removal of six BTB positive cattle from a family should be seen as strong side from public health point of view.

7.2. Limitations

Purposive sampling of the study areas with purposive sampling methods, lack of a comparative or control groups for tuberculosis infection assessment and failure to resample negative chronic coughers, smaller sample size in all cases and inability to recheck dubious cattle were the limitations of this study. Moreover, although 3 *Mycobacterium tuberculosis* lineages were recorded in this study failure of isolation of any of *Mycobacterium tuberculosis complex species* from skin test positive cattle should be seen as a limitation of this study since the aim of the study was to see the possible role of cattle in the epidemiology of human tuberculosis by determining the presence of cross infections with detecting the same strain in the same house. Besides, geographical locations, withdrawals from participation after giving written, in most of the areas maintaining cold chain system were difficult due to power interruption and unwillingness to use the institute cold chain materials, lack of finance, inability to buy all positive cattle for tubercle lesion detection and then culturing, failure to retest cattle with

inclusive test results and Refusal to participate by some TB confirmed individuals were the limitations which could affect the power of the study.

7.3. Challenges

Some of the problems faced during the working times are summarized as follows. Getting inadequate confirmed pulmonary TB human patients who own cattle were the prime setback. Frequent relocation of those laboratory technicians who agreed to collect AFB positive sputum samples were somehow contributed for a lower sample size. Besides, getting households as control/comparative group were challenging. The work was labor intensive and cattle kick as well as horn attacks were also the usual occurrence during skin test and sample taking. Driving purchased positive cattle to slaughtering places were also tough. Leaving test positive and inclusive cattle within the herd was also an ethical challenge since these animals could serve as a future source of infections. Owners do have a right to know the status of their cattle after each test and telling the result, especially when the animal is doubtful or positive was a source of frustrations to the owner, thereby leading him/her to sell the animal/s as a means of culling and facilitating further disease spread to another area, which paves ways to additional/new disease epidemiology.

8. CONCLUSION

TB is a bacterial infection and one who is latently infected can develop active pulmonary TB. The current study can tell us the advantage of culturing since all of the culture positive chronic coughers were smear negatives. In this study, four households (3.2%) having different family size were benefited since treatment for the cases and screening to the families were implemented. The findings show that atypical *Mycobacterial species* in humans should be seen as a problem in North *Wollo Zone*. According to the findings, the cattle owned by presumptive TB cases individuals were affected with Bovine TB with a prevalence of 2.63% and more than two-third of the individuals who usually drink raw milk suffered from chronic cough. Positive cattle owned by these individuals will remain source of infections. The findings of the current study could serve as a springboard for future possible cross infection assessment of *Mycobacterial species* between the two populations.

The current comparative study indicates the presence and importance of BTB whereby the odds of cattle owned by pulmonary TB households was nearly three times compared to those cattle owned by non-pulmonary TB households in North *Gondar* and *Wollo Zones* of Amhara regional state. This denotes the possible transmission/spread of the disease in the studied rural areas. In both *Zones* bovine TB dubious cattle were recorded. The existing cattle management practices, the practice of free and mixed grazing as well as sharing common water sources and free livestock movement in the study area is likely to facilitate the expansion of the disease in the areas. BTB eradication from livestock is expensive, but it is possible to minimize the possible spread of the disease by regular testing/surveillance, segregation and creation of awareness among livestock owners.

Human tuberculosis is mainly caused by *M. tuberculosis* and at times by *M. bovis*. In this study bovine TB has been seen as an exposure to human pulmonary TB occurrence. Prolonged close contact between human and cattle could contribute for the transmission of the disease. Apart from it, drinking unboiled milk and urinating on animal feeds were found as a factor for the occurrence of human TB. Those who did not share their house with their cattle were less likely to reveal TB.

From acid fast bacilli positive sputum samples, 3 *Mycobacterium tuberculosis* lineages, namely; the primitive, intermediate between the modern lineages and modern were identified in the study areas. Among the isolates, 61.7 % (29/47), (including the new isolates to the denominator) were clustered indicating the presence of recent tuberculosis transmission in both areas.

9. RECOMMENDATIONS

Households and the community: The households as well as the community should construct a separate house for animals and humans. They should also use a separate utensil for the two populations. Moreover, they should avoid using common water sources for animals and themselves, and if it is a must they should exercise using the water after boiling. In most of the areas there is a habit of consuming uncooked meat and drinking unboiled milk and these should be prohibited in order to avoid possible risk of zoonoses. They should also develop health institute visiting in case of chronic cough occurrences. The house should have adequate ventilation.

District health departments and health institutes: They should strengthen the health extension activities, which is already in place. They should create awareness to the community regarding diseases like tuberculosis transmission from animals to humans. Health institutes should screen presumptive TB cases and subject to further laboratory examinations. In addition, they should improve active case detection.

Zonal health department and regional health bureau: These bodies should give training and work on capacity building, monitoring and support activities related to TB. They should seek financial support to run research activities. The presence of Lineage-7 in North *Wollo Zone* should be given emphasis since it result in prolonged patient delay in seeking treatment.

Researchers/research institutes: Research is key to curb TB situations in our country and at large to the globe. Further exploration in a larger scale and better design should be implemented. The role of animals to human TB is an area that should be investigated in detail. There were occurrences of extra-pulmonary TB development after complete anti-TB treatment and this should be addressed. Although it is advocated, researches related to poverty reduction should be given more emphasis. Since there is resistance difference between the exotic and local cattle to TB infection reaction the cutoff value to skin test results to our cattle need to be standardized. The contribution of atypical *Mycobacteria* to human pulmonary TB as well as cattle should be assessed in detail. Researchers should give weight to skin test doubtful results. Determination of the new SITs isolates is suggested. Moreover, the occurrence of lineage 7 (Ethiopia) in

Woldeyia area should be investigated in accordance with the socio-cultural aspect of the community. Research on causes of BTB in cattle/animals should be carried out.

Ministry of health and agriculture: Since these are decision makers, they should work in coordination to mitigate TB situation in the country. Ministry of Agriculture and rural development should practice regular BTB testing/surveillance.

Policy Makers: In order to avoid further bovine TB transmission, test positive animals should be removed from the herd or the households by following a test and slaughter policy, which actually requires compensations to the owners, which needs policy. Allocation of funds for future research.

10. FUTURE STUDIES

- ☞ Verification of the role of cattle in the occurrence of human pulmonary TB and role of humans for cattle BTB positivity.
- ☞ Importance of atypical *Mycobacterial species* as a cause of chronic cough in humans
- ☞ Prevalence of extra pulmonary TB Development after anti-pulmonary TB treatments
- ☞ Zoonotic disease knowledge assessment in the rural community
- ☞ Epidemiological study on Bovine tuberculosis in *Kobo Girana* rift valley, North *Wollo Zone*, Eastern Amhara

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13. ANNEX

Annex13.1: Subject Information Sheet and individual consent form in English with equivalent Amharic versions

13.1. 1. Information Sheet

Dear respondent

Good morning/Good afternoon. Thank you for your interest in talking with me today. I am _____ who is a member of a team conducting a study to assess bovine tuberculosis as a Risk Factor for the occurrence of human pulmonary tuberculosis in your locality. The study is conducted as part of a PhD research conducted under the school of public health, College of Health Sciences, Addis Ababa University.

The purpose of my visit today is to take information from you on the aforementioned issue. The screening of you as a tuberculosis patient will be done by the physician. After screening, if you are willing to participate in the study, I will collect about 5ml of sputum sample from you and I will ask you few questions lasting for about 15-20 minutes. All the information given will be confidential. If your result is positive for tuberculosis you will be referred soon as possible to nearby health institution to get an appropriate treatment. In addition, I will conduct intradermal tuberculin skin test on your cattle and read them after 72 hours for the possible reactions. Your cattle will receive Anthelmintics at the recommended dosage after testing is done. There is no harm to your cattle up on testing them except possibility of hypersensitivity reactions that might be seen in few cattle. However, swelling at the injection site is observed if your cattle is positive to the test and this swelling disappears through time. I will take about 30ml of milk samples from your positive cows and buy some positive male cattle with a market price to scarify and collect tubercle lesions from them. The samples taken from you and your cattle will only be used for culturing and then typing, which is the central point of the research. The findings obtained after processing the samples will be communicated to you.

Your name will not be written on this form and will never be used in connection with any of your information. You do not have to answer any question that you do not feel comfortable with,

and you may end this task any time. However, your honest answers to these questions will help me a lot. Your participation in the study is fully based on your interest and choice and hence no financial payment will be made for your participation. Your participation or non-participation will not be related with the health service that you will get from governmental and non-governmental institutions. If you have any un-clarity on my visit you can ask me now so that I can elaborate it. If you come across with any concern during my stay with you, you can stop me and raise it anytime you want to. It is also possible to communicate the principal investigator through the telephone address +25191175 2959 or the institutional review boards which approved the study through the telephone address +251115538734 and ALERT/AHRI Review Committee secretary 251-0113211567.

Data collector: Please encourage him to raise questions

13.2. Informed Consent Form

I have been well aware that this research undertaking is a PhD research project to be conducted by Araya Mengistu the principal investigator coordinated by Addis Ababa University school of public health and Armauer Hansen Research Institute.

- ✓ I have been fully informed in the language I understand about the research project objectives. I knew that the interview will take 15-20 minutes. I was also well informed that all the information I provide will be kept confidential. I understood that my examination will be done by physician.
- ✓ I knew sputum samples will be collected from me without any harm and skin tests will be conducted on my cattle resulting in swelling at the injection site if they are positive for the test and some of my cattle might be sensitized with no threat to the life while taking the test. I knew that my cattle will get medicaments after tests and they will be examined after 72hrs for the results.
- ✓ In addition I knew that milk samples will be taken from my cow if it is positive to the test. I agreed to sell my male cattle with a market price if it is positive to the test. I knew that I have the right to withdraw from the study at any time and to withhold information. I was also enlightened that none of my health benefits will be affected by not participating in the study. I have read this form, or it has been read to me in the language I

comprehended and understand the condition stated above, therefore, I am willing and confirm my participation by signing the consent.

Participant

Name _____ Signature _____ Date _____

Interviewer

Name _____ Signature _____ Date _____

Witnesses

1. _____ 2. _____

Supervisors/Researcher

Name _____ Signature _____ Date _____

Note: In case of unclarity you can communicate

Principal investigator (Araya Mengistu): +25191175 2959 or Supervisor: 0912459707 or

The institutional review boards which approved the study through the telephone address +251115538734 and ALERT/AHRI Review Committee secretary 251-0113211567.

Addis Ababa University, School of Public Health: P.O. Box: 9086; Tell: 011-5-157701, Fax: 011-5-517701.

የመጠይቁ መለያ ቁጥር: _____

በአማራ ብሄራዊ ክልላዊ መንግስት በሰሜን ጎንደርና በሰሜን ወሎ ዞኖች የከብቶች ቴቢ ለሰው የሳንባ ቴቢ መከሰት ሊኖራቸው የሚችለውን አጋላጭነት ሚና ለማጥናት የቀረበ መጠይቅ

1. መረጃ መስጫ ቅጽ

ወደ ተሳታፊ፤

እንደምን አደሩ/ዋሉ። ከእኔጋ ለመወያየት ፍቃደኛ በመሆንዎ አመሰግናለሁ። እኔ _____ ስሆን የሰው ቴቢ በሽታ አምጭ የሆነው ተውሃስ ከሰው ወደ ዕንስሳ ወይም ከዕንስሳ ወደ ሰው ዕንዲተላለፍ አጋላጭ ምክንያቶችን ለማጥናት የመጣዉ የጥናት ቡድን አባል ነኝ። ጥናቱ የሶስተኛ ዲግሪ ትምህርት አካል ሆኖ በአዲስ አበባ ዩኒቨርሲቲ ተማሪ የሚከናወን ነዉ።

የዛሬው ጉብኝቱ ከላይ በጠቀስኩት ጉዳይ ከእርሰዎ መረጃ ለመውሰድ ነው። ምርመራዎ በሀኪም ይከናወናል። በምርመራ ከተለዩ በኋላ ከእርሰዎ 5ሚሊ የሚሆን የአክታ ናሙና ዕውስዳለሁ። በዚህ ጥናት ለመሳተፍ ፍቃደኛ ከሆኑ ከ 15-20 ዳቂቃዎች የሚወስድ ቃለ-መጠይቅ አደርግለዎታለሁ። የሚሰጡት መረጃ ሁሉ በሚስጢር የሚያዝ ነው። በምርመራው ወቅት የቴቢ በሽታ ከተገኘብዎ አስፈላጊውን ህክምና እንዲያገኙ ወዲያውኑ ወደ አቅራቢያዎ የጤና ተቋም ይላካሉ። በዕርሰዎ ከብቶችም ላይ በቆዳ ስር በሚሰጥ የቴቢ በሽታ መኖር አለመኖሩን ዕመረምራለሁ። ከ72 ሰዓታታ በኋላም ተመልሽ በመምጣት ከከብቶች ላይ ሊኖር የሚችለውን ውጤት አነባለሁ። በቆዳ ስር የሚሰጠው በከብቶችም ላይ አልፎ አልፎ ሊከሰት የሚችል የቆዳ መቆጣት ከማጋጠም ውጭ የሚያደርሰው ጉዳት የለም። ይሁን እንጂ መድሃኒቱ በተሰጠበት አካባቢ ከብቶቹ በበሽታው ከተያዙ እብጠት ይከሰታል፤ እብጠቱም ከቆይታ በሀኪላ ይጠፋል። ከብቶችም ለጥናቱ የሚሆነውን ከተወጉ በኋላ የፀረ-ተውሳክ መድሃኒት በመጠናቸው ልክ ይሰጣቸዋል።

በበሽታው የተያዙ ላሞች ከእርሰዎ ከተገኙ 30ሚሊ የሚሆን የወተት ናሙና ዕውስዳለሁ። በተጨማሪም በበሽታው የተያዙ ወንድ ከብቶች ከተገኙ በገበያ ዋጋ ገዝቸ ካረዱኩ በኋላ የቴቢ በሽታ ምልክት ያለበትን የአካል ክፍል ናሙና እወስዳለሁ። የተወሰደውም ናሙና በላቦራቶሪ ውስጥ በመዝራት ለጥናቱ ማዕከላዊ ለሆነው የቴቢ በሽታ መንስኤ የሆነውን ለመለየት አገልግሎት ብቻ ይውላል። የተገኘውም ውጤት ለእርሰዎ ይገለፅለዎታል።

ስምዎ ከቅጹ ላይ አይጻፈም፤ ከእርሰዎ መረጃ ጋርም በፍጹም ተያይዞ ጥቅም ላይ አይውልም። በመጠይቁ ጊዜ ለመመለስ የማይፈልጉት ጥያቄ ካለ ያለመመለስ መብት ያለዎት ሲሆን ጥናቱንም በፈለጉት ጊዜ ማቋረጥ ይችላሉ። ሆኖም የሚሰጡን ታማኝ መልስ ለእኔ ብዙ ይረዳኛል። በጥናቱ ላይ የሚኖረዎት ተሳትፎ ሙሉ በሙሉ በእርሰዎ ፍላጎትና ምርጫ ላይ የተመሰረተ በመሆኑ ለሚሳተፉበት የገንዘብ ክፍያ የለም። በጥናቱ መሳተፍ ወይም አለመሳተፍ ቢመርጡ መንግስታዊ ወይም መንግስታዊ ካልሆኑ ድርጅቶች ከሚያገኙት ማንኛውም የጤና እንክብካቤ ጋር ግንኙነት የለውም።

ጥናቱ በሚከናወንበት ጊዜ ምንም አይነት ሀሳብ ቢገባዎት ጥናቱን አስቁመው ሊጠይቁኝ ይችላሉ። በዛሬው ጉብኝቱ ላይ ግልጽ ያልሆነሎት ነገር ካለ ይግለጹልኝና ላብራራ። ከዚህም በተጨማሪ ባስፈለግዎት ጊዜ የዚህን ጥናት ዋና ተመራማሪ በ +2511752959 ወይም ደግሞ ጥናቱን ያፀደቁትን በ +251115538734 ወይም 251-0113211567 ደዉለዉ በመጠየቅ ተጨማሪ መረጃ ማግኘት ይችላሉ።

መረጃ ሰብሳቢ፡ ማንኛውንም ጥያቄ እንዲያነሱ ያበረታቷቸዉ።

2. የጥናት ተሳፍሮ ፍቃድ የመጠየቂያ ቅጽ

- ይህ ምርምር በሶስተኛ ደረጃ ድግሪ ተማሪ በአርዳያ መንግስቱ ተመራማሪነት በማህበረሰብ ጤና አጠባበቅ ትምህርት ቤትና በአርማውር ሀንሰን የምርምር ተቋም አስተባባሪነት እንደሚከናወን አውቁአለሁ። ስለ ምርምሩ ዓላማዎች በሚገባኝ ቋንቋ ተነግሮኛል። መጠይቁ ከ15-20 ደቂቃዎች ሊፈጅ እንደሚችል አውቁአለሁ።
- የምስጢው መረጃ በሙሉ በሚስጢር እንደሚያገዝ ተነግሮኛል። ምርመራውም በሀኪም እንደሚከናወን ተረድቻለሁ። የአክታ ናሙና ከእኔ ያለምንም ጉዳት እንደሚወሰድ እና በዳልጋ ከብቶቸ ላይ በቆዳ ስር የሚሰጥ የቲቢ በሽታ መለያ እንደሚኖርና ከብቶቸ ላይ ከተወጉበት ቦታ የቲቢ በሽታ ካለባቸው እብጠት ሊያጋጥም እንደሚችልና መድሃኒቱም ሲሰጥ አንዳንድ እንስሳት ላይ ለህይወት መጥፋት አደጋ ያልሆነ የቆዳ መቆጣት ሊጋጥም እንደሚችል አውቁአለሁ።
- ከብቶቸ መድሃኒቱን ከተወጉ በሀኪላ አስፈላጊ መጠን ፀረ-ተውሳክ እንደሚሰጣቸውና ከተወጉ ከ72 ሰዓታት በኋላ እንደሚታዩ አውቁአለሁ። በተጨማሪም በሽታው ካለባቸው ላሞች 30ሚሊ የሚሆን ወተት እንደሚወሰድ አውቁአለሁ። በበሽታው የተያዙ ወንድ የዳልጋ ከብቶች ከእኔ ከተገኙም በገበያ ዋጋ ለመሸጥ ተስማምቻለሁ።
- በፈለጉት ጊዜ ጥናቱን ለማቋረጥ መብት እንዳለኝና መረጃም ላለመስጠት እንደምችል አውቁአለሁ። በጥናቱ ባለመሳተፌ ከጤና ተቋምም ይሁን መንግስታው ካልሆነ የጤና ተቋም የማገኘው ጥቅም ችግር እንደማይገጥመው ተገልጿልኛል። ከዚህ በላይ ያለውን ቅፅ አንብቤ ወይም በሚገባኝ ቋንቋ ተነቦልኝ የተረዳሁ ስለሆነ በምርምሩ ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዎ አረጋግጣለሁ።

ስም _____ ፊርማ/የጣት
 አሻራ _____ ቀን _____

የመረጃ ሰብሳቢው

ስም _____ ፊርማ _____ ቀን _____

የነበሩ እማኞች

- 1.
- 2.

የተቆጣጣሪው/ተመራማሪው

ስም _____ ፊርማ _____ ቀን _____

ማሳሰቢያ - ግልፅ እንዲሆን የሚፈልጉት ነገር ካለ ከዚህ በታች የተጠቀሱትን ማነጋገር ይችላሉ። ዋና ተመራማሪውን፡ ስም አርአያ መንግስቱ፤ ስልክ ቁጥር 0911752959 ወይም የጥናቱን ተከታታይ 0912459707 ወይም ጥናቱን ያፀደቁትን ኮሚቴዎች በ 251115538734 እና አለርት/አህሪ 251-0113211567. አዲስ አበባ ዩኒቨርሲቲ፤ የህብረተሰብ ጤና ት/ቤት፤ ፖ.ሳ.ቁ. 9086፤ ስልክ፡ 011-5-157701፤ ፋክስ፡ 011-5-517701

INFORMED CONSENT

Parent/guardian name -----

- I, whose name is written above, have decided to allow my child to participate in the study after getting the following information explained clearly.
- That if I am volunteer to allow my child to give specimen, my child will know his TB status. I knew that test will be free of charge. I have been told that specimen taken from my child is sputum. If test result confirms that he/she is TB positive, he/she will get free treatment services for TB whether I will participate in study or not.
- I have been told that he/she will be asked about potential risk factors for manifestation of TB. I have been told that if study unrelated conditions are found during examination, my child will be given consultation by a health professional and forwarded to a duty physician or health facility where he/she can get a health care. I have been told that intradermal skin test will be conducted on my cattle to know the status of TB in my cattle. I have been told that I have the right not to take part in the study and that I can terminate participating in the study whenever I wish so, and that this will not affect in any way my child from getting treatment from health facilities for any health problem. I knew that I shall get no incentive for my participation in the study.
- I knew that the confidentiality of any information obtained from the laboratory examination and reply will be secured. After giving thorough thoughts on the issues explained above, I

hereby ascertain that I have accepted the invitation and am willing for participating my child in the study.

Parent/guardian Signature: _____ Date: _____

Name of supervisor/researcher: _____ Sig. _____ Date: _____

Name of witnesses: 1. _____ Sig. _____ Date: _____

2. _____ Sig. _____ Date: _____

N.B. If you have any question, please communicate Dr Araya Mengistu (0911752959), Dr Fikre Enqusellassie (0912459707), AAU, Medical Faculty IRB 0115538734 or AHRI IRB 011311334.

Addis Ababa University-Faculty of Medicine, School of Public Health, P.O. Box 9086, Tele: 011-5-157701, Fax: 011-5-517701

INFORMED ASSENT (15-17 years)

- I, whose name is written below, have decided to take part in the study after getting the following information explained clearly.
- That if I am volunteer to give specimen, I will know my TB status. Test will be free of charge. I have been told that the specimen taken from is sputum. If test result confirms that I am TB positive, I will get free treatment services for TB whether I will participate in study or not. I have been told that I will be asked about potential risk factors for manifestation of TB.
- I have been told also if study unrelated conditions are found during examination, I will be given consultation by a health professional and forwarded to a duty physician or health facility where I can get a health care. I knew that I have the right not to take part in the study and that I can terminate participating in the study whenever I wish so, and that this will not affect in any way my getting treatment from health facilities for any health problem. I have been told that I shall get no incentive for my participation in the study. I have been told that I shall get no payment for my participation in the study. Rather, I shall get TB test done free of charge with pre and post-test counseling and that the result will be sent to my physician.
- I understood that the confidentiality of any information obtained from my laboratory examination and reply will be secured. After giving thorough thoughts on the issues explained above, I hereby ascertain that I have accepted the invitation and am willing to participate in the study.

Name of the participant: _____ Sig: _____ Date: _____

Name of data collector: _____ Sig: _____ Date: _____

Name of supervisor/researcher: _____ Sig: _____ Date: _____

Name of witnesses 1. _____ Sig: _____ Date: _____

2. _____ Sig: _____ Date: _____

N.B. If you have any question, please communicate Dr Araya Mengistu (0911752959), Dr Fikre Enquesselassie (0912459707), AAU, Medical Faculty IRB 0115538734 or AHRI IRB 011311334.

Addis Ababa University-Faculty of Medicine, School of Public Health, P.O. Box 9086, Tele: 011-5-157701, Fax: 011-5-517701.

ለሳንባ በሽታ ምርመራ የስምምነት መግለጫ

የወላጅ/ያሳዳጊ ስም -----

- እኔ ስሜ ከላይ የተገለጸው ወላጅ/ሕጋዊ አሳዳጊ ከዚህ በታች ባገኘሁት መረጃ መሰረት ልጄ የሳንባ በሽታ ምርመራ እንዲደረግለት ወስኛለሁ።
- ለጥናቱ ልጄ ናሙና እንዲሰጥ ፈቃደኛ ከሆንኩ ልጄ ስለ እራሱ ሳንባ በሽታ ሁኔታ ያውቃል። ምርመራ የሚደረግለትም በነጻ እንደሆነ አውቄአለሁ። ከልጄ የሚወሰደው የአክታ ናሙና እንደሆነ ተነግሮኛል። እኔ በጥናቱ ተሳተፍኩም አልተሳተፍኩም በምርመራው ከልጄ የሳንባ በሽታ ከተገኘበት በነጻ የህክምና አገልግሎት እንደሚያገኝ አውቄአለሁ። ልጄ ለሳንባ በሽታ መከሰት አጋላጭ ሊሆኑ ስለሚችሉ ምክንያቶች እንደሚጠየቅ ተነግሮኛል።
- በሳንባ በሽታ ምርመራ ወቅት ተጓዳኝ በሽታዎች ከልጄ ከተገኙበት/ባት የምርመራ ውጤቱና ልጄ ወደ ተረኛ ሃኪም ወይም ጤና ድርጅት ቀርቦ/ባ ምክርና የጤና አገልግሎት እንደሚያስፈልግ/ታገኝ ተነግሮኛል። በእኔ የዳልጋ ክብቶች ላይም የሳንባ በሽታ ሁኔታቸውን ለማወቅ በቆዳ ስር በመስጠት የሚደረግ ምርመራ እንዳለም ተነግሮኛል። በጥናቱ ያለመሳተፍ መብቴ የተጠበቀ መሆኑ፤ በጥናቱ ላይ መሳተፍ ከጀመርኩ በኋላም በማንኛውም ጊዜ ከጥናቱ ማቋረጥ የምችል መሆኔንና በጥናቱ ባለመሳተፌ ወይም ደግሞ በማቋረጤ በልጄ ላይ የሚደርስ ጉዳት እንደሌለና ከጤና ተቋማትም ለማንኛውም የጤና ችግር ህክምና ማግኘትን እንደማይጎዳ ተነግሮኛል።

➤ በጥናቱ በመሳተፊ የማገኘው የገንዘብ ክፍያ እንደሌለ ተረድቻለሁ። ማንኛውም የሚገኝ የምርመራ ውጤትና መረጃ በሚስጢር የሚያዝ መሆኑን አውቄአለሁ። ይህን ሁሉ ግምት ውስጥ በማስገባትና በማገናዘብ ልጅ የጥናቱ ተሳታፊ እንዲሆን/ድትሆን የቀረበልኝን ግብዣ የተቀበልኩ ሲሆን ልጄም እንዲሳተፍ/ድትሳተፍ መፍቀዴን በፊርማዬ አረጋግጣለሁ።

የወላጅ/ያሳዳጊ ስም----- ፊርማ -----ቀን -----

የመረጃ ሰብሳቢ ስም -----ፊርማ -----ቀን -----

የተቆጣጣሪ/ተመራማሪ ስም: -----ፊርማ -----ቀን -----

የእማኞች 1. ስም-----ፊርማ -----ቀን -----

2. ስም -----ፊርማ -----ቀን -----

ማሳሰቢያ - ግልፅ እንዲሆን የሚፈልጉት ነገር ካለ ከዚህ በታች የተጠቀሱትን ማነጋገር ይችላሉ። ዋና ተመራማሪውን፡ ስም አርአያ መንግስቱ፤ ስልክ ቁጥር 0911752959 ወይም የጥናቱን ተከታታይ 0912459707 ወይም ጥናቱን ያወደቁትን ኮሚቴዎች በ 251115538734 እና አለርት/አህሪ 251-0113211567. አዲስ አበባ ዩኒቨርሲቲ፤ የህብረተሰብ ጤና ት/ቤት፤ ፖ.ሳ.ቁ. 9086፤ ስልክ: 011-5-157701፤ ፋክስ: 011-5-517701

ለሳንባ በሽታ ምርመራ የስምምነት መግለጫ (15-17 አመት ላሉ)

- እኔ ስሜ ከዚህ በታች የተገለጸው የዚህ ጥናት ተባባሪ ለመሆን በፍላጎቴ ስወስን የሚከሉትን ሁኔታዎች በግልጽ በመረዳት ነው።
- ለጥናቱ ናሙና ለመስጠት ፈቃደኛ ከሆንኩ ስለ ሳንባ በሽታ ሁኔታዬ አውቃለሁ። ምርመራ የሚደረገው በነጻ እንደሆነ አውቄአለሁ። የሚወሰደው የአክታ ናሙና መሆኑን ተነግሮኛል። እኔ በጥናቱ ተሳተፍኩም አልተሳተፍኩም በምርመራው የሳንባ በሽታ ከተገኘብኝ በነጻ የህክምና አገልግሎት እንደማገኝ አውቄአለሁ።
- ለሳንባ በሽታ መከሰት አጋላጭ ሊሆኑ ስለሚችሉ ምክንያቶች እንደምጠየቅ ተነግሮኛል። በሳንባ በሽታ ምርመራ ወቅት ተጓዳኝ በሽታዎች ከተገኙብኝ ወደ ተረኛ ሃኪም ወይም ጤና ድርጅት እንደምቀርብና ምክርና የጤና አገልግሎት እንደማገኝ ተነግሮኛል። በጥናቱ

ያለመሳተፍ መብቱ የተጠበቀ መሆኑ፤ በጥናቱ ላይ መሳተፍ ከጀመርኩ በኋላም በማንኛውም ጊዜ ከጥናቱ ማቋረጥ የምችል መሆኔንና በጥናቱ ባለመሳተፌ ወይም ደግሞ በማቋረጫ በእኔ ላይ የሚደርስ ጉዳት እንደሌለና ከጤና ተቋማትም ለማንኛውም የጤና ችግር ህክምና ማግኘትን እንደማይጎዳ ተነግሮኛል። በጥናቱ በመሳተፌ የማገኘው የገንዘብ ክፍያ እንደሌለ ተረድቻለሁ።

➤ ማንኛውም የሚገኝ የምርመራ ውጤትና መረጃ በሚስጢር የሚያዝ መሆኑን አውቄአለሁ። ይህን ሁሉ ግምት ውስጥ በማስገባትና በማገናዘብ እኔ የጥናቱ ተሳታፊ እንድሆን የቀረበልኝን ግብዣ የተቀበልኩ ሲሆን በጥናቱም ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

የተሳታፊ ስም -----ፊርማ -----ቀን-----

የመረጃ ሰብሳቢ ስም ----- ፊርማ ----- ቀን-----

የተቆጣጣሪ/ተመራማሪ ስም----- ፊርማ ----- ቀን-----

- የእማኞች
1. ስም ----- ፊርማ ----- ቀን -----
 2. ስም ----- ፊርማ ----- ቀን -----

ማሳሰቢያ:- ግልፅ እንዲሆን የሚፈልጉት ነገር ካለ ከዚህ በታች የተጠቀሱትን ማነጋገር ይችላሉ። ዋና ተመራማሪውን፡ ስም አርአያ መንግስቱ፤ ስልክ ቁጥር 0911752959 ወይም የጥናቱን ተከታታይ 0912459707 ወይም ጥናቱን ያፀደቁትን ኮሚቴዎች በ 251115538734 እና አለርት/አህሪ 251-0113211567. አዲስ አበባ ዩኒቨርሲቲ፤ የህብረተሰብ ጤና ት/ቤት፤ ፖ.ሳ.ቁ. 9086፤ ስልክ፡ 011-5-157701፤ ፋክስ፡ 011-5-517701.

Annex13. 2: Questionnaires

School of Public health, AAU

Armauer Hansen Research Institute

Questionnaire to Assess bovine tuberculosis as a Risk Factor for the occurrence of human pulmonary tuberculosis in North Gondar and North Wollo Zones, in Amhara National Regional State, Ethiopia.

SN	Questionnaire	Detailed to be responded	Skip
A	Socio-demographic information		
1	Name: _____ Age: _____	Date _____ Sex: Male (1) Female (2) House No____ Marital status: Married (1) Single (2) Divorced(3) Widowed (4)	
2	Dwelling	Urban (1) Rural (2) Periurban (3)	
3	Agroclimatic Zone	Dega (1) W/dega (2) Kolla (3)	
4	Number of families	Male_____ Female _____ Children under 5yrs: Male_____ Female _____	
5	Education status	Illiterate (0) Elementary (1) High school (2) Collage (3) Informal (4) Others (5), specify_____	
6	Religion	Orthodox(1) Muslims (2) Other (3), Specify_____	
7	Ethnicity	Amhara (1) Tigray (2) Agew/Agawi (3) Others (4), specify	
B	Animal information		
1	Types of animals	Breed types (use number): Local_____ Cross_____ Cattle (No) Ox _____ Cow _____	

	Heifers ____ Bull ____	Calves <6mths ____ >6mth-1year ____	
	Sheep: Male ____ Female ____	Goats Male ____ Female ____	
	Equines:	Horse ____ Mule ____ Donkey ____	
2	Purpose of keeping	Draught (1) Prestige (2) Milk (3) Meat (4)	
	If for milk use, Do you sale milk?	No (0) Yes (1); if yes:	
	For whom do you sale?	Local (1) Trader (2) Civil (3) I do not Know (4)	
3	If you boil or cook, do you know the reason?	No (0) Yes (1), could you Mention the reason ____	
4	Feeding habit	Raw milk: No (0) Yes (1) Boiled milk: No (0) Yes (1)	
		Raw meat: No (0) Yes (1) Cooked meat: No (0) Yes (1)	

School of Public health, AAU

Armauer Hansen Research Institute

Questionnaire to Assess bovine tuberculosis as a Risk Factor for the occurrence of human pulmonary tuberculosis in North Gondar and North Wollo Zones, in Amhara National Regional State, Ethiopia.

SN	Questionnaire	Detailed to be responded	Skip
C	Cattle management/husbandry		
1	Cattle management/feeding system	Free grazing (1) Stall feeding (2) Mixed (3)	
2	Your cattle keeping (day time)	Keeping alone (1) Mixing with others (2)	
	Keeping during night time	House sharing (1) Separate house (2) Other (3), Specify _____	
		If it is a separate house, mention the distance (meters) from the main house _____	
		If it is a separate house, who look after them? Son (1)) Father (2) Hired (3) Other (4), Specify _____	

3	Herding of your animals	All together (1) Separate(2)	
4	Housing of your animals	All together (1) Separate (2)	
5	Watering point for animals (WP)	Different wP (1) The same WP (2)	
	Did you use the same water source for home consumption? No (0) yes (1)		
	If the same source, do you treat the water? No (0) yes (1)		
	If you treat, what is the method used? Chemicals (1) Boiling (2) Filtration (3)		
6	Did you add cattle to your herd recently? No (0) Yes (1) . If yes, for what purpose?		If no skip
7	Did you sale cattle? No (0) yes (1) . If yes, why?		
	Did you sale a sick cattle? No (0) Yes (1), If yes		If no skip
	Did you tell the reason why you are selling? No (0) Yes (1). IF yes, why?		
8	Do you know disease/s transmitted from animals to human? No (0) Yes (1)		If no skip
	If yes, could you mention them?		
9	Currently do you have animals having cough? No (0) Yes (1).		If no skip
	If yes, Male (1) ___ Female (2)___		
	Duration of cough < 3months (1) 3- 6 months (2) 6mths-1yr (3) >1year (4)		
	Have you tried treatment? No (0) Yes (1). If yes, How was the response? No (0) Good (1)		
	How is the body condition of the animal/s? Good (1) Deteriorating (2) No change (3)		
	Do you have animals with wasting conditions? No (0) Yes (1)		
	Do you isolate the animal/s? No (0) Yes (1)		
Do you keep such animal in your house? No (0) Yes (1)			
What is your decision, if not cured? Slaughter (1) Sale (2) Gift (3) Other (4) specify			

Questionnaire to Assess bovine tuberculosis as a Risk Factor for the occurrence of human pulmonary tuberculosis in North Gondar and North Wollo Zones, in Amhara National Regional State, Ethiopia.

SN	Questionnaire	Detailed to be responded	Skip
D	Tuberculosis knowledge/history		
1	Did you know tuberculosis before?	No (0) Yes (1)	If no skip
	Where do you think it comes from?	I have no idea (1) Air (2) Dust (3) Other (4), specify	
2	For how long did you stay with the disease before diagnosis?	<6mths (1) >6mths-1yr (2) >1yr (3)	
3	Have you tried traditional medicine?	No (0) Yes (1). If yes, Where did you get?	If no skip
4	Do you have other family member affected with TB?	No (0) Yes (1) How many?_____	If no skip
5	Where did you acquire the infection?	From family (1) From guest (2) from neighbor (4) I do not know (5) Other (6), specify	
6	Do you know the type of TB the patient affected with?	No (0) Yes (1) , If yes, EPTB (1) PTB (2)	If no skip
7	Have you got the appropriate Treatment?	No (0) Yes (1)	
8.1	Did you complete the course?	No (0) Yes (1)	
8.2	If your answer is no for No 8.1 why?	Lack of money (1) Lack of awareness (2) Other (3) Specify	
9	Have you ever come with relapse?	No (0) Yes (1)	
10	If TB positive cattle obtained in your herd what you are going to do?	Sell (1) Slaughter(2) Keep the animal (3)	

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SN	Questionnaire	Detailed to be responded	Skip
E	Wildlife interface		
1	Are there wild animals around?	No (0) Yes (1), if yes	If no skip
	Could you list them?		
	If yes for No 1- Is there contact between wild and domestic animals	No (0) Yes (1)	
2	What are the common contact places	Grazing place (1) Water points (2)	
F	Possible human contact with cattle		
1	Do you share utensils with cattle?	No (0) Yes (1); if yes	If no skip
	Could you mention them?		
2	Do you have a habit of drenching medicaments with your mouth to cattle? Or giving after chewing?	No (0) Yes (1)	
3	Do your cattle have behaviour of licking human urine?	No (0) Yes (1)	
4	Do you give your urine to cattle?	No (0) Yes (1)	
5	Do you urinate on hay/straw?	No (0) Yes (1)	
6	Do animals lick your hand/head?	No (0) Yes (1)	
7	Do you have a habit of spitting sputum in the house? Animal feed?	No (0) Yes (1)	
G	Cattle House type and sanitary/hygienic issues		
1	House/pen type: Roof	Corrugate (1) Hat (2) Open air(3)	

2	Floor	Concert (1) Stone (2) Soil (3)	
3	Grass mulching	No (0) Yes (1)	
4	House ventilation	Closed (0) Have windows/fenestration (1) Holes (2)	
5	House(pen) cleaning habit	Per day (1) Once in 2-3 days (2) Twice a wk (4) Once in a week (3) Occasionally (5) No cleaning at all (6)	
6	Personal hygiene	washing after cleaning house/pen (1) Washing before milking (2) Washing after milking cows (3) Occasional washing after/before activities (4)	
7	Do you use detergents?	No (0) Yes(1); if yes	If no skip
8	What type of detergent you are using?	Modern (1) Local/traditional (2)	

ሀብረተሰብ ጤና ሳይንስ ት/ቤት ፣ አዲስ አበባ ዩኒቨርሲቲ

አርማውር ሀንሰን የምርምር ተቋም

በአማራ ብሄራዊ ክልላው መንግሥት በሰሜን ጎንደርና ሰሜን ወሎ ዞኖች የዳልጋ የከብቶች ቲቢ ለሰው የሳንባ ቲቢ መከሰት ሊኖራቸው የሚችለውን አጋላጭነት ሚና ለማጥናት የቀረበ መጠይቅ

ተ.ቁ	መጠይቅ	የሚመለስ ዝርዝር መጠይቅ	የሚዘለል
ሀ	አጠቃላይ መረጃ		
1	ስም: _____ ዕድሜ: _____	ቀን _____ ፆታ: ወንድ (1) ሴት (2) የቤት ቁጥር _____ የጋብቻ ሁኔታ: ያገባ/ች (1) ያላገባ/ች (2) የፈታ/ች (3) የሞተበት/ባት(4)	
2	መኖሪያ	ከተማ (1) ገጠር (2) ከተማ ዙሪያ (3)	
3	የአየር ሁኔታ	ደጋ (1) ወይና ደጋ (2) ቆላ (3)	
4	የቤተሰብ አባላት ብዛት	ወንድ _____ ሴት _____ ዕድሜያቸው ከ5 ዓመታት በታች: ወንድ _____ ሴት _____	
5	የትምህርት ሁኔታ	ያልተማረ (0) የመጀመሪያ ደረጃ (1) ሁለተኛ ደረጃ (2) ከፍተኛ ትምህርት (3) መደበኛ ያልሆነ ትምህርት (4) ሌላ (5)፤ ይገለፅ _____	
6	ሃይማኖት	ኦርቶዶክስ (1) ሙስሊም (2) ሌላ (3)፤ ይገለፅ _____	
7	ብሄረሰብ	አማራ (1) ትግሬ (2) አገው (3) (3), ሌላ (4)፤ ይገለፅ _____	
ለ	የእንስሳት መረጃ		
1	የእንስሳት ዓይነት	የእንስሳት ዝርያ (በቁጥር): የሀገር ውስጥ _____ የተዳቀሉ _____ ዳልጋ ከብት(በቁጥር): በሬ _____ ላም _____ ገደር _____ ወይፈን _____	
		ጥጃ ከ6ወር በታች _____ ጥጃ ከ6ወር በላይ-1ዓመት _____	
	በግ (ቁጥር): ወንድ _____ ሴት _____	ፍየል (ቁጥር): ወንድ _____ ሴት _____	
	የጋማ ከብቶች (ቁጥር)	ፈረስ _____ በቅሎ _____ አህያ _____	
	ከብቶችን የሚያረቡበት	ለእርሻ (1) ለዝና (2) ለወተት (3) ለስጋ (4)	

2	ምክንያት		
	ለወተት ጥቅም ከሆነ፤ ወተቱን ይሸጣሉ?	አልሸጥም (0) እሸጣለሁ (1); ከሸጠ:	
	ለማን ወተቱን ይሸጣሉ?	ለአካባቢው (1) ለነጋዴ (2) ለመንግስት ሰራተኛ (3) አላውቅም(4)	
3	ወተት ያፈላሉ ወይም ስጋን የሚቀቅሉ ከሆነ ምክንያቱን ያውቃሉ?	አላውቅም (0) አውቃለሁ (1), የሚያውቁ ከሆነ ምክንያቱን ይግለፁ _____	
4	የአመጋገብ ሁኔታ	ያልተፈላ ወተት: አልወስድም (0) እወስዳለሁ (1)	
		ጥሬ ስጋ: አልወስድም (0) እወስዳለሁ (1)	

ህብረተሰብ ጤና ሳይንስ ት/ቤት ፣ አዲስ አበባ ዩኒቨርሲቲ አርማውር ሀንሰን የምርምር ተቋም

በአማራ ብሄራዊ ክልላው መንግስት በሰሜን ጎንደርና ሰሜን ወሎ ዞኖች የዳልጋ ከብቶች ቲቢ ለሰው የሳንባ ቲቢ መከሰት ሊኖራቸው የሚችለውን አጋላጭነት ሚና ለማጥናት የቀረበ መጠይቅ

ተ.ቁ	መጠይቅ	የሚመለስ ዝርዝር መጠይቅ	የሚዘለል
ሐ	የዳልጋ ከብቶች አያያዝ ሁኔታ		
1	የዳልጋ ከብቶች አያያዝ/አመጋገብ	ግጦሽ (1) አስሮ መመገብ (2) ቅልቅል (3)	
2	በቀን የዳልጋ ከብቶች አጠባበቅ	ለየብቻቸው (1) ከሌሎች ከብቶች ጋር በመቀላቀል (2)	
	በሌሊት የዳልጋ ከብቶች አጠባበቅ	ከሰው ጋር የተያያዘ (1) የተለየ የከብቶች ቤት (2) ሌላ(3)፤ ይገለፁ _____	
	ማደሪያቸው የተለየ ቤት ከሆነ እርቀቱ ከዋናው ቤት በሜትር ስንት እንደሆነ ይገለፁ፤ _____		
	ማደሪያቸው የተለየ ከሆነ ማን ነው የሚጠብቃቸው ? ወንድ ልጅ (1)) አባት (2) ደመወዝተኛ (3) ሌላ (4)፤ ይገለፁ _____		
3	ከብቶችን መንጋ አጠባበቅ	ሁሉንም እንስሳ ባንድ ላይ (1) ለየብቻቸው (2)	
4	የከብቶች አዳር	ሁሉንም እንስሳ ባንድ ላይ (1) ለየብቻቸው (2)	
5	የውሃ መጠጫ	የተለየ ውሃ (1) ለሁሉም እንደ የውሃ ምንጭ (2)	
	ለሰውና ለእንስሳ አንድ የውሃ ምንጭ ትጠቀማላችሁ? የለም (0) አዎ (1)		የለም ከሆነ ይዘለል

	የሰውና የእንስሳ ምንጩ አንድ ከሆነ ወሃውን ታክሙታላችሁ? የለም (0) አዎ (1)	
	የምታክሙት ከሆነ በምን ታክሙታላችሁ? በኬሚካል (1) በማፍላት (2) በማጣራት (3)	
6	በቅርብ የዳልጋ ከብት ወደ መንጋዎ ቀላቅለዋል? የለም (0)አዎ (1)አዎ ከሆነ , ምክንያቱን ቢገልጹ?	የለም ከሆነ ይዘለል
7	በቅርብ የዳልጋ ከብት ሸጠዋል? የለም(0) አዎ(1) አዎ ከሆነ ለምን?	
	የታመመ እንስሳን ይሸጣሉ? የለም (0) አዎ (1): መልሱ አዎ ከሆነ:-	የለም ከሆነ ይዘለል
	እንስሳውን ለምን እንደሚሸጡት ምክንያቱን ይናገራሉ? አልናገርም (0) እናገራለሁ (1). የሚናገሩ ከሆነ ለምን?	
8	ከእንስሳት ወደ ሰው የሚተላለፍ በሽታ ያውቃሉ? አላውቅም (0) አውቃለሁ (1) የሚያውቁ ከሆነ ቢጠቅሷቸው?	የለም ከሆነ ይዘለል
9	አሁን የሚሰሉ ከብቶች አሉዎት? የለም (0) አዎ (1). አዎ ከሆነ, ወንድ (1) ሴት (2)---	የለም ከሆነ ይዘለል
	ሳሉ ለስንት ጊዜ ቆየ < 3 ወራት (1) 3- 6 ወራት (2) ከ6ወራት-1 ዓመት (3) ከ1 ዓመት በላይ (4)	
	ህክምና ሞክረዋል? የለም(0) አዎ(1). አዎ ከሆነ፤ , የህክምናው ውጤት እንዴት ነበር? ለውጥ የለም (0) ጥሩ(1)	
	የእንስሳው/ች የሰውነት ሁኔታ እንዴት ነው? ጥሩ (1) እየከሰ/ሱ ነው (2) ለውጥ የለም (3)	
	ሰውነቱ/ታቸው የሚከሰ/ሱ እንስሳ/ሳት አለ/ሉ? የለም (0) አለ (1)	
	የታመመ/ሙ እንስሳን/ትን ይለያሉ? አለይም (0) እለያለሁ (1)	
	የታመመ/ሙ እንስሳ/ዎችን ቤተሰብ ውስጥ ያስቀምጣሉ? አላስቀምጥም (0) አስቀምጣለሁ (1)	
	የታመመ/ትን እንስሳ/ት አሳክመው ባይድን/ኑ ምን ያደርጋሉ? አርደዋለሁ (1) እሸጠዋለሁ (2) ለሰው እሰጠዋለሁ(3) ሌላ(4)፤ ይገለፅ----	

ህብረተሰብ ጤና ሳይንስ ት/ቤት ፣ አዲስ አበባ ዩኒቨርሲቲ

አርማውር ሀንሰን የምርምር ተቋም

በአማራ ብሄራዊ ክልላዊ መንግሥት በሰሜን ጎንደርና ሰሜን ወሎ ዞኖች የዳልጋ ከብቶች ቲቢ ሰሎው የሳንባ ቲቢ መከሰት ሊኖራቸው የሚችሉውን አጋላጭነት ሚና ለማጥናት የቀረበ መጠይቅ

ተ.ቁ	መጠይቅ	የሚመለስ ዝርዝር መጠይቅ	የሚዘለል
መ	የቲቢ ዕውቀት/ታሪክ		
1	የቲቢ በሽታን ከዚህ በፊት ያውቁታል?	አላውቅም (0) አውቃለሁ (1)	ካላወቁ ይዘለል
	በሽታው ከየት መጣ ብለው ያስባሉ?	ሀሳብ የለኝም (1) በነፋስ/አየር (2) በአባሪ (3) ሌላ (4)፤ ይገለፅ -----	
2	የቲቢ በሽታው ከመታወቁ በፊት ከበሽታው ጋር ለምን ያህል ጊዜ ቆይተዋል?	ከ6 ወራት ያነሰ (1) ከ6 ወራት-1 ዓመት(2) ከ1 ዓመት በላይ(3)	
3	ለበሽታው ባህላዊ ህክምና ሞክረዋል?	አልሞክርኩም (0) ሞክራለሁ (1)፤ ከሞከሩ, መድኃኒቱን ከየት አገኙት?	ካልሞከሩ ይዘለል
4	ከቤተሰብዎ በቲቢ በሽታ የተያዘ ሌላ ሰው አለ?	የለም (0) አለ (1) ካለ ስንት? _____	የለም ካሉ ይዘለል
5	የቲቢ በሽታውን ከየት አገኙት?	ከቤተሰብ (1) ከእንግዳ (2) ከጎረቤት (4) አላውቅም(5) ሌላ (6)፤ ይገለፅ-----	
6	በሽተኛው የተጠቃበትን የቲቢ በሽታ ዓይነት ያውቁታል?	አላውቅም (0) አውቃለሁ (1) ካወቁት፤ የሳንባ ውጭ ቲቢ (1) የሳንባ ቲቢ (2)	ካላወቁ ይዘለል
7	እርስዎ ለቲቢ በሽታው ተገቢውን ህክምና አግኝተዋል?	አላገኘሁም (0) አግኝቻለሁ (1)	
8.1	ህክምናውን በጊዜ ገደቡ ጨርሰዋል?	አልጨረስኩም (0) ጨርሻለሁ (1)	
8.2	ለመጠይቅ 8.1 መልሰዎ አልጨርሰኩም ከሆነ ለምን?	የገንዘብ እጥረት(1) ካለማወቅ (2) ሌላ (3)፤ ይገለፅ-----	
9	የቲቢ ግርሻ አጋጥሞዎት ነበር?	አላጋጠመኝም (0) አጋጥሞኛል (1)	
10	በቲቢ የተያዘ ከብት ከመንጋዎ ቢገኝ ምን ያደርጋሉ?	እሽጠዋለሁ (1) አርደዋለሁ(2) ከመንጋው ጋር ይኖራል (3)	

ሀብረተሰብ ጤና ሳይንስ ት/ቤት ፣ አዲስ አበባ ዩኒቨርሲቲ

አርማውር ሀንሰን የምርምር ተቋም

በአማራ ብሄራዊ ክልላው መንግሥት በሰሜን ጎንደርና ሰሜን ወሎ ዞኖች የዳልጋ ከብቶች ቲቢ ለሰው የሳንባ ቲቢ መከሰት ሊኖራቸው የሚችለውን አጋላጭነት ሚና ለማጥናት የቀረበ መጠይቅ

ተ.ቁ	መጠይቅ	የሚመለስ ዝርዝር መጠይቅ	የሚዘለል
ሰ	ክዳር እንስሳት ጋር ያለ ግንኙነት		
1	በአካባቢው የዳር እንስሳት አሉ?	አሉ (0) የሉም (1)፤ ካሉ	የሉም ከሆነ ይዘለል
	ስማቸውን ሊዘረዝሯቸው ይችላሉ?		
	ለመጠይቅ 1 መልሰዎ አሉ ከሆነ በዳርና በቤት እንስሳት መካከል ግንኙነት አለ?	የለም (0) አለ (1)	
2	የጋራ መገናኛ ቦታዎች የትኛዎቹ ናቸው?	የግጦሽ ቦታዎች (1) የውሃ መጠጫ ቦታዎች (2)	
ረ	የሰውና የእንስሳት ንክኪ		
1	ቁሳቁሶችን ከከብች ጋር በጋራ ይጠቀማሉ?	አልጠቀምም (0) እጠቀማለሁ (1)፤ ከተተቀሙ	ካልተጠቀሙ ይዘለል
	ስማቸውን ሊዘረዝሯቸው ይችላሉ?		
2	ለከብቶች በአፈዎ መድሃኒት በአፋቸው ይሰጣሉ? ወይም አኝከው ይሰጣሉ?	አልሰጥም (0) እሰጣለሁ (1)	
3	ከብቶች የሰው ሽንት የመላስ ባህሪ አላቸው?	የላቸውም (0) አላቸው (1)	
4	እርሶዎ ለከብቶች ሽንተዎን ይሰጣሉ?	አልሰጥም (0) እሰጣለሁ (1)	
5	እርሶዎ ድርቆሽ ወይም ገለባ ላይ ሽንተዎን ይሸናሉ?	አልሸናም (0) እሸናለሁ (1)	
6	ከብቶች እጆቻቸውን ወይም እራስዎን ይልሳሉ?	አይልሱም (0) ይልሳሉ (1)	
7	አክታዎን ቤት ውስጥ ወይም ከእንስሳት መኖሩ ላይ ይተፋሉ?	አልተፋም (0) እተፋለሁ (1)	
ሠ	የከብቶች ማደሪያና ንፅህና ሁኔታ		
1	የከብቶች ቤት ወይም በረት ፣ ጣራ	ቆርቆሮ(1) የእሳር ጎጆ(2) ክፍት (3)	
2	ወለሉ	ስሚንቶ(1) ድንጋይ (2) አፈር (3)	

3	እሳር መጎዘጎዘ	አልጎዘጎዘም (0) እጎዘጎዘለሁ(1)	
4	የቤቱ አየር ዝውውር	ዝግ (0) መስኮት/ወንፊት(1) ቀዳዳ (2)	
5	ቤታቸውን/በረታቸውን ማፅዳት	በየቀኑ (1) አንድ ጊዜ በ 2-3 ቀናት (2) በሳምንት ሁለት ጊዜ (4) በሳምንት አንድ ጊዜ (3) አልፎ አልፎ (5) አይፀዳም (6)	
6	የግል ንፅህና አጠባባቅ	ቤታቸውን/በረታቸውን ካፀዳሁ በኋላ መታጠብ (1) ከማለብ በፊት መታጠብ(2) ካለቡ በኋላ መታተብ (3) ከስራ በፊት/በኋላ አልፎ አልፎ መታጠብ (4)	
7	ማፅዳቶችን ይጠቀማሉ ?	አልጠቀምም (0) እጠቀማለሁ(1)፤ ከተጠቀሙ	ካልተጠቀሙ ይዘለል
8	ምን አይነት ማፅዳ ይጠቀማሉ?	ዘመናዊ (1) ባህላዊ (2)	

Study Title: The Epidemiological Role of Cattle in the Occurrence of Human Pulmonary Tuberculosis in the Rural Community of Northwest and Northeast Parts of Amhara Region, Ethiopia

Questionnaires used to collect data from chronic coughers in North Wollo Zone.

1. Area: Region_____ Zone_____ District_____ Kebele _____
Got_____ EcoZone _____

2. Socio-demographic Data: Name (head) _____ Sex Male 1—Female 2
Age _____ Religion _____ Filed IDNo _____ AHRI IDNo _____
Family size: _____ Male _____ Female _____
Age: Under 5 (1) >5 -15 (2) >15-25 (3) >25-35(4)
 >35-45(5) >45-55 (6) >55 (7)

Education Illiterate (0) Primary education (1) Secondary education (2)
University (3) Informal (4)

3. Was there a history of tuberculosis in the household? No (0) Yes (1)

4. If yes, did the patient get appropriate treatment? No (0) Yes (1)

5. Did the patient complete his course of treatment? No (0) Yes (1)

6. Number of individuals having cough in the household _____

7. Duration of cough (More than 2wks): _____

8. Action taken to combat the cough No action taken (0) Local/traditional treatment (1) Modern (2)

9. If action was taken, Conditions after taking the treatment: No change (0) Improved (1)
Aggravated (2)

10. Habit of drinking unboiled milk: No (0) Yes (1)

11. Habit of eating uncooked meat: No (0) Yes (1)

12. History of contact with tuberculosis patient: No (0) Yes (1)

በአማራ ብሄራዊ ክልላዊ መንግሥት በሰሜን ወሎ ዞን ለሰው ቲቢ በሽታ የዳልጋ ከብቶች የሚኖራቸውን ሚና ለማወቅ በቲቢ በሽታ ለተጠረጠሩ ሰዎች የቀረበ መጠይቅ

1. አካባቢ: ክልል _____ ዞን _____ ወረዳ _____ ቀበሌ _____ ጎጥ _____ የአየር ንብረት _____
2. አጠቃላይ መረጃ: ስም _____ ያታ _____ ወንድ (1) ሴት (2) ዕድሜ: _____
 ሃይማኖት _____
 የቤተሰብ ብዛት: _____ ወንድ _____ ሴት _____
 ከዓምስት ዓመት በታች (1) >5 -15 ዓመት (2) >15-25 (3) >25-35(4) >35-45(5)
 >45-55 (6) >55 (7)
 የትምህርት ሁኔታ: ያልተማረ (0) አንደኛ ደረጃ (1) ሁለተኛ ደረጃ (2) ከፍተኛ (3)
 መደበኛ ያልሆነ (4)
3. በቤት ውስጥ የቲቢ በሽታ ነበር? አልነበረም (0) አዎ (1)
4. መልሰዎ አዎ ከሆነ፣ በቲቢ ታማሚው ተገቢውን ህክምና አግኝቶ ነበር? አላገኘም (0)
 አግኝቷል (1)
5. ታካሚው የህክምና ጊዜውን ጨርሷል? አልጨረሰም (0) ጨርሷል (1)
6. በቤትዎ ውስጥ የቆየ ሳል ያለባቸው ስንት ሰዎች አሉ? _____
7. ሳሉ ለምን ያህል ጊዜ ቆይቷል? (ከሁለት ሳምንታት በላይ) _____
8. ለሳሉ ምን እርምጃ ተወስደ? አልተወሰደም (0) ባህላዊ ህክምና (1) ዘመናዊ (2)
9. ለሳሉ እርመጃ ከተወሰደ፣ ከህክምና በኋላ የሳሉ ሁኔታ፣ ለውጥ የለውም (0) ለውጥ አለው (1) ተባብሷል (2)
10. ያልተፈላ ወተት የመጠጣት ልምድ አለዎት? የለኝም (0) አለኝ (1)
11. ያልተቀቀለ ስጋ መብላት ልምድ አለዎት? የለኝም (0) አለኝ (1)
12. በቲቢ ከተያዘ ሰው ጋር ግንኙነት ነበረዎት? አልነበረኝም (0) ነበረኝ (1)

Format 3: Case Record Form-Sputum Sample

Name of the health institute: _____

Date _____

Centre Card No. _____ Code No. _____ AHRI No _____

1. Age _____

2. Sex: 1. Male 2. Female

3. Address: 1. Urban / Rural _____ Keb /PA _____ House No _____
Got _____

4. Occupation: 1. Merchant 2. Student 3. Housewife

4. Government employee 5. Peasant

6. Abattoir worker 7. others specify _____

5. Educational Level: 1. Illiterate 2. Primary School 3. Secondary School

4. Diploma 5. Degree 6. Others specify _____

6. Martial status: 1. Single 2. Married 3. Divorced 4. Widowed

5. Living with Partner

7. Family size (number of people living in one household): _____

8. Ethnic group: 1. Amhara 2. Oromo 3. Tigre

4. Agew 5. Guragie 6. Others specify _____

9. Religion: 1. Christian (Orthodox, protestant, catholic) 2. Muslim

3. others specify _____

10. History of contact with tuberculosis patients: 1. Yes 0. No

If yes, who was it? 1. Family member 2. Neighbours 3. Friends

4. Others _____

11. History of BCG vaccination (for children): 1. Yes 0. No

12. How many cattle do you have? _____

ቅፅ 3: ለቲቢ በሽተኛ አክታ ለመሰብሰብ የቀረበ መጠይቅ

የህክምና መስጫ ተቋም ስም _____

ቀን _____

የበሽተኛው የካርድ ቁጥር _____ የበሽተኛው የሚስጢር መለያ ቁጥር _____ የአህሪ ቁጥር _____

1. ዕድሜ _____ 2. ፆታ _____ 1. ወንድ 2. ሴት

3. አድራሻ: ከተማ/ገጠር _____ ቀበሌ/ቀበሌ ገ/ማህበር _____ የቤት ቁጥር _____ ጎጥ _____

4. ስራ: 1. ነጋዴ 2. ተማሪ 3. የቤት ዕመቤት 4. የመንግስት ሰራተኛ

5. አርሶ አደር 6. ቁራ ሰራተኛ 7. ሌላ (የገለፅ) _____

5. የትምህርት ደረጃ: 1. ያልተማረ 2. 1ኛ ደረጃ 3. 2ኛ ደረጃ 4. ዲፕሎማ 5. ድግሪ 6. ሌላ (የገለፅ) _____

6. የጋብቻ ሁኔታ: 1. ያላገባ/ች 2. ያገባ/ች 3. የፈታ/ች 4. የሞተበት/ባት 5. በጓደኝነት መኖር

7. የቤተሰብ ብዛት _____

8. ብሄረሰብ: 1. አማራ 2. ኦሮሞ 3. ትግራይ 4. አገው 5. ጉራጌ 6. ሌላ (ይገለጽ) _____

9. ሀይማኖት: 1. ክርስትና (ኦርቶዶክስ፣ ፕሮቴስታንት፣ ካቶሊክ) 2. ሙስሊም 3. ሌላ (ይገለጽ) _____

10. ከቲቢ ታማሚ ጋር ንክኪ/ግንኙነት ነበረ ወይ? 0. የለም 1. አዎ

አዎ ካል ከማን ጋር ነበር? 1. የቤተሰብ አባል 2. ጎረቤት 3. ጓደኛ 4. ሌላ (ይገለጽ) _____

11. ህጻናት የመከላከያ ክትባት ወስደው ነበር? 1. አዎ 0. የለም

12. ስንት የዳልጋ ከብቶች አሉዎት?-----

Annex 13. 3: Formats used for different purposes

Study Title: The Epidemiological Role of Cattle in the Occurrence of Human Pulmonary Tuberculosis in the Rural Community of Northwest and Northeast Parts of Amhara Region, Ethiopia

Form- I: Format used to record PPD skin test on cattle

Zone _____ District _____ Kebele _____ Got _____

Date _____

S/no	Owner name	Owner Id	Cattle history							Skin thickness in mm				DI F	IN T
										Avian		Bovine			
			Breed	IDNo	Sex	Age	Color/ name	Source	B.C	PRT	PT	PRT	PT		
1															
2															
3															
4															
5															
6															
7															
8															
9															

Source:

HB: Homebred
P: Purchase
G: Gift

Body Condition (B.C)

L: Lean
M: Medium
F: Fat

PRT: Pre-test

PR: Posttest

DIF: Measurement difference between Bovine and avian test

sites INT: Interpretation

Study Title: The Epidemiological Role of Cattle in the Occurrence of Human Pulmonary Tuberculosis in the Rural Community of Northwest and Northeast Parts of Amhara Region, Ethiopia

Form-II: Format used to collect tissue/milk samples from cattle

Date	Owner Name	Owner ID	District	Kebele	Animal history							ST-M	Lesion type	Sample type
					Breed	IDNo	Sex	Age	Color /name	Source	B. C			

NB:

If the sample was tissue: specified as Lung, lymph node, Head lymph node, Liver, Intestine

Type of lesion: Characterize as Generalized, local, calcified

BC: Body Condition score

ST-M: Skin Test Measurement

Addis Ababa University, School of Public health

Armauer Hansen Research Institute

Study Title: **The Epidemiological Role of Cattle in the Occurrence of Human Pulmonary Tuberculosis in the Rural Community of Northwest and Northeast Parts of Amhara Region, Ethiopia**

Form III. Contact Slip: For Sputum AFB Positive Individuals

1. Name of the health institute: _____ *Zone* _____
District _____
2. Date _____ Centre Card No. _____ Code No. _____
3. Name of the patient (head) _____ Sex Male 1—Female 2__
4. Specific area to contact: *Kebele* _____ Got/Village _____
Liyu Got _____ Cell Phone _____
5. Estimated distance from the health institute (could be explained in Hours) _____
6. Road accessibility: Yes ___ No _____
7. Name of the *Kebele* Leader (any locally authorized person who will know the patient)
_____ Phone Number _____
8. Preferred days to contact you and conduct SKIN test on your cattle

9. Could you arrange persons having cattle to carry out skin test on their cattle? Yes ___ No__

Addis Ababa University, School of Public health

Armauer Hansen Research Institute

Study Title: **The Epidemiological Role of Cattle in the Occurrence of Human Pulmonary Tuberculosis in the Rural Community of Northwest and Northeast Parts of Amhara Region, Ethiopia**

Form- IV: Format used to collect sputum sample from Tuberculosis Patients (AFB positives)

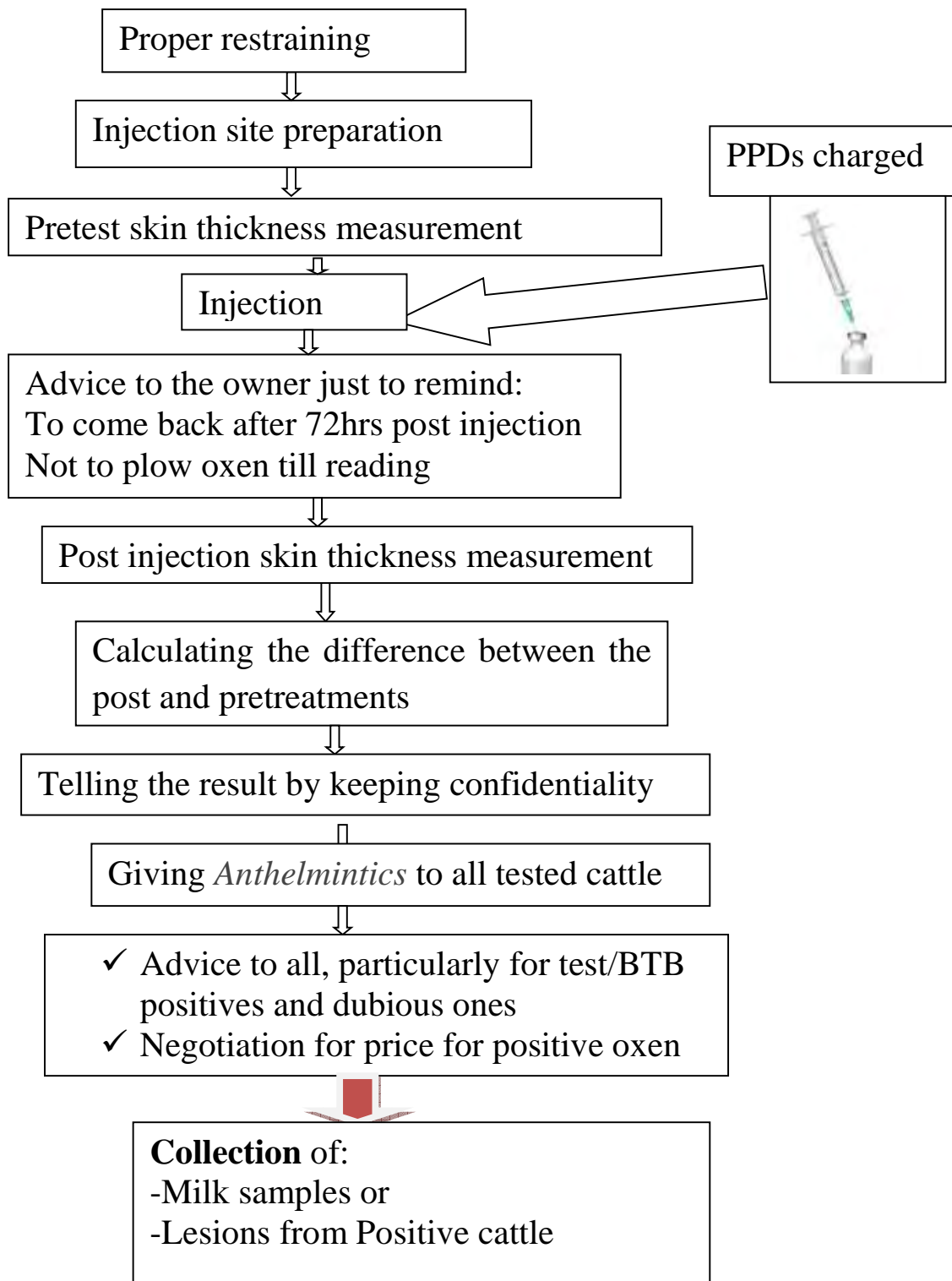
Date _____	Zone _____	District _____	Kebele _____	Got _____
Patient Name _____	<i>EcoZone</i> _____	Hospital ID _____	Possible contact person _____	
Age _____ Sex _____	Sample code _____	AHRI No _____	Type of Tb (pulmonary) _____	
Responsibility in the house _____	New case _____	Relapse _____	Suspicion of MDR (Yes/No) _____	
	If relapse treatment given _____		Sample collected by Name _____ Sig _____ Date _____	
Date transported to AHRI _____	Received by _____	Sig _____	Storage site _____	
Possible remarks: _____				

Study Title: The Epidemiological Role of Cattle in the Occurrence of Human Pulmonary Tuberculosis in the Rural Community of Northwest and Northeast Parts of Amhara Region, Ethiopia

Form- V: Format used to record laboratory results

Date submitted to AHRI _____	Zone _____ District _____	Kebele _____ Got _____
Patient ID _____	Sample type (Sputum) Sample code _____	AHRI No _____ Date processed _____
	Culture positivity Glycerol____ Pyruvate____ Both____	AFB Stain
	DNA amount _____ Deletion typing____ Species _____	Spoligotyping_____ Species_____ Further test_____
	<i>Possible remarks</i>	
Date submitted to AHRI _____	Zone _____ District _____	Kebele _____ Got _____
Owner Name	Sample type (Milk or tissue) Sample code _____	AHRI No _____ Date processed _____
Cattle Name	Culture positivity Glycerol____ Pyruvate____ Both____	AFB Stain _____
	DNA amount _____ Deletion typing____ Species _____	Spoligotyping_____ Species_____ Further test_____
<i>Possible Remarks</i>		

Annex13. 4: Intradermal Skin Test Procedure



Annex 13. 5: List of manuscripts

- I. Mengistu A, Enquasselasie F., Mulatu F., Hailu E., and Beyene D. 2013. **Tuberculosis Infection in Cattle and Cattle Owners in North Western parts of Ethiopia.** (Status: Accepted by Journal of Biology and Medical Sciences).
- II. Mengistu A, Enquasselasie F., Aseffa A. and Beyene D. 2014. **Bovine Tuberculosis in rural Ethiopia: A comparative Cross-sectional Study on Cattle Owned by Households with and without Tuberculosis (TB).** (Status: Submitted to African Health Sciences-African Journals-online. Recommend to publish with minor comments/revisions and the revised version is resubmitted).
- III. Araya Mengistu, Fikre Enquasselasie, Abraham Aseffa and Demissew Beyene. 2014. **Bovine Tuberculosis (BTB) as a risk factor for developing Tuberculosis in humans in the rural community of Ethiopia: A Case-control study.** (Status: Published on Ethiopian Medical Journal: *Ethiop Med J, 2015, Vol. 53, No. 1*).
- IV. Araya Mengistu, Fikre Enquesselassie, Elena Hailu, Abraham Aseffa, and Demissew Beyene. 2014. **Identification and Characterization of *Mycobacterium Tuberculosis* Isolated from Cattle Owners in North Western and North Eastern Parts of Rural Ethiopia.** (Published on International Journal of Innovation and Applied Studies, ISSN 2028-9324 Vol. 10 No. 1 Jan. 2015, pp. 85-94).

Tuberculosis Infection in Cattle and Cattle Owners in North Eastern parts of Ethiopia

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Abstract

Introduction: Ethiopia is one of the highest TB burden countries in the world. Transmission of *M. tuberculosis* between humans is well known, however, little is known about the transmission of *M. tuberculosis* complex species between human and animals, in particular cattle. The purpose of this study was, therefore, to investigate the status of bovine tuberculosis infection in cattle owned by tuberculosis suspected households and isolate *M. tuberculosis* complex species from sputum of chronic coughers in North Wollo Zone of the Amhara National Regional State, Ethiopia.

Method: A cross-sectional study was conducted in selected *Kebeles* of North Wollo Zone. An intradermal tuberculin skin test was carried out on 381 cattle and sputum samples collected from 124 chronic coughers. Sputum samples were cultured and positive ones were identified by polymerase chain reaction using RD4 and RD9 as a marker.

Result: Of the 124 sputum samples cultured, 4/ 124 (3.2%) were positive. Polymerase Chain Reaction using has confirmed that 2/4 (50%) were found to be *M. tuberculosis* while the rest was atypical *Mycobacterial species*. Of the 381 tested cattle 5/381 (1.31%) and 10/38 (2.63%) were found positive according to manufacturer recommendation (with a cutoff value > 4mms) and a cutoff value > 2mms, respectively. About 67% (6/9) of the individuals who owned positive cattle were having the habit of drinking raw milk. Among the ten positives, five of them resided in lowland (*Kolla*) areas (1300-1500mabs). However, none of the owners of tuberculin positive cattle were found to be TB positive.

Conclusion: This study has shown 3.2% of culture positivity and of these two were found to be *M. tuberculosis*. The sputum sample culturing from suspected chronically coughing individuals would help detection of the TB causative agents. According to the current findings BTB is considerable in Ethiopia and cattle should be seen as a potential source of zoonotic TB to humans.

Key words: bovine/cattle, bovine tuberculosis, cough, North Wollo, skin test, tuberculosis.

Introduction

Human TB, caused by *M. tuberculosis (M.tb)*, is among the oldest diseases in the world and is the seventh leading cause of death worldwide [1]. WHO [2] reported that 98% of tuberculosis deaths occurred in the developing world, affecting mostly young adults in their productive ages. Despite efforts made, globally or nationally to curb the effects of TB, different studies have shown that the trend of pulmonary TB remained as a health problem in the world [3-7]. In 2013, WHO [8] reported a decline in the impact of tuberculosis by the year 2012. The challenge of TB is becoming complicated by the occurrence of HIV/AIDS, the emergence of multi-drug resistant TB and extremely multi-drug resistant and to some extent with totally multidrug resistant strains reported in some parts of the globe [9]. TB is still killing nearly 5000 people every day and 1.8 million deaths in 2008 alone [10]. If TB is left unchecked within 20 years, it will kill 35 million people [9].

Ethiopia ranks third in Africa, 8th among the 22 highest TB burdened countries in the world and the prevalence of all forms of TB is estimated at 261 per 100 000 population, leading to an annual mortality rate of 64 per 100 000 population [11]. In 2008 the Amhara National Regional State Health Bureau reported 1.6% and 3.5% TB case detection and TB death rates, respectively [12]. The sources of infection for *M. tb* are mainly those humans who are developing active TB [13]. However, *M. tb* has been isolated from different animal species, including bovines/cattle, which should be seen as a challenge to further move in the prevention or controlling effort made to tackle the disease. In Ethiopia, little is known about the transmission of *M. tb complex* from cattle to humans. The purpose of this study is therefore, to investigate/determine the prevalence of BTB and see the possible role of cattle in the epidemiology of human TB and isolate *Mycobacterium tuberculosis complex (MTC)* species in North *Wollo Zone*, the Amhara National Regional State.

Methodology

Study area

This study was conducted in North *Wollo Zone*, Amhara National Regional State (ANRS). *Woredas* namely; *Meket*, *Gidan*, *Gubalafto/Woldeya* and *RayaKobo*, were included in the study. The *Kebeles*

comprised of lowland, mid-altitude and highlands. The *Zone* has a total population of 1,500,303 and a total of 355,974 households were counted and the cattle population was 836697 [14].

Study Design and study setting: The study was Cross sectional and community based, which was conducted from December 5-24/2012.

Study population, sampling method and sample size

In this study, individuals suffering from chronic cough for a duration of two or more weeks were suspected as tuberculosis cases [15, 16]. Sputum samples were collected from TB suspected individuals who own cattle and comparative cervical intradermal tuberculin test (CCITT) were conducted on their cattle. Single population proportion sample size determination was used, with the assumptions, prevalence of 50% *M. tuberculosis* and [17, 18] 5% precision and 95% confidence interval. Based on this, 314 bovines were required to be tested. *Gidan*, *Gubalafte*, *Rayakobo* and *Meket Woredas* were selected purposefully and 314 cattle's were distributed proportional to sample size to each *Woreda*. Based on these 39, 79, 79 and 117 cattle were planned to be tested in *Gidan (Eyela Kebele)*, *Gubalafte (Woynye and Sibilkay Kebeles)*, *RayaKobo (Addis-alem and Robit area Kebeles)* and *Mekete (033 and 044 Kebeles) Woredas*, respectively. Assuming an average of 3 cattle owned by a household in Amhara region, approximately 105 households were required to participate in the study. The seven *Kebeles* listed above (study sites), were selected based on the report having a high TB occurrence or being suspected to have TB by the local authorized personnel.

Intradermal Skin test on Cattle

Comparative cervical intradermal tuberculin test was conducted as manufacturer's recommendation [19]. The operation was performed on either side of the animal middle neck region. Two circular areas of about 2.5cm diameter, about 12–15 cm apart, on the cervical area of the skin, were shaved using a scalpel blade. The initial skin thickness was measured with a digital caliper [20]. Measurements were read to the nearest millimeter. Lelystad (Netherlands) products of 0.1 ml (20 000 IU/ml) of bovine tuberculin PPD at one site and 0.1 ml (25 000 IU/ml) of avian tuberculin PPD at the other site were injected into the dermis using BD1/2 tuberculin syringe [21]. Correct injection was confirmed by palpating a small pea-like swelling at each site of injection. Skin thickness was measured again at both injection sites after 72 hrs \pm 4hrs. The reaction at each site was derived by calculating the difference of

the two tests. The results were interpreted according to the manufacturer recommendations [19] using the cutoff point for positivity of the test, that is >4mms difference, calculated as the difference between skin thicknesses of bovine tuberculin (B) and avian tuberculin (A) injection sites (B – A). But, Ameni *et al.*, (2008) [22] reported >2mm difference as positive in Ethiopia. Based on his report, the test was considered positive if the difference was greater than 2 mm.

Cattle body weight estimation

For the purpose of this study the method mentioned by Nicholson and Butterworth, (1986) [23] with modifications was used to determine the body condition of the zebu cattle.

Sputum Sample collection and processing

For *M. tuberculosis* isolation sputum samples were collected from individuals suffering from chronic cough, according to Cheesbrough [24]. Suspected cases were asked to produce an “on spot” sputum specimen in a 20 ml screw plastic container under the supervision of a trained laboratory technician in the open air. Those cases who produced sputum were asked to give additional sputum samples on the second day and then on the spot. The sputum samples obtained from each patient was put in an ice box with ice packs (4°C), and immediately after collection, it was transferred to the hospital laboratory to be stored at -20°C. The samples collected from each site transported to Armauer Hansen Research Institute/AHRI TB laboratory to be processed.

Briefly, an equal amount of Phosphate-buffered saline solution was added to the sputum and then decontaminated, digested with equal volume of 4% sodium hydroxide for 15 minutes. Then vortexing was done in a closed tube till the mixture becomes homogeneous. The homogenate was centrifuged at 3000 RPM for 15 minutes. Neutralization done by using concentrated hydrochloric acid. In order to monitor neutralization 1 or 2 drops of phenol red was added. The supernatant was decanted and the sediment was inoculated onto Lowenstein-Jensen medium (tubes containing glycerol and sodium pyruvate) using the drop method. The tubes were incubated at 37°C and examined for growth weekly for 12 weeks [25, 26]. Cultures were considered negative if there was no mycobacteria growth after 12weeks of incubation. Positive cultures were sub-cultured onto another set of media and incubated for another 3–4 weeks for further identification. Polymerase chain reaction using RD4 and RD9 as a marker was conducted. Armauer Hansen Research Institute (AHRI) *M. tuberculosis* (H37Rv) and *M. bovis* (AHRI No.1632) isolates used as a positive control.

Data management, entry, analysis and data quality

Good data management practices such as study monitoring, timely data archiving and transferring, sorting and filing were done. The collected data were coded and entered into EpiInfo version 3.2.1 [27]. Data cleaning and analysis were done by using SPSS software version 16.0 [28]. Descriptive statistics like frequency distributions, percentages, mean and standard deviations were used to describe the findings. Tables were used to present the results. The chi-square test was conducted to assess associations. All statistical tests were two-sided and significance was considered at a p-value <0.05. All data were collected in collaboration with experts and checked regularly while collecting the data by the principal investigator. All tuberculin tests were conducted by the same person and sputum sample collections and culturing done by laboratory personnel.

Exclusion criteria

Individuals having unproductive cough, less than 2 weeks duration of cough, those who have no cattle as well as under TB treatment and below 15 years were excluded. If sputum was not produced within 15 minutes he/she was exempted. Female cattle either recently delivered or at their last trimester, newly added, and those less than 6 months of old as well as aggressive cattle were not included in the study.

Ethical considerations

Ethical clearance was obtained from the college of health sciences, Addis Ababa University institutional review board (IRB) and AHRI/ALERT (Armauer Hansen Research Institute) ethics committee. Permission was obtained from the Amhara Regional Health Bureau. The aim of the study was explained to the patient who was complaining chronic cough with one or more additional clinical signs and his/her willingness were asked to participate in the study. If he/she is willing to participate, consent were taken and sputum samples were collected. Confidentialities were maintained and to maintain confidentiality codes was used rather than participants' names. Based on the results individuals who own positive cattle were advised and those individuals whose sputum samples were cultured positives communicated through health institutes to receive appropriate treatments and conduct family screening.

Results

The study covered *Dega* (highland) 3 (42.9%), *Woina Dega* (midland) 3 (42.9%) and *Kolla* (lowland) 1 (14.2%) agroecological *Zones*. One hundred twenty four individuals (households) were reported to have a chronic cough in four words. Of the study participants, 76% were males **Table 1**.

Table-1: Characteristics of TB suspected cases in North *Wollo Zone*, Ethiopia, 2013.

Characteristics (n=124)		No (%)	Characteristics		No (%)
Sex	Male	91 (73.4)	Religion	Orthodox	114 (91.9)
	Female	33 (26.6)		Muslim	10 (8.1)
Age in years	18-28	10 (8.1)	Education	Illiterate	86 (69.4)
	29-39	19 (15.3)		Primary	30 (24.2)
	40-50	37 (29.8)		Secondary	4 (3.2)
	51-61	27 (21.8)		University	1 (0.8)
	>62	31 (25.0)		Traditional	3 (2.4)

The mean age of participants was 50 (\pm 15) and nearly one third of them aged between 40-50 years. The majority (nearly 60%) of the individuals was having family size ranging from 1year-5years age. About 92% of the participants reported to be followers of Orthodox Christianity. Of the participants about 69.4% were illiterate (**Table-1**).

Table-2: Tuberculosis history, feeding habit and sputum culture result of TB suspected cases in North Wollo Zone, Ethiopia, 2013.

Characteristics (n=124)		No (%)
Household TB history	No	102 (82.3)
	Yes	22 (17.7)
Receiving Treatment	No treatment	105 (84.7)
	Treated	19 (15.3)
Completing anti-TB Rx course	Incomplete	105 (84.7)
	Completed	19 (15.3)
Cough duration	≥ 2wks-1month	37 (29.8)
	>1month-2 months	13 (10.5)
	>2months	74 (59.7)
Action taken to combat cough	No action	95 (76.6)
	Modern	1 (0.8)
	Traditional	28 (22.6)
Household contact with TB patient	Yes	109 (88.0)
	No	15 (12.0)
Feeding habit	Drinking unboiled milk	91 (73.4)
	Drinking boiled milk	33 (26.6)
	Eating raw meat	64 (51.6)
	Eating cooked meat	60 (48.4)
Sputum culture result	Negative	120 (96.8)
	Positive	4 (3.2)

As it is presented in **Table-2**, among the participants, 17.3% and 15.3% had a TB history in their household and the patients were received antituberculosis treatment, respectively. Among the treated ones, only 15.3% completed a course of antituberculosis treatment. Chronic cough is not seen as a serious problem in the study area and hence nearly 77% of them were not taking any action and only 1% was seeking modern treatment for their ailments. Nearly 60% of the participants were suffering from chronic cough for more than 2 months. Nearly three fourth (73.4%) and more than half (nearly 52%) of the participants had the habit of taking unboiled milk and raw meat, respectively. Of the participants only 12% were having contact with TB diagnosed patients. Although there is no association all culture positive individuals were having the habit of taking unboiled milk as well as raw meat. From the collected 124 TB suspected sputum samples 4 *Mycobacterial species*, which accounted about 3.2%, were grown in LJ media. Polymerase chain reaction using deletion method revealed that 2/4 (50%) of the isolates were *M. tuberculosis*. Distribution of sputum culture positive results with some predictors is presented in **Table-3**.

Table-3: Distribution of sputum culture positive results with some predictors, 2013.

Category	Total	Sputum culture results(n=124)		
		Positive (%)	Negative (%)	
Sex	Male	93	4(3.2)	89(71.8)
	Female	31	0 (0)	31 (25.0)
EcoZone	Highland	55	1 (0.8)	54 (43.5)
	Midland	55	2 (1.6)	53 (42.7)
	Lowland	14	1 (0.8)	13 (10.5)
Education:	Illiterate	86	2 (1.6)	84 (67.8)
	Formal & informal education	38	2 (1.6)	36 (29.0)
Age (mean=50 ± 15):	Below the mean	68	1 (0.8)	67 (54.1)
	Above the mean	58	3 (2.4)	53 (42.7)
Cough duration:	≥2wks-1month	37	1 (0.8)	36 (29.0)
	>1month	87	3 (3.2)	84 (67.8)
TB history in the household:	No	102	4 (3.2)	98 (79.0)
	Yes	22	0 (0)	22 (17.8)
Taking appropriate treatment	No	105	4 (3.2)	101(81.5)
	Yes	19	0 (0)	19 (15.3)
Completing treatment course	No	105	4 (3.2)	101 (81.5)
	Yes	19	0 (0)	19 (15.3)
Habit of drinking raw milk :	No	33	0 (0)	33 (26.6)
	Yes	91	4 (3.2)	87 (70.2)
Habit of taking raw meat:	No	60	0 (0)	60 (48.4)
	Yes	64	4 (3.2)	60 (48.4)
Contact with TB patient:	No	109	4 (3.2)	105 (84.7)
	Yes	15	0 (0)	15 (12.1)

As indicated in **Table-3**, all positive cultures (100%) were belonging to males and out of which 2 (50%) of them lived in the mid-altitude. Equal positive cultures were recorded in those who were illiterate and attending formal and informal education. Using the mean score (50yrs as a mean), three fourth of the culture positives where aged beyond 50 years. Besides, three fourth of the culture positives had a cough duration of more than 1month and 75% of them took traditional treatments to curve cough situation. All culture positive individuals were not having a TB history in their house and there was no contact history. Polymerase Chain Reaction (PCR) using RD 9 as a marker indicated that 2 (50%) among 4 sputum culture positives were *M. tuberculosis* while the rest were atypical *Mycobacterial species*.

Of the total participants who allowed their cattle to be tested 73.4% and 51.6% of them consumed unboiled milk and uncooked meat, respectively. In the actual work 381 cattle owned by these households were tested intra-dermal by using APPD (Avian purified protein derivative) and BPPD (Bovine purified protein derivative). Of the total cattle tested males contribute 192 (50.4%). The mean age of cattle was 6.0341 ± 3.5246 and the majority of cattle 63.5% (almost two-third) were having

medium body condition. Almost 96% of the cattle tested were local zebu and 75% of them were home-bred (**Table-4**).

Table 4: Characteristics of tested cattle and skin test results in selected *Kebeles* of North Wollo Zone, Ethiopia, 2013.

Categories	Cattle No (%)	Skin test result (n=381)		
		Positive ¹ No (%)	Negative ² No (%)	Inclusive ³ No (%)
Breed [*] : Local	365 (95.8)	10 (2.6)	359 (94.3)	12 (3.1)
Cross	16 (4.2)	0 (0)	0 (0)	0 (0)
Source ^{**} : Home-bred	286 (75.1)	10 (2.6)	359 (94.3)	12 (3.1)
Purchase	95 (24.9)	0 (0)	0 (0)	0 (0)
Sex : Male	192 (50.4)	5 (1.3)	183 (48.0)	5 (1.3)
Female	189 (49.6)	5 (1.3)	176 (46.2)	7 (1.8)
Body condition ^{***} : Lean	65 (17.1)	0 (0)	66 (18.4)	0 (0)
Medium	242 (63.5)	6 (60)	227 (63.2)	80 (66.7)
Fat	74 (19.4)	4 (40)	66 (18.2)	4 (33.3)
EcoZone ^{****} : <i>Dega</i> ⁴	173 (45.4)	4 (1.1)	166(43.6)	3 (0.8)
<i>WoinaDega</i> ⁵	151 (39.6)	1 (0.3)	148 (38.9)	2 (0.6)
<i>Kolla</i> ⁶	57 (15.0)	5 (1.3)	45 (11.8)	7 (1.8)

*: refers to indigenous zebu cattle and crosses of Holstein-Frisian in the study area

** refers to the origin of cattle to the owner,

***: indicates the nutritional status of cattle during the time of testing

****: indicates the three major agro-climatic Zones in North Wollo Zone

¹: differences in skin fold measurement greater than 2mms; ²: differences in skin fold measurement ranges between greater 1mms- <2mms; ³: differences in skin fold measurement less than 1mms

⁴: *Dega*: equivalent to high land area; ⁵: *WoinaDega*: Equivalent to Mid altitude; ⁶: *Kolla*: equivalent to low land

Based on manufacturer recommendation [19] 5 (1.31%) cattle were found positive. As it is shown in Table- 4, according to previous cut off proved by Ameni *et al.*, 2008 [22] of the tested cattle 10 (10/381) (2.63%) were positive for the test. Highest positivity (with a reading >4mms) was recorded in Raya-Kobo *Woreda*, in *Adis alem Kebele*, where 5 (~8. 8%) cattle revealed BTB out of 57 tested cattle. The total households in the *Adisalem Kebele* were 312, of which 14 households (4.5%) were reported as having chronic cough and of these reactor cattle were recorded in four households, which is about 28.8%. According to the manufacturer recommendation 12 (about 3.2%) inconclusive test results were obtained. Of the positives females contribute 50% of skin test positivity. In general, according to Ameni

et al., 2008 [22] report among the ten positive cattle, 4 (40%), 1 (10%) and 5 (50%) cattle were positive for the test in *Dega*, *WoinaDega* and *Kolla Woredas*, respectively.

From the tested cattle nearly two-third (63.5%), and among the positive once 60% of them were having medium body condition. Lean cattle did not show positive reaction. The majority of cattle (359/381), which accounted about 94.2%, were negative for the test. Excluding inconclusive test result cattle, Fisher's exact test (**Table-4**) showed a significance difference ($P < 0.05$, $\chi^2 = 12.13$ with 2 df) in skin test reaction among different agro-climatic *Zones*.

Bovine tuberculosis

According to the Purified Protein Derivative manufacturer's recommendation [19] from 381 tested cattle 5 of them showed positive results, in particular in *Rayakobo Woreda*, *Addis-alem Kebele*, which is characterized by *kolla* (equivalent to low land) agroclimatic *Zone* and this, contributes about 1.31%. Based on this, the prevalence of BTB in this study was 0.013 (95% CI, 1-03%). In another scenario when the cut of skin fold measurements is taken as >2 mm, according to *Ameni et al.*, (2008) [22] the number of positive cattle recorded lifted to 10, which in turn raises the prevalence to 2.63%. Six (6/9, nearly 67%) individuals among nine who owned positive cattle and reported having a chronic cough were having the habit of drinking unboiled milk.

Discussion

Although the role of *M. bovis* in human TB in Africa is not well known, its global contribution has been estimated about 3.1% of all human TB cases [29]. But the agent is isolated in some African countries including Ethiopia from human sputum samples [30-43]. Reports made in countries like Egypt, Nigeria, Zaire and Tanzania confirmed bovine type human tuberculosis from their patients [44-47].

Of 124 sputum samples collected from chronic coughers 4 (3.2%) culture positives (2 of them were *M. tuberculosis* based on PCR), were isolated. A tuberculosis survey conducted from October 2010-June 2011 in Ethiopia, indicated 0.2% (110/51667) culture positivity from pulmonary TB suspected cases [48]. A study conducted in the rural community of South-west Ethiopia revealed a culture positivity of ~4% (17/482) [49]. The current result lies within the limits of the two findings. The variability between these results might arise from differences in the quality of sputum sample, time of sputum sample collections, cold chain systems during collections and transportations as well as the time of processing

after collection. Fifty percent of the isolates confirmed as atypical *Mycobacterial species*. As it is indicated in literature people could be infected with atypical Mycobacteria or Mycobacteria other than tuberculosis [MOTT] and some of them could lead to disease development in different parts of the body including the lungs [50].

Majorities suffered from a chronic cough for more than 2 months and more than three-fourth (77%) of participants did not take any action to cure their cough as well as 75% of the culture positives had cough duration of more than 2 months. These factors will increase the chance of disease transmission; if the individual is infected with tuberculosis since smear negative-culture positive cases are responsible for 20% of tuberculosis transmission [51, 52]. Three-fourth of the culture positive individuals were aged beyond 50 years old and this might be related to the decline in host immunity as age increases. Chain and colleagues 1995 [53] reported that immunologic abnormalities in elders led to tuberculosis development.

Among the coughing individuals, 74 % (nearly three fourth) were having the habit of taking raw/unboiled milk. Taking unboiled/raw milk could be a potential source of infection to humans as described above since infected cows excrete *M. bovis* via milk [54]. Polymerase chain reaction conducted from Albanian milk samples in Tirana university revealed the presence of the MTC gene in 36/50 (72%) of the tested samples would support the possibility of ingesting the disease causing agent [55].

In the current study, 381 cattle owned by chronic coughers were tested and prevalence of 2.63 was recorded. The prevalence of BTB in *Meskanmareko*, *Woldeya*, *Bakogazer Woredas* and *Bale* mountains was 7.9%, 1.2%, 4.3% and 2.0%, respectively with an overall prevalence of 3.1% [56]. The prevalence in small scale dairy production systems ranges from 3.5% in *Asella* to 14.2% in *Wolaita Sodo* area [57, 58]. The prevalence in intensive dairy farms ranged from 4.5%- 73.6% in *Desie* area [59, 60], whereas in the traditional management system, it ranged from 3.4% (*West Wolega*) to 22% (*Kombolcha*) [59, 61]. The present finding is a bit higher to the findings of *Woldeya* area, otherwise, it is in agreement with the overall findings reported by Tschopp *et al.* (2009) [56] and fall within the ranges of her findings. The difference in *Woldeya* area might be attributed to the time difference in conducting intradermal skin test, the area covered as well as the sampling techniques. The current finding difference from different

management systems might be described to the differences in breed types/composition, the way of management of cattle, herd size, the environment and the body condition of the cattle during testing time, which is related to feed availability.

All cattle showed a positive result for the test was local zebu breed and 100% home-bred, having a longer contact period (with a mean age of 6.0341 ± 3.5246) to the household. Longer contact period might act as a source of infection to the family members as well as cattle including other animal species and at large to the community. As a result of longer duration of stay with the household, females, particularly lactating ones will serve as a permanent source of infection since it has been reported that a single cow can excrete sufficient viable mycobacteria in milk to make even pooled milk infective [54] [64] and infection of the udder has been found in varying proportions of tuberculous animals as high as 5.4% [54, 62].

The difference between *Dega*, *WoinaDega* and *Kolla* areas in BTB was significant ($P < 0.05$, $\chi^2 = 12.13$ with 2 df). Report in Ethiopia indicated that the prevalence is relatively high in the highlands, having an altitude of >2000 masl [63]. Indeed the presence of difference in skin test positivity in geographical locations is described by another author [64]. However, this needs further study since altitude in human TB showed a higher TB prevalence in low altitudes [65, 66]. Purposive sampling of the study areas, lack of a comparative study, focusing on chronic coughers as well as failure to resample negative chronic coughers could be seen as a limitation of this study.

Conclusion and recommendations

Despite the current finding in this study looked small and lacked representativeness, the work can tell us the advantage of culturing since all of the culture positive chronic coughers were smear negatives. These culture positive ones are active pulmonary TB cases and hence the spread of the agent to the family or those who had/have contact with them is more likely. In this study four were found positive culture, of which half were atypical Mycobacteria. The finding of atypical *Mycobacterial species* in humans should be seen as a problem in the study areas. The presence of skin test positive cattle in different herds owned by different households should be seen as an indication as to the possible source of infection to humans, in particular zoonotic TB. In this study, more than two-third of the individuals suffered from chronic cough and owned positive cattle practiced consuming raw milk, whereby infected cows excrete the bacilli via milk chance of taking the BTB causative agent. A prevalence of 2.63% was recorded and those positive cattle might remain as a source

of infection for the family, to the neighbors and at large to the community as well as to other animal species. Even though bovine TB was not found from culture positive chronic coughers, the current approach to see the BTB situation on their cattle owned by them will serve as a springboard for future possible cross infection assessment of *Mycobacterial species* to both populations.

Early detection of chronic coughers, identification of the causes of chronic cough, family members screening for TB, treatment for active pulmonary TB cases, repeated sputum sample collection and processing for the chronic coughers as well as a means of exclusion or culling of positive cattle to break the possible cycles of BTB in the study area and continual community awareness with further research based on altitudes are recommended. Besides, to have a better picture as to the prevalence of the disease among individuals who suffered from chronic cough case control study is suggested.

Competing interests

The authors have declared that they have no competing interests.

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Bovine Tuberculosis in rural Ethiopia: A comparative Cross-sectional Study on Cattle Owned by Households with and without Tuberculosis

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Research Work place: In the rural communities of North eastern and north western parts of Ethiopia.

Bovine Tuberculosis in rural Ethiopia: A comparative Cross-sectional Study on Cattle Owned by Households with and without Tuberculosis

Abstract

Background: Bovine tuberculosis (TB) is endemic in Ethiopia. Intimacy of cattle and humans in rural farming communities may transmit *Mycobacterium bovis* to humans. However, there is little information about the possible transmission of pulmonary tuberculosis between humans and cattle. The current study aimed at investigating the likelihood of bovine TB among livestock in households with a confirmed pulmonary TB cases.

Method: The households studied were those where TB had been diagnosed in any household member (35 pulmonary TB case households) in comparison to households without a person diagnosed with pulmonary TB (105 comparison households). Cattle owned by both households were tested with the comparative intradermal skin test (CIDT) to collect primary data using forms. Logistic regression was used to assess the factors associated with CIDT reactivity in both households.

Results: The study revealed 23.6% (n=140) of an overall household/herd and 8.7% (n= 481) an individual cattle apparent prevalence. The odds of bovine TB among cattle owned by households with pulmonary TB was about three times (2.90, 95% CI: 1.50-5.54) higher compared to those cattle owned by households without pulmonary TB.

Conclusion: The study implicated possible transmission of TB between humans and cattle. It is necessary to investigate whether the pathogen responsible is *M. tuberculosis* or *M. bovis*.

Key words: *bovine tuberculosis, cattle, household, tuberculosis.*

Background

Tuberculosis (TB), one of the oldest and among the most devastating of human diseases, is a bacterial infection mainly caused by *Mycobacterium tuberculosis* (*M.tb*). In 2012, 8.6 million people were infected and 1.3 million died from TB¹ Nine million people become ill with TB each year and around 5000 people die in a single day¹⁻⁴.

Ethiopia ranks 3rd in Africa and 8th amongst the 22 highest TB burden countries in the world. The prevalence of all forms of TB is estimated at 224 per 100 000 population and an annual mortality rate of 18 per 100 000 population. The incidence rate of all forms of TB is estimated at 247 per 100 000 population, while the smear positivity rate is about 32.0%. TB case detection rate, the treatment success rate and TB cure rate are 64%, 90% and 89%, respectively⁵.

Although *M. tuberculosis* is the main cause of human pulmonary TB (PTB), the main causative agent of bovine TB, *M. bovis*, is well described to infect humans, primarily through close contact with infected cattle or consumption of contaminated animal products such as unpasteurized milk. Globally, most cases of zoonotic TB are caused by *M. bovis*, and cattle are the major sources^{5,6}. Infection to humans can also occur through wound contamination during slaughtering or inhalation of bacteria in the air exhaled by infected animals. Direct transmission from animals to humans through the air is thought to be rare, but *M. bovis* can be spread directly from person to person when people with the disease cough or sneeze⁷. Likewise, transmission of *Mycobacterium tuberculosis* from human TB patients to cattle could also be possible⁸.

Bovine TB is endemic in Ethiopia with a prevalence of 10% to 54%^{9, 10}. In humans, *M. bovis* causes TB disease that can affect the lungs, lymph nodes, and other parts of the body⁷. However, the relationship of human pulmonary tuberculosis mainly caused by *Mycobacterium tuberculosis* to cattle skin test positivity owned by households with pulmonary TB is not well understood. Still pulmonary TB is a problem in the country. Since *Mycobacterium tuberculosis* is isolated from cattle¹¹; they may serve as a source of *Mycobacterium tuberculosis* infections to humans, indicating the possibility of reverse zoonosis and therefore, this study was carried out to assess prevalence of bovine TB status in cattle owned by individuals with confirmed human pulmonary

TB cases compared to those owned by pulmonary TB free households in the rural northwestern and northeastern Ethiopia.

Methodology

Study setting and design

This study used a comparative cross-sectional design and was conducted in North Gondar and North Wollo zones of Amhara National Regional State, north western and north eastern Ethiopia, where the livelihood is mainly mixed farming. Dembia, Chilga, Dabat, Debark, Adarkay and Wegera from North Gondar zone and Meket, Gubalafto, Habru and Raya Kobo in North Wollo zone were the study districts where those willing to conduct tuberculin skin test on their cattle confirmed PTB human cases living. Based on the 2007 Ethiopian Census¹², the study site covered an estimated area of 58,117.13 square kilometers with a total human population of 4,429,931. Rural dwellers accounted for about 90% and 85% in North Wollo and North Gondar zones, respectively¹². The data collection was carried out between September and November 2013. Health institutions of the two zones were used as a source to identify individuals diagnosed with pulmonary TB.

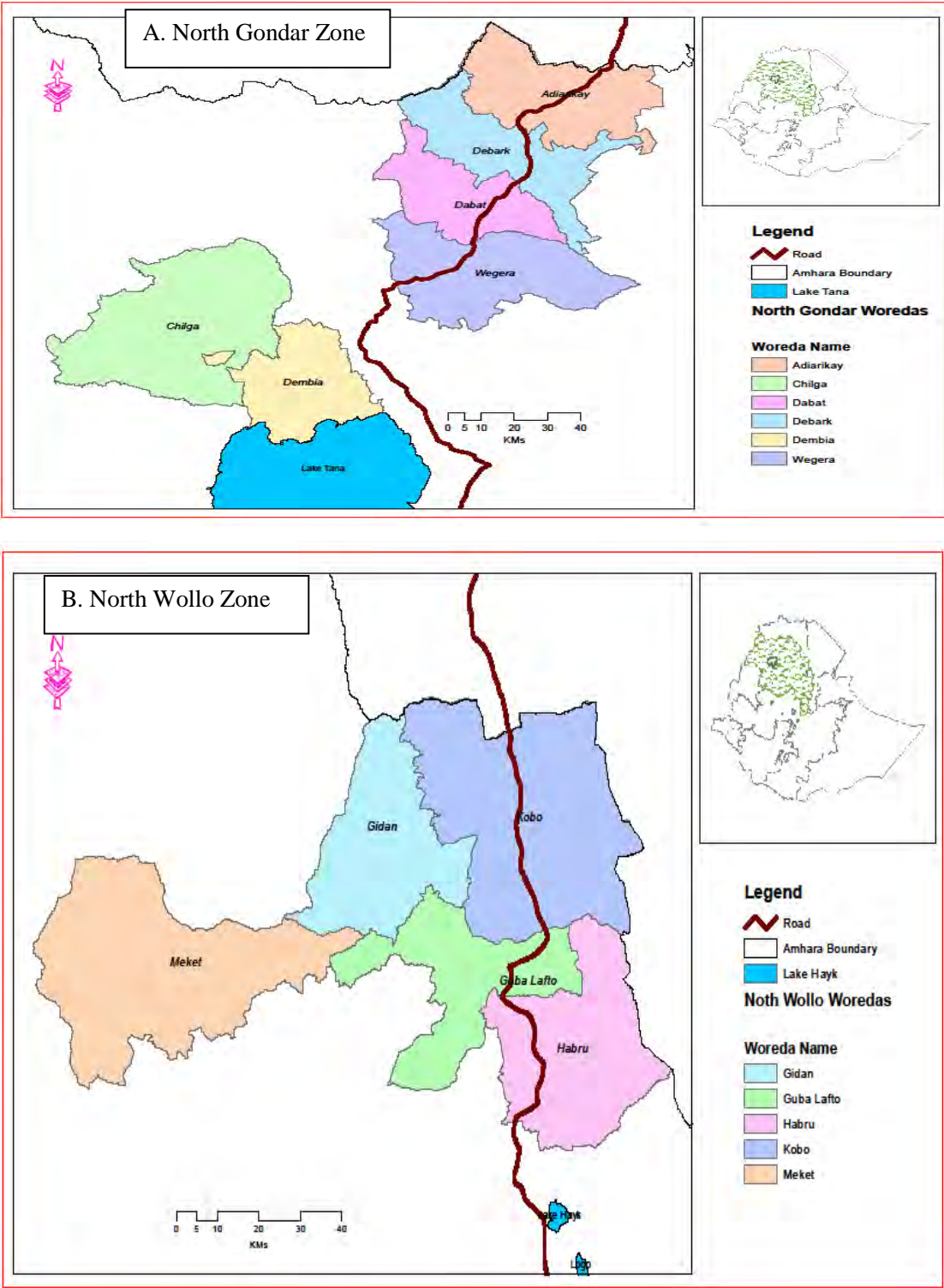


Fig 1: Map of the study sites

Study subjects/populations

TB patients who owned cattle were identified in their respective health institutes (purposefully selected public health centers as well as hospitals) and traced back to their home guided by health workers or veterinarians in the respective sites. A human TB case was defined as a smear positive adult pulmonary TB patient (aged 18 and above years) diagnosed at the respective health institutes in the study zones while pulmonary TB negatives were apparently healthy individuals (aged 18 and above years, as a household head) who did not have any clinical signs suggestive of pulmonary TB or had no history of TB in the family in the past as well as no chronic cough, which lasted for more than two weeks prior to data collection. All cattle older than six months of age in herds owned by households with and without TB cases were tested using a comparative cervical intradermal tuberculin test. Cattle included in the study were local zebu breeds and some crosses kept under extensive management system. For each tested cattle, sex, breed, age, source (homebred or purchased) and body condition score (BCS) were collected and recorded at the time of the test. The body condition of each of the study cattle was scored using guidelines established by Nicholson and Butterworth (1986)¹³. Cattle which were under 6 months of age, new additions to the herd (less than 6 months duration), pregnant cows near to term, sick cattle during the time of the visit, cattle under treatment, highly aggressive cattle and oxen used for plowing during the time of study days were excluded from tuberculin skin testing.

Sample size determination

The sample size was calculated taking into consideration a comparative cross-sectional design with the assumption of 15% bovine TB among the non-pulmonary TB households and 41.38% among the pulmonary TB households, confidence interval of 95% with a power of 80%, a ratio of cases to comparison group of 1:3; (findings of 15.4% *Mycobacterium tuberculosis* and 44.1% *Mycobacterium bovis* from collected milk samples by Fetene *et al.*,¹¹ were used to calculate the sample size). Adding 10% non-response the required sample sizes were 35 households with pulmonary TB and 105 households without pulmonary TB cases. The distribution of the samples was based on the identified, confirmed pulmonary TB cases during the follow up periods in both study zones. Those eligible cattle owned by both households were tested.

Bovine TB diagnosis procedure: Tuberculin intradermal skin test

Comparative cervical intradermal skin test was performed to know the status of bovine tuberculosis in the study areas. For this test AVITUBAL-25000 IU/ml-inj. ads us.vet, *Mycobacterium avium* (strain D4 ER, 25000IU/ml) and BOVITUBAL (*Mycobacterium bovis*, strain AN-5 25.000 IU/ml) PPD antigens were used at a dose rate of 0.1ml each intradermally¹⁴. First the injection sites; for injection of avian tuberculin about 10 cm from the crest of the neck and bovine tuberculin about 12.5cm lower on a line roughly parallel with the line of the shoulder or on different sides of the neck in young cattle, if there is no room to separate the two, were shaved and cleansed. Then a fold of skin within each clipped area taken between the forefinger and the thumb was measured using digital caliper and the test antigen injected using a BD1/2 tuberculin syringe (graduated) based on manufacturer recommendations¹⁴. A correct administration was confirmed by palpating a small pea-like swelling at each site of injection. Evaluation of results was done 72 ± 4 hrs after injection. The result was considered as positive, if the reaction number to bovine exceeds the reaction to avian tuberculin by more than 2mm and 4mm; or clinical symptoms such as diffuse or extensive edema, exudation, necrosis soreness or inflammatory reaction of relevant lymphatic vessels or lymphatic nodes were discovered in the application place of bovine tuberculin. Results considered inclusive¹⁵ if the reaction measurement for bovine TB is from 1-2 mm and 1-4mm greater than the reaction to avian tuberculin or no clinical symptoms were discovered. If the reaction number is the same or lower than for avian tuberculin with no clinical symptoms were observed in either site; the result considered as negative.

Ethical considerations

Ethical clearance was obtained from the Institutional Review Board (IRB) of the College of Health Science, Addis Ababa University and AHRI/ALERT (Armauer Hansen Research Institute) ethics committee. Permission was obtained from the Amhara Regional Health Bureau and from North Gondar and North Wollo Zones as well as respective health departments. Permission was also obtained from Zonal department of agriculture and agricultural offices in each zone. Written consent or assent was taken from each participant TB cases and comparative groups. Confidentiality was maintained by using codes instead of participants' names. During the

study period advice was given to those households identified as bovine TB positive or doubtful. Individuals still had cough and other TB related signs after treatment were consulted to go to health centers for possible checkups. All participants were given health education on safe food practices. All tested cattle received antiparasitic drugs as per the recommended dosage. All participants were enrolled only upon giving written consent, which was previously approved by the ethics committee.

Data management and analysis

During the study period, good data management practices (including survey monitoring as well as on site supervision, timely data archiving and transfer, sorting and filing) were implemented. The collected data were coded and entered into EpiInfo version 3.2.1¹⁶ and exported to SPSS¹⁷ for analysis. The manufacturer¹⁴ >4mms and Ameni *et al.*,¹⁸ >2mms cut-off values of the skin test measurements were used to determine the skin test result. Descriptive statistics was done using frequency distributions, percentages, mean and standard deviations. Multiple logistic regression was used to assess factors associated with positive bovine tuberculosis. During the analysis a household/herd was considered positive if one head of cattle among others was positive for the skin test.

Results

Of the total 481 cattle tested for bovine TB, 76% and 1:1.2 were from North Gondar zone and with male to female ratio, respectively. The mean age of cattle was 5.6 ± 3.0 and 5.2 ± 3.7 years for those cattle owned by households with pulmonary TB and without pulmonary TB, respectively. Among them, 76% (n=481) cattle were homebred (reared at home) and 87.1% were local; 61.3% had medium size, 24.1% were lean and 14.6% fat (**Table-1**).

Using >2mms as a cut-off value, 4.6% (22/481) and 4.2% (20/481) had skin test positive cattle in households with PTB and without PTB, respectively. With the same cut-off value, the individual cattle prevalence of bovine TB was 15.3% (95% CI: 9.4 -21.2) and 5.9% (95% CI: 3.4-8.5) among PTB and non-PTB households, respectively. In this study an overall individual apparent prevalence of 8.7% (95% CI: 6.2-11.3) was recorded.

On the other hand, using >4mms cut-off value, the individual cattle prevalence of bovine TB was 5.6% (95% CI: 1.8-9.3) and 0.6 % (95% CI: 0.2-1.4) among PTB and non-PTB households,

respectively, making the overall individual cattle prevalence of 2.1%, 95% CI: 0.8-3.4). About 30% (42/140) of the households/herds and 13.5% (65/481) of the individual cattle had inclusive bovine TB results based on >4mms cut-off value, of which 31.4% (11/35) were from PTB households and 29.5% (31/105) without PTB households.

Table-1: Characteristics of tested cattle in North Gondar and North Wollo Zones, Ethiopia, 2014.

Category	Households (n=481)					
	With PTB		Without PTB		Total	
	No	%	No	%	No	%
Study Area						
North Gondar	117	81.2	247	73.3	364	75.7
North Wollo	27	18.8	90	26.7	117	24.3
Sex						
Male	75	52.1	145	43.0	220	45.7
Female	69	47.9	192	57.0	261	54.3
Cattle age (Mean) in years	5.6 ± 3.0		5.2 ± 3.0			
Breed						
Local	126	87.5	293	87.0	419	87.1
Cross	18	12.5	44	13.0	62	12.9
Source						
Homebred	103	71.5	262	77.7	365	75.9
Purchase	41	28.5	75	22.3	116	24.1
Body condition						
Lean	28	19.5	88	26.1	116	24.1
Medium	85	59.0	210	62.3	295	61.3
Fat	31	21.5	39	11.6	70	14.6
Bovine tuberculosis						
>4mm						
Inclusive	20	13.9	45	13.4	65	13.5
Negative	116	80.5	290	86.0	406	84.4
Positive	8	5.6	2	0.6	10	2.1
>2mm						
Inclusive	6	4.2	27	8.0	33	6.9
Negative	116	80.6	290	86.0	406	84.4
Positive	22	15.2	20	6.0	42	8.7

As it is shown in **Table- 2**, households with PTB showed 20% (7/35) (95% CI: 6.7-33.7) and 48.6% (17/35) (95% CI: 32.0-65.1) while those without PTB revealed 2% (2/105) (95% CI: 0.7-4.5) and 15.2 (16/105) (95% CI: 8.4-22.1) bovine tuberculosis using >4mms and >2mms cut-off

values, respectively. An overall household/herd apparent prevalence of 6.4% (9/140) (95% CI: 2.4-10.5) and 23.6% (33/140) (95% CI: 16.5-30.6) were registered using >4mms and >2mms cut-off values, respectively.

Table -2: Skin test result at household/herd level in North Gondar and North Wollo Zones, Ethiopia, 2014.

Measurements	Test Result	Household with TB (n=35)		Household without TB (n=105)	
		No	%	No	%
		BTB >4mms	Negative	28	80
	Positive	7	20	2	1.9
BTB >2mms	Negative	18	51.4	89	84.8
	Positive	17	48.6	16	15.2

As presented in **Table- 3**, based on >2mms as a cut-off value adjusted for age, body condition, source, sex and breed of the individual animal the odds of bovine TB that cattle owned by PTB cases was about 3 times (2.90, 95% CI: 1.50-5.54) higher compared to those cattle owned by without PTB households and the difference was highly significant ($p<0.05$). In this study, sex, age, breed, body condition and source of the cattle were not associated with the occurrence of bovine TB.

Table-3: Bovine Tuberculosis in North Gondar and Wollo Zones based on >2mms as a cut-off value, Ethiopia, 2014.

Category	BTB (n=481)			P-value	AOR	95% CI	
	Positive	Negative	Tot.			Lower	Upper
Sex							
Male	18	202	220	1			
Female	24	237	261	0.399	1.346	0.674	2.690
Mean cattle Age[§]	5.6 ± 3.0	5.2 ± 3.0		0.813	0.987	0.888	1.098
Breed							
Local	34	385	419	1			
Cross	8	54	62	0.431	1.421	0.592	3.411
Source							
Homebred	28	337	365	1			
Purchase	14	102	116	0.214	1.603	0.761	3.377
Body condition							
Lean	6	110	116	1			
Medium	29	266	295	0.174	1.895	0.753	4.765
Fat	7	63	70	0.446	1.575	0.489	5.078
Household TB status							
Negative	20	317	337	1			
Positive	22	122	144	0.002	2.876	1.493	5.543

[§]: age was not categorized

Discussion

The current study, which mainly conducted on local zebu cattle and some cross breeds, indicated the importance of bovine tuberculosis (BTB), particularly in cattle owned by human PTB diagnosed households in the study areas. Based on >4mm measurement difference 6.4% an overall household/herd and 2.1% of an individual cattle apparent prevalence of BTB was documented. Herd (household) and individual cattle apparent prevalence of 20% and 5.6% registered, respectively in PTB households (cases). In the comparative groups 2% and 0.6% of herd and individual cattle apparent prevalence, respectively were recorded. Using >2mms as a cut-off value 23.6% and 8.7% of an overall household/herd and individual cattle apparent

prevalence of bovine TB was observed. Using the same cut-off value 48.6% in households with PTB and 15.2% in households without PTB apparent prevalence of bovine tuberculosis were documented.

Bovine TB is recorded in different animal species in Ethiopia. In Afar area (Ethiopia) an individual animal BTB prevalence of 0.4% and 0.2% were recorded in Camels and Goats, respectively¹⁹. Sera of wild-lives tested with rapid test in five regions of Ethiopia revealed 23% (20/87) of BTB²⁰. A herd prevalence of 19%²¹, 44%²² and 51.4%²³ of BTB were reported in Boji district, Afar and Jima dairy herd, respectively, and the difference might be attributed to the epidemiological factors that favor the transmission of BTB, which include herd sizes, communal grazing and watering of diverse species of animals²². A study conducted in and around Mekelle area, Tigray regional state, Ethiopia, revealed 54% of herd BTB prevalence¹⁰.

Similarly a cross sectional study of BTB conducted in Ambo and Toko Kutaye districts, Ethiopia, showed an overall individual and herd prevalence of 1% and 7.02%, respectively²⁴; and this is more or less agrees with the current finding. The authors also showed that BTB was more prevalent in cattle owned by tuberculosis households (1.36% at an individual and 12% at herd levels) compared to non-tuberculosis households (nearly 0.6% and 3.13% at individual and herd levels), respectively, which in general supports the current finding. However, contrary to the above mentioned study from cattle owned by the TB patient's higher prevalence of BTB at herd level was registered in this comparative cross-sectional study.

Adjusted for age, sex, body condition, breed and source, at an individual cattle level the risk of a cattle to be positive for bovine TB, when owned by PTB affected individuals were about three times (2.9) using >2mms as cut-off, higher compared with those cattle owned by non-PTB households. Studies done in different parts of Ethiopia indicated the presence of high prevalence of BTB in cattle owned by PTB diagnosed cattle owners than PTB negative ones at herd and individual cattle levels. For instance, of 11 TB households, 4 (4/11, 36.4%)²⁵ and 5 (5/8, 62.5%)²³ in northwest Ethiopia and Jimma dairy herd, respectively, had BTB reactor herds. Fetene *et al.*,¹¹ reported a significantly higher prevalence of BTB in cattle owned by TB patients than in cattle owned by non-TB owners and at the same time ascertained by isolating *M. tuberculosis*

(*M.tb*) and *M. bovis* from sputum and fine needle aspiration specimens of TB patient cattle owners. Besides, *M. tb* was isolated in grazing cattle in Central Ethiopia. In Nigeria 11.8% prevalence was reported in cattle owned by TB positive owners²⁶ and Aishatu²⁷ denoted that the high TB prevalence trend among human patients in Nigeria has similar trend among cattle populations.

The presence of higher BTB reactor cattle in cattle owned by PTB positive households than PTB negative ones could suggest that either of them could be a source of infection for the other creating a sort of vicious cycle⁶. From the current study and other literatures human TB may be transmitted to cattle or that cattle TB may be acquired from humans^{19,24,28} and, therefore, *M. tb* might be a possible cause of the skin test positivity in cattle. A study carried out, in and around Mekele reported that those who owned cattle had 5% TB patients in their house compared to 3% TB patients in the house for those who did not own cattle¹⁰. A report in Spain indicated *M.tb* infection of cattle and the source of infection was human TB patient²⁹. Apart from the possible contribution of *M.tb* for skin test positivity, the difference in skin test result might be related to the differences in the cattle husbandry system, nutritional status of the animal, breed differences⁶ number of PTB case households, number of cattle (herd size)³⁰ owned by the PTB cases, extent of study area coverage as well as cattle duration of cattle stay in the households, the use of proper cold chain systems and technical disparity as well as cattle immunity/circulating antibodies³¹.

Based on the current comparative intradermal skin test referring to >4mms 30% (42/140) of the herds as well as 13.5% (65/481) of individual cattle seems important because of the fact that such cattle could serve as a source of infection to other cattle or humans, as this was evidenced by culturing of *Mycobacterium species* from suspected BTB lesions³² and milk samples²⁷. Besides, a study conducted in India showed 26.7% doubtful/inclusive skin test result, and among the inclusive cattle 66.7% revealed positive results with interferon assay³³. Purposive selection of the study sites, smaller tuberculosis cases, inability to recheck inclusive cattle as well as a lack of determination/identification of the causal agent to establish possible cross infections were some of the limitations of the this study.

Conclusion and Recommendations

The current study indicates the presence and importance of BTB in North Gondar and Wollo zones of Amhara National Regional State and denotes the possible transmission of the disease in the studied rural areas. Existing cattle management practices, the free and mixed grazing as well as using common water sources and free livestock movement in the study area is likely to facilitate the expansion of the disease in the areas. BTB eradication from livestock is expensive, but it is possible to minimize the possible spread of the disease by regular testing/surveillance, segregation and creation of awareness among livestock owners. In addition the investigation of the BTB situation in humans in the study areas should be given emphasis to see the possible cross infections or reverse zoonoses verification. The possible contribution of *M. tuberculosis* to cattle skin test positivity in those cattle owned by households with a TB case needs to be verified by tubercle lesion or milk sample collection and culturing with subsequent molecular typing. BTB finding in Deberak (where Ras Dashen Mountain harboring endemic wild lives is located) adjacent Kebeles could be seen as a risk to wildlife, thereby hampering the tourism industry and this might require additional studies to highlight the possible spread of BTB at the wildlife-domestic animal interface. The current finding obtained by comparing one case with three comparative groups is strong and this will help in developing policies related to animal husbandry systems in the country. At this moment test and slaughtering strategy might be difficult for Ethiopia and therefore, frequent education and creation of awareness among the farming communities should be strengthened to prevent the public from possible BTB infections. The contribution of BTB inclusive cattle was high and this should be given attention in future studies.

Conflict of interest: none

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ORIGINAL ARTICLE

BOVINE TUBERCULOSIS (BTB) AS A RISK FACTOR FOR DEVELOPING TUBERCULOSIS IN HUMANS IN THE RURAL COMMUNITY OF ETHIOPIA: A CASE-CONTROL STUDY.

Araya Mengistu,¹ Fikre Enqueselassi,² Abraham Aseffa³, Demissew Beyen³

ABSTRACT

Aim: The current study aimed at assessing BTB as a possible risk factor for human TB in the rural community of North Eastern and Western parts of Ethiopia.

Method: A case-control design was conducted among cattle owning households with TB and without TB. Comparative cervical intradermal test using purified protein derivatives were used to test cattle. Reading of the reaction was done 72 ± 4hrs after antigen injection. Based on the skin test reaction measurement, cattle categorized as negative, doubtful and positive. Questionnaires were used to collect the required factors.

Results: Thirty-five with TB and 105 households without TB participated in this study of which 49.3% and 61.4% had the habit of drinking raw milk and eating uncooked meat, respectively. About 70.7% knew about the chance of disease transmission from animals to humans. Among the TB households 31.43% shared their house with their cattle. Of the attendants, approximately 38% shared utensil. Based on >2mm as a cutoff value 23.6% an overall apparent bovine tuberculosis (BTB) and 48.6% apparent BTB in households with TB were recorded. The odds for households having bovine TB in their cattle to get tuberculosis was more than 8 times (95% CI; 2.82-24.60) higher than those owned by households without TB.

Conclusion: Bovine TB has been seen as an exposure to human pulmonary TB occurrence. A separate house for cattle should be constructed to minimize the fear of cross infections and further study regarding the possible infection of cattle with *M. tuberculosis* is suggested.

Key words: bovine tuberculosis, households, human TB, *M. tuberculosis*, risk.

INTRODUCTION

Bovine tuberculosis is a debilitating disease mainly affecting cattle. The disease causing agent, *Mycobacterium bovis* (*M. bovis*) could infect a wide range of hosts including humans [1-3]. Humans could be infected by mainly taking raw milk and to some extent aerosol or droplet nuclei inhalation in situations of close contact [4,5]. Humans infected with *M. bovis* revealed extrapulmonary and to some extent pulmonary TB [1]. Research works revealed that cattle owned by TB households showed higher bovine tuberculosis skin test results [6-8]. This might be due to the possible chance of harboring *Mycobacterium tuberculosis* (*M. tb*). *Mycobacterium tb* is isolated from cattle lesions as well as milk samples in different parts of the world, including Ethiopia [6,9]. *Mycobacterium tb* and *M. bovis* are very similar genetically [10-12] and purified protein derivatives (PPD) antigens shared by members of *Mycobacterium species* [13] cattle harboring *M. tb* could react positively to skin tests. In this case cattle could be considered as false positives, since the antigen (purified protein derivative, PPD) for cattle skin test purpose prepared from *Mycobacterium bovis*,

strain AN-5 [14]. Cattle could get the *M. tb* infections when there is close contact with TB patients and have an access to their discharges containing the infective bacilli. Cattle may get the agent directly from feed materials contaminated with the agent as well as with aerosols [15, 16] when the patient sneezes or coughs. Drinking and feed materials could be contaminated by the disease causative agent if an infected person spits his sputum, which contains infective bacilli, particularly before treatment commences. Besides the main route of aerosol transmission, ingestion of contaminated water, feed as well as fomites have been recognized [17,18]. To look for possible risk factors that contribute to the development of human TB in households with bovine tuberculosis, the status of bovine tuberculosis was assessed in the rural community of North Eastern and Western parts of Ethiopia. For this purpose cattle owned by confirmed TB cases were compared to those cattle owned by individuals without TB.

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MATERIALS AND METHODS

Study area and design: This study used a case-control design in North Gondar and Wollo zones of Amhara regional state, North Western and Eastern Ethiopia, where the livelihood mainly is mixed farming. Dembia, Chilga, Dabat, Debark, Adarkay and Wegera from North Gondar zone and Meket, Gubalafto, Habru and Raya Kobo Districts from North Wollo zone were included in the study. Based on 2007 Census [19], the study place covered an estimated area of 58,117.13 square kilometers with a total population of 4,429,931. Of whom majorities are rural dwellers accounted about 90% and 85% in North Wollo and North Gondar zones, respectively [19]. The data collection was carried out between September and November 2013.

Study Population: Households owning cattle with at least one family member of TB case were considered as cases while those without TB were used as controls. For each case three control households owning cattle were selected. In the study populations houses could be shared and for this purpose a house share is defined as a house shared by cattle/animals and human/s or families to live together in the same house or separate house having the same main door for entrance.

Sample size determination: The sample size was calculated taking into consideration case-control with the assumption of 15%, where a study done in three districts of west Gojjam zone reported detection of 14.9% of *Mycobacterium bovis* from cattle owners [6] bovine TB among the control households and 41.38% among the cases, confidence interval of 95% with a power of 80%, a ratio of cases to control group of 1:3. Adding 10% non-response the required sample sizes were 35 households with TB and 105 households without TB.

Comparative cervical intradermal skin test: Comparative cervical intradermal skin test was performed to know the status of bovine tuberculosis (which is defined as a disease of bovine caused by *Mycobacterium bovis* and tested positive for intradermal tuberculin skin tests) in the study area. For this test AVITUBAL-25000inj. ads us.vet, *Mycobacterii avium* (strain D4 ER, 25000TU/ml) and BOVITUBAL (*Mycobacterium bovis*, strain AN-5 25.000 IU/ml) PPD antigens were used at a dose rate of 0.1ml each intradermally [14]. First the injection sites for the antigens were shaved and cleansed.

Then a fold of skin within each clipped area taken between the forefinger and the thumb was measured using digital caliper and the test antigen injected using a BD1/2 tuberculin syringe (graduated) [20] based on manufacturer recommendation [14]. A correct administration was confirmed by palpating a small pea-like swelling at each site of injection. Evaluation of results was done 72 ± 4hrs after injection. The result was considered as positive, if the reaction number to bovine exceeds the reaction to avian tuberculin by >2mms and if the reaction number is the same or lower than for avian tuberculin the result considered as negative.

Questionnaire: Questionnaires were used to collect the relevant information to identify the possible factors that could be associated with the occurrence of higher BTB reaction in TB households. Most of the questionnaire was closed and organized to collect data concerning Socio-demographic, livestock related, human knowledge of zoonoses diseases as well as tuberculosis, consumption habits and human-livestock interface. The questionnaires were pretested before the actual study.

Data analysis: The data entered into access using double data entry method and transported to SPSS version 20 [21] for edition, recoding and analysis. Multivariable logistic regression was applied and a P<0.05 was considered as significant.

RESULTS

Socio-demographic issues: On average the age of participants was 42.13 ±13.70, ranging from 15-82 years old, male contributes about 88% (120/140) and among the case groups more than half were females 51.4% (18/35). Eighty point seven percent (113/140), nearly 58% (81/140), 95% (133/140) and 97.1% (136/140) were married, illiterate and orthodox Christianity followers, respectively. Roughly a quarter, 23.6% (33/140) of herds were positive for bovine tuberculosis (Table-1).

Table-1: Socio-demographic characteristics of participants in two zones of Amhara region, Ethiopia
N= 140 households

Category	Households				Total	
	With tuberculosis (N=35)		Without tuberculosis (N=105)			
	No	%	No	%	No	%
Socio-demographic						
Sex						
Male	17	48.6	103	98.1	120	85.7
Female	18	51.4	2	1.9	20	14.3
Age (Mean)	42.13 ±13.70					
Marital status						
Married	15	42.9	98	93.3	113	80.7
Single	16	45.7	6	5.7	22	15.7
Divorced	0	0	1	1.0	1	0.7
Widowed	4	11.4	0	0	4	2.9
Educational status						
Illiterate	16	45.7	65	61.9	81	57.9
Primary	13	37.2	24	22.9	37	26.4
High school	4	11.4	6	5.7	10	7.1
College	2	5.7	2	1.9	4	2.9
Informal	0	0	8	7.6	8	5.7
Religion						
Orthodox	34	97.1	99	94.3	133	95.0
Others	1	8.9	6	5.7	7	5.0
Household (HH) BTB*						
HH with BTB	17	48.6	16	15.2	33	63.6
HH without BTB	18	51.4	89	84.8	107	76.4

*BTB: bovine tuberculosis

Purpose of cattle keeping, food consumption habit and disease knowledge: All farmers were keeping cattle for the purpose of draught and milk use. Nearly half, 49.3% (69/140) and 61.4% (86/140) were having the habit of drinking raw milk and consumption of uncooked meat, respectively. More than three fourth, 67.1% (94/140) of the participants did not know the benefit of drinking boiled milk. Among the individuals, 70.7% (99/140) and 85.7% (120/140) knew the possible transmission of disease from animals/cattle to human and human tuberculosis, respectively (**Table-2**).

Cattle related data: Individuals practiced mixed type of agriculture like other communities in the area. Twenty nine point three percent, (41/140) and 21.4% (30/140) of cattle owners responded that there was the presence of cattle having cough and showing wasting conditions, respectively. Of the participants, 78.6 % (110/140) did not isolate their cattle when they get sick and 87.1% (122/140) keep sick cattle with them (**Table-2**).

Table-2: Purpose of cattle keeping, feeding habit and disease knowledge in two zones of Amhara region, Ethiopia.

Category	Households				Total	
	With tuberculosis (N=35)		Without tuberculosis (N=105)		No	%
	No	%	No	%		
Purpose of cattle keeping						
Draught	35	100	105	100	140	100
Milk	35	100	105	100	140	100
Consumption of raw milk/meat						
Raw Milk						
No	10	28.6	61	58.1	71	50.7
Yes	25	71.4	44	41.9	69	49.3
Raw meat						
No	23	65.7	31	29.5	54	38.6
Yes	12	34.3	74	70.5	86	61.4
Knowledge assessment						
Consumption of boiled milk benefit						
No	22	62.9	72	68.6	94	67.1
Yes	13	37.1	33	31.4	46	32.9
Knowing zoonoses diseases						
No	17	48.6	24	22.9	41	29.3
Yes	18	51.4	81	77.1	99	70.7
Knowing Tuberculosis						
No	7	20.0	13	12.4	20	14.3
Yes	28	80.0	92	87.6	120	85.7
Cattle related						
Cattle that cough						
No	22	62.9	77	73.3	99	70.7
Yes	13	37.1	28	26.7	41	29.3
Cattle with wasting						
No	21	60.0	92	87.6	113	80.7
Yes	14	40.0	13	12.4	27	19.3
Sick cattle isolation						
No	28	80.0	82	78.1	110	78.6
Yes	7	20.0	23	21.9	30	21.4
Keeping sick cattle						
No	4	11.4	14	13.3	18	12.9
Yes	31	88.6	91	86.7	122	87.1

Possible risk factors: Among the participants, 22.1% (31/140) shared a house with their cattle/animals. Of individuals with tuberculosis 31.43% (11/35) used house sharing. Assessing some possible risk factors, nearly 18% (25/140) were using common water source, ~38% (53/140) sharing utensil, 30.0% (42/140) were giving their urine to cattle or cattle licked their urine, 7.1% (10/140) individuals had the habit of urinating on cattle feed/hay, 25.7% (36/140) answered cattle lick their hands, 32.9% (46/140) were spit sputum in the house and 44.3% (62/140) of the houses were having ventilations (window or holes) (**Table-3**).

As it is presented in **Table- 3** adjusted for taking un-boiled milk, sharing house, cattle licking human urine, man urinating on cattle feed, human spitting sputum in the house, house ventilation, using the same water source and knowing zoonoses diseases, the odds of households with positive bovine tuberculosis to acquire tuberculosis was more than 8 (AOR=8.32, 95% CI; 2.82-24.60) times higher compared to bovine TB negative households. Raw milk taking (OR=8.80, 95%CI; 2.60-29.81), human urinating on cattle's feed (OR=12.10, 95% CI; 1.80-81.20) and house ventilation (OR=5.50, 95%CI; 1.60-18.9) were also the contributing factors for the possible circulation of *Mycobacterium tuberculosis complex* in the households.

Table-3: Risk factors associated with the occurrence of Bovine tuberculosis in two zones of Amhara region, Ethiopia using Ameni *et al.*, 2010, as cut off value.

Category	Households Human TB		Total	Sig	AOR	95% CI	
	Controls (105)	Cases (35)				Lower	Upper
Bovine TB							
Negative	89 (84.8)	18 (51.4)	107 (76.4)		1.0		
Positive	16 (15.2)	17 (48.6)	33 (23.6)	0.0001	8.32	2.82	24.60
House sharing							
No	85 (81)	24 (68.6)	109 (77.9)		1.0		
Yes	20 (9)	11(31.4)	31 (22.1)	0.05	0.31	0.01	0.99
Urinating on hay/feed							
No	100 (95.2)	30 (85.7)	130 (92.9)		1.0		
Yes	5 (4.8)	5 (14.3)	10 (7.1)	0.01	12.10	1.8	81.22
Urine licking							
No	76(724.4)	22(62.9)	98 (70.0)		1.0		
Yes	29 (27.6)	13(37.1)	42 (30.0)	0.90	1.10	0.32	3.60
Cattle licking hand							
No	78 (74.3)	26 (74.3)	104 (74.3)		1.0		
Yes	27 (25.7)	9 (25.7)	36 (25.7)	0.008	0.14	0.03	0.60
Sharing utensils							
No	67 (63.8)	20 (57.1)	87 (62.1)		1.0		
Yes	38 (36.2)	15 (42.9)	53 (37.9)	0.22	1.97	0.70	5.90
Spitting sputum in the house							
No	75 (71.4)	19 (54.3)	94 (67.1)		1.0		
Yes	30 (28.6)	16 (45.7)	46 (32.9)	0.08	2.64	0.90	7.70
House ventilation							
No	61 (58.1)	17 (48.6)	78 (55.7)		1.0		
Yes	44 (41.9)	18 (51.4)	62 (44.3)	0.007	5.50	1.60	18.9
Using same water source							
No	89 (84.8)	26 (74.3)	115 (82.1)		1.0		
Yes	16 (15.2)	9 (25.7)	25 (17.9)	0.40	1.80	0.51	6.11
Habit of taking unboiled milk							
No	61 (58.1)	10 (28.6)	71 (50.7)		1.0		
Yes	44 (41.9)	25 (71.4)	69 (49.3)	0.0001	8.80	2.6	29.81

Number in brackets represents percentages.

DISCUSSION

As bovine tuberculosis (BTB) is endemic to Ethiopia, reaching to herd prevalence of 62.5% (7), in this case control study, nearly a quarter (23.6%) of households showed BTB. Looking BTB as a risk factor to human pulmonary tuberculosis, the adjusted odds ratio showed that households with BTB to get tuberculosis was more than 8 (AOR= 8.32, 95% CI: 2.82-24.60) times higher compared to households without BTB. As close contact is a predisposing factor for TB transmission between humans (22, 23), prolonged close contact (24-28) between the cattle with TB and human could result in longer duration of exposure that would probably lead to higher risk for the disease transmission between the two populations. Most of the houses in the study areas were not having adequate ventilation or completely closed. Having house ventilation was found as a contributing factor in the occurrence of human TB in households with bovine BTB. The odds of house ventilation for the occurrence of human TB in households with bovine TB was 5.50 (95% CI: 1.60-18.90) times to households without BTB. It is known that inadequate ventilation could result in insufficient dilution or removal of infectious droplet nuclei which enhance the transmission of *M. tuberculosis complex* (22,23). However, in this study the definition used for ventilation and the perception of ventilation by respondents might cause a bias and hence presence of ventilation seen as contributing factor for the occurrence of human TB.

Awareness about the disease transmission between cattle and humans could play a great role in the occurrence of diseases like TB. The more peoples' awareness about the diseases the better chance of reducing disease transmission. Based on this case control study, among the participants, 70.7% knew disease transmission from cattle to humans and mentioned some of the diseases, particularly anthrax and rabies, while their knowledge regarding BTB was limited to literates (few in number). Ameni and his colleagues reported 38.3% of the respondents knew about BTB (4). This difference of awareness of BTB might be attributed to lower participation of veterinary personnel's in the public health issues, little attention given by health extension workers or less literate peoples' participation in the current study compared to Wuchalie-Jida (Ethiopia) districts (4). Similarly, the study also indicated that of the households 85.7% knew human tuberculosis. The higher percentage of awareness about humans TB might be related to the existing health extension activities practiced and implemented in the country as well as in the study areas.

Since there are food-borne diseases, feeding habits of the individuals could also serve as a vehicle for disease transmission. Taking raw milk and uncooked meat is well practiced in different parts of the country. In this study, nearly half, which is 49.3% of the participants,

was having a habit of consuming raw milk and 61.4% of them were eating uncooked meat. Besides, in this study more than three-fourth (67.1%) of the respondents did not know the benefit of using boiled milk. In a study conducted in rural Torodi (Niger), more than 68% of the household families consumed unpasteurized milk (29) and a study carried out in Wuchalie-Jida (Ethiopia) districts showed 52.1% of the interviewed households used to drink raw milk and 90% ate raw as well as cooked meat (4). Relatively a lower percentage of raw meat and milk consumption habit was recorded in the current study when we compared to studies done in Niger while a relatively closer figure is registered in Wuchalie-Jida area and this might be related to having similar cultural settings between the study sites in Ethiopia while the higher figure in Niger could be justified as having different soil-cultural settings between the two countries. The habit of raw meat consumption in the current study was very low compared to the findings from Wuchalie-Jida district and this might be a reflection of the effects of increased community awareness about the use of boiled milk and cooked meat consumption. As Ameni and his colleagues(4) reported that 44.7% of individuals practiced consumption of boiled fresh milk, individuals interviewed in the current study areas who own cattle used to take boiled milk but 67.1% of them did not know the advantages of consuming boiled milk. As a factor for the occurrence of human pulmonary TB in households with BTB, unboiled milk drinking was associated with the manifestation of TB where the risk was 8.80 (AOR= 8.80, 95%CI: 2.6-29.81) higher compared to households without BTB.

Since TB is a contagious disease, close contact or proximity between cattle and humans could create favorable condition for TB transmission. In this case control study, 22.4% (31/140) households were practicing house sharing. Although house sharing was not seen as a contributing factor in the occurrence of human TB in households with BTB, cattle which harbor *Mycobacterium tuberculosis complex species* could serve as a source of infection to humans by releasing droplet nuclei during coughing (30). Similarly Ayele and co-authors (31) reported that inhalation of dust particles or bacilli containing aerosols released from cattle could cause pulmonary TB in humans. The survival of the bacilli in the dark (while house sharing) for hours will facilitate transmission of *M. tuberculosis complex species* (23). The more droplet nuclei, space, ventilation, air circulation as well as air pressure are the factors that could increase the probability of *M. tuberculosis complex* transmission (22). The current study revealed that those households who did not share a house with their cattle and those who owned cattle that do not have the habit of hand licking were 31% (95% CI: 10-99%) and 14% (95% CI: 3-60%) less likely acquire TB infection than their counterparts, respectively.

Malpractices could also play their own role in the disease circulation. In this study, about 7.1% of the participants were urinating (Males) on animal feed and this was found to be as a contributing factor (AOR: 12.10, 95%CI: 1.80-81.20) for the occurrence of human TB in

households with BTB. As evidenced by different author's urine could contain *Mycobacterium* bacilli when a person is infected with either disseminated or localized TB. Mortier and colleagues (32) isolated *M. tuberculosis* from human urine samples and (33) detect *Mycobacterium tuberculosis* complex in HIV-infected and uninfected pulmonary and extrapulmonary tuberculosis patients in Burkina Faso. In Italy [34] *Mycobacterium tuberculosis* complex was also detected in urine using rapid gen-probe amplification test. The possible explanation of this contribution is that when infected individuals urinate on animal feed, it will be contaminated by tubercle bacilli and taken by cattle. Infected cattle might serve as a continuing source of infection to the owners thereby re-infection or new infection could be possible despite the patients' having received adequate treatment and cured of the disease.

In a nutshell, the risk of bovine TB to the occurrence of human TB in the current study could be seen in light of the detection of 6.2% and 7.4% *M. tuberculosis* prevalence in cattle in Algeria and Sudan, respectively (35, 36). Besides, *Mycobacterium species* could be excreted in the milk (37). This might suggest the possible role of cattle in the maintenance as well as transmission of tuberculosis to humans. Using 3 controls per case could have been seen as strength, however, lack of isolating the disease causing agent either from cattle owners or positive cattle's should be seen as a limitation of this study.

Conclusion: Human tuberculosis is mainly caused by *M. tuberculosis* and at times by *M. bovis*, which mostly occurred as extrapulmonary TB. In this study bovine TB has been seen as an exposure to human pulmonary TB occurrence. Prolonged close contact between human and cattle could help the disease transmission. Apart from it drinking unboiled milk and urinating on animal feeds were found as a contributing factor for the occurrence of human TB. Those who do not share their house with their cattle were less likely to reveal TB. The participant knowledge about zoonoses and TB diseases should be valued for future TB control activities. Further study regarding the possible isolation of the disease causing agent in both populations is mandatory. A separate house for cattle should be constructed to minimize the fear of cross infections. In addition to these the habit of eating raw meat and taking unboiled milk should be discouraged through vigorous health extension activities and rising the community knowledge about the risk of zoonotic diseases is paramount important.

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Identification and Characterization of *Mycobacterium Tuberculosis* Isolates from Cattle Owners in North Western and North Eastern Parts of Rural Ethiopia

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ABSTRACT: Tuberculosis (TB) is a major global public health problem resulting in a considerable morbidity and mortality worldwide. Ethiopia ranks 8th among the 22 high TB burden countries. Establishing an appropriate and improved intervention strategy to prevent and control tuberculosis requires the presence of evidence based data on the genetic diversity of its causative agent. The current research work, therefore, was to differentiate strains of *Mycobacterium tuberculosis* isolated from pulmonary tuberculosis patients who own cattle in North Eastern and North Western parts of Ethiopia using molecular techniques. Sputum samples were collected from Acid Fast Bacilli (AFB) positive pulmonary tuberculosis patients and cultured on Lowenstein-Jensen (LJ) medium containing glycerol and sodium pyruvate. Deoxyribonucleic acid (DNA) was extracted from each positive culture, spoligotyping and single nucleotide polymorphisms were performed to further differentiate strains of *M. tuberculosis*, after deletion typing PCR confirmed that all the isolates were *Mycobacterium tuberculosis*. The mean age of study participants was 35.7 years (18-63 years) ± 13.24. The majority (55.7%) were from North Gondar zone. Spoligotyping revealed that (47/50) 94% had interpretable patterns and 3 lineages namely; East-Africa-Indian (57.4%), Euro-American-African (EAA lineage- Lineage 4) 38.3% and Ethiopian (lineage-7) 2/50 (4.3%). Lineage 7 was registered in North Wollo zone only. In this study 8 clusters (with cluster size ranging from 2-8), 8 unique and 10 new patterns were recorded. Spoligotype International Types (SIT) (21, 25, 26, 35, 53, 109, 149 and 289), were found as clusters and of these SIT 25 (7) and SIT 289 (8) were the predominant ones. Our study proved that 3 *Mycobacterium tuberculosis* lineages, namely; the ancient, intermediate between the modern lineages as well as modern were identified. Besides, considerable clustering was seen, which indicates the presence of current TB transmission in the study areas.

KEYWORDS: Ethiopia, Lineages, *Mycobacterium tuberculosis*, SIT, Tuberculosis, Typing.

1 INTRODUCTION

Tuberculosis (TB) as a disease traveled for several decades with humans [1], [2], [3] and yet remains as a major global public health problem [4] despite the availability of anti-TB drugs. In 2012 there were 8.6 million new TB cases and 1.3 million deaths [5]. According to WHO 2013 report, Ethiopia ranked 8th among 22 TB high burden countries with an estimated 247 (183–321) (in thousand population) incident rate including HIV positives. According to Federal Ministry of Health (FMoH), Ethiopia, report TB is one of the leading causes of morbidity, the fourth main cause of hospital admission, and the second largest cause of hospital deaths (after malaria) [6] and TB is endemic in the Amhara region state as well. A study that has collected sputum samples from different health institutes in the region were cultured and among 240 positive cultures, 237 were *Mycobacterium tuberculosis* based on RD9 based PCR analysis [7]. Similarly, in South Wollo zone, Desie referral hospital (Neighbor to North Wollo zone, Woldeya) 6.2% smear positive cases were reported among diabetic patients [8]. Among 250 prisoners in North Gondar zone 26 (10.4%) were found to have tuberculosis [9]. The disease in humans is mainly caused by *Mycobacterium tuberculosis* and to some extent by other species of *Mycobacterium tuberculosis complex* [10], [11], [12],

[13], [14]. Now day's molecular technique has become a powerful tool and is widely used to type *Mycobacterium tuberculosis* complex species to know TB transmission dynamics [15], [16], [17], [18], [19]. Understanding TB epidemiology, determining either TB relapse or exogenous reinfection, outbreak investigation and knowing laboratory cross contaminations as well as identification of the spread of clones is possible using molecular genotyping [20], [21], [22], [23], [24], [25]. For the purpose of this study pulmonary tuberculosis patients who own cattle were considered to isolate *Mycobacterium tuberculosis complex species* from them and test their cattle for bovine tuberculosis. Therefore, the current research work aimed to look the genetic biodiversity of *Mycobacterium tuberculosis* isolates recovered from pulmonary tuberculosis patients who own cattle in North Eastern and North Western parts of Ethiopia using molecular techniques.

2 ETHICAL CONSIDERATIONS

Ethical clearance was obtained from the institutional review board (IRB) of the College of Health Sciences, Addis Ababa University and AHRI/ALERT ethics review committee (AAERC). Letter of support was obtained from the Amhara Regional Health Bureau and from North Gondar and North Wollo Zones as well as from the respective woreda health Bureaus. Written consent was obtained from each TB case study participants. Confidentiality was maintained by using codes instead of participants' names. Confirmed TB cases were referred to TB clinics and received all the necessary anti-TB treatments with counseling by professionals.

3 METHODS AND MATERIALS

3.1 STUDY AREA, SETTING AND DESIGN

This study was conducted in North Gondar and Wollo zones of Amhara regional state, North Western and North Eastern Ethiopia, where the livelihood is mainly mixed farming. Dembia, Chilga, Dabat, Debark, Adarkay and Wegera from North Gondar zone and Meket, Gubalafto, Habru and RayaKobo Districts from North Wollo zone were included in the study. Based on 2007 Census, the study place covered an estimated area of 58,117.13 square kilometers with a total population of 4,429,931. Of these, the majority are rural dwellers accounted for about 90% and 85% in North Wollo and North Gondar zones, respectively [26]. Acid fast bacilli (AFB) positive sputum samples were collected cross sectional from August 2012-August 2013 from TB patients who own cattle at hospitals and health centers in both zones.

3.2 STUDY POPULATIONS

TB patients having cattle were identified at their respective health institute and used as a study population. A human TB case was defined as a smear positive adult pulmonary TB patient diagnosed at the respective health institutes in the study zones.

3.3 SAMPLE SIZE

During the follow up study period, we encountered willing 70 AFB positive TB patients who own cattle in both zones. A questionnaire used to collect information and willingness to participate in the study was the main criteria to identify participants.

3.4 SPUTUM SAMPLE COLLECTION AND CULTURING

For *Mycobacterium tuberculosis* isolation sputum samples were collected from Acid Fast Bacilli (AFB) positive patients. The Sputum samples were collected according to Cheesbrough *et al.*, [27]. Briefly, the aim of the study was explained to the patients who were active pulmonary tuberculosis cases with smear positive findings and his/her willingness was asked to participate in the study. If he/she is willing to participate, sputum samples were collected from the patient. Patients were asked to produce an "on spot" sputum specimen in a 20 ml screw plastic container under the supervision of a trained laboratory technician in an open air. If sputum was not produced within 15 minutes, the patient was excluded from the study. Those patients who produce sputum was asked to give additional sputum samples on the second and third day. The sputum samples obtained from each patient put in a cooler with ice packs (4°C) and immediately after collection it was transferred to the hospital laboratory to be stored in -20⁰c or refrigerator. The samples collected from each site put in a cooler with ice packs (4°C) to transport to the AHRI tuberculosis laboratory and then sputum samples were processed and cultured in the same laboratory.

Briefly, an equal amount of Phosphate-buffered saline (PBS) solution was added to the sputum and then was decontaminated, digested with equal volume of 4% sodium hydroxide for 15 minutes. Then vortexing was done in a closed tube for 1-3 (5) minutes till the mixture becomes homogeneous. The homogenate was centrifuged at 3000 Revolution Per Minute (RPM) for 15 minutes. Neutralization was done by using concentrated hydrochloric acid. In order to monitor neutralization 1 or 2 drops of phenol red was added. The supernatant was decanted and the sediment was inoculated onto Lowenstein-Jensen medium (tubes containing glycerol and sodium pyruvate) using the drop method. The tubes were incubated at 37°C and examined for growth weekly for 12 weeks [28]. Cultures were considered negative if there is no mycobacteria growth after 12 weeks of incubation. Growth of mycobacteria was confirmed by detection of a typical colonial morphology and by microscopy for AFB after Ziehl-Neelsen staining. Positive cultures were sub-cultured onto another set of media and incubated for another 3–4 weeks for further identification. To characterize the isolates polymerase chain reaction (PCR) (deletion typing), spacer oligonucleotide typing (spoligotyping; which analyses polymorphism of direct repeat [(RD), RD patterns is important for typing systems for epidemiological and evolutionary studies of *M. tuberculosis*] region, a reliable technique and used to detect and type *M. tuberculosis*) were applied [29].

3.5 DNA EXTRACTION

Briefly, a loop full of bacterial colonies were taken from culture positive Lowenstein-Jensen media and were transferred to the 1.5-ml tube containing 200 µl of 1% Tris-EDTA (TE) buffer. The re-suspended bacteria was heated in a dry bath at 90°C for 40 minutes and centrifuged at 10,000x g for 10 minutes. The supernatant was processed for target DNA isolation in the laboratory using the method described by Chakravorty and Tyagi [30].

3.6 PCR USING THE DIRECT REPEAT DELETION TECHNIQUE

Differentiation of *Mycobacterium tuberculosis* complex species is made by PCR amplification of species -specific DNA fragments. Polymerase Chain Reaction (PCR), which encompasses denaturation, annealing and extension, was performed for all culture positive samples to identify the species of the *M. tuberculosis* complex causing tuberculosis in human using both RD4, RD9 and RD 10 regions as a marker with their respective specific forward, reverse and internal primers. The amplification products were analyzed by electrophoresis on 1.5% (W/V) agarose gel containing 1x ethidium bromide and were visualized with Ultraviolet rays [30]. To run the PCR-deletion typing the standard operation procedure used at AHRI, which is adopted from the Veterinary Laboratory agency (VLA), UK, was followed. Materials like Mycobacteria positive control strains: *M. tuberculosis* H37RV (ATCC 25681) and *M. bovis* AF2122/97 (ATCC BAA-935), ATCC ([http: / WWW.lgcpromochem-atcc.com.](http://WWW.lgcpromochem-atcc.com)), PCR Thermocycler, laminar flow cabinet, cleaning agent DNA away/equivalent), pipettes, filter tips, nuclease free water (Qiagen), HotStar Tagmaster Mix kit (Qiagen; Product catalog No 203445, which includes DNA polymerase, buffer, MgCl₂, and dNTPS), stock solutions of oligonucleotide primers, namely; RD4-FlankFW, RD4-FlankRev, RD4-interanlFW, RD9-FlankFW, RD9-InternalRev, RD9-FalnRev, RD10- FlankFW, RD10- InternalRev, RD10- FlankRev, heat killed cells that need to be detected and materials like Agarose gel electrophoresis equipments, DNA ladder and loading dye, agarose gel, 1XTAE running buffer and Ethidium bromide were used.

3.7 SPOLIGOTYPING

In order to further differentiate the *Mycobacterium tuberculosis* detected by PCR, spoligotyping (spacer oligonucleotide typing, Kamerbeek *et al.*, [29] was performed by following the protocols used for AHRI spoligotyping. Spoligotyping, which used to detect and type bacteria of the genus *Mycobacterium tuberculosis* complex (MTC) includes three main methods, namely; PCR amplification of a specific spacer sequence of the strain, hybridization to a spoligomembarne and detection.

PCR was performed using DNA obtained from heat killed cells. The PCR amplification of the spacers was accomplished by using the primers RDa (5'-GGT TTT GGG TCT GAC GAC-3') and RDb (5' -CCG AGA GGG GAC GGA AAC-3'), which anneal to all repeat sequences and thereby enables for amplification of all spacers that occur in the DR region of the specific strain. The PCR products were labeled with biotin since the DRa primer was biotinylated. The PCR products amplified were loaded onto the membrane at right angle to the 43 parallel spacer lines by using a miniblottedter and left for hybridization. After hybridization the membrane was washed with buffer to remove the non-hybridized and non-specific bound PCR products. The step was followed by incubation with conjugated streptavidin-peroxidase, of which streptavidin binds to the biotin labeled PCR products.

The presence or absence of spacers in specific strains was then detected by an enhanced chemiluminescence (ECL) detection system onto an autorad (photographic film). When the membrane was exposed to ECL a substrate for peroxidase, the autorad will detect light signals where hybridization has occurred and thereby produced a pattern that allows for

spoligotyping of an isolate. Acceptable results include clearly defined squares on a clear background ensuring that the controls have worked, especially the negatives (water) control. Materials such as all reagents needed for PCR amplifications, baths, glass bottles, measuring cylinders, scales, magnetic stirrers, pH meter, thermocyclers, autorad and gelelectrophoresis as well as among the consumable stock solutions, buffer solutions (10% SDS, Sodium dodecyl sulphate), 0.5M EDTAX₂H₂O and other chemicals/salts needed for hybridization and detection were utilized. In addition positive and negative controls and samples to be typed were used.

3.8 SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) TYPING

After predicting the lineages of the isolates by Spoligotyping final confirmation for Lineage 3 and Lineage 4 was done by SNPs typing using real-time PCR. We performed the TaqMan Real-time PCR (Rotor Gene) SNP assay for lineage 4 (Euro-American, E-A lineage) and lineage 3. The lineage 4 was defined by katG 463 SNP and the assay was performed as described [31]. The lineage 3 was defined by Rv3804c_0012s SNP and assay was performed using standard procedures [32].

4 RESULTS

The age of participants ranged from 18-63 years old with a mean age of 35.7 years \pm 13.24. Of them, 45 (64.3%) were between 18-40 years. Majorities (55.7%) were from North Gondar zone with 1:1 male to female ratio. Among the participants, 64.3%, 82.9%, 41.5%, 60.0%, 38.6%, 20.0%, 52.9%, 38.6% and 71.4% were illiterate, rural dwellers, farmers, married, had contact with TB patients before, treated with anti-TB drugs before, took unboiled milk as well as uncooked meat and revealed culture positive results, respectively (**Table-1**).

Of the 70 AFB positive sputum samples cultured, 28/70 (40%) and 22/70 (31.4%) were culture positive from North Gondar and North Wollo zones, respectively. As shown in Table-1 among culture positives, 33/70 (47.1%), 17/70 (24.3%), 10/70 (14.3%), 26/70 (37.1%) and 23/70 (32.8%) were married, had previous contact with TB patients, received anti-TB treatment before, experienced drinking unboiled milk and consuming uncooked meat.

Table-1: Sociodemographic characteristics of AFB positive TB patients and sputum culture result in Northwest and Northeast Ethiopia, 2014.

N=70

Category	Frequency	Percent	Culture result	
			Negative	Positive
Zone				
North Gondar	39	55.7	11(15.7)	28(40.0)
North Wollo	31	44.3	9(12.9)	22(31.4)
Sex				
Male	35	50	13(18.6)	22(31.4)
Female	35	50	7(10.0)	28(40.0)
Educational status				
Illiterate	45	64.3	11(15.7)	34(48.6)
Primary school	13	18.6	6(8.6)	7(10.0)
Secondary school	6	8.6	1(1.5)	5(7.2)
College	4	5.7	2(2.8)	2(2.8)
Informal	2	2.8	0	2(2.8)
Dwelling				
Urban	12	17.1	4(5.7)	8(11.4)
Rural	58	82.9	16 (22.9)	42 (60.0)
Occupation				
Merchant	5	7.2	2 (2.9)	3(4.3)
Student	14	20.0	7 (10.0)	7 (10.0)
Housewife	19	27.1	5 (7.1)	14(20.0)
Government employee	3	4.3	0 (0)	3(4.3)
Farmer	29	41.5	6(8.6)	23(32.8)
Marital status				
Single	19	27.1	7(10.0)	12(17.1)
Married	42	60.0	9 (12.9)	33 (47.1)
Divorced	5	7.2	2 (2.8)	3(4.3)
Widowed	3	4.3	2(2.8)	1(1.5)
With parents	1	1.4	0(0)	1(1.5)
History of contact with TB patients				
No	43	61.4	10(14.3)	33(47.1)
Yes	27	38.6	10 (14.3)	17(24.3)
Anti-TB treatment before				
No	56	80.0	16(22.9)	40 (57.1)
Yes	14	20.0	4(5.7)	10 (14.3)
Taking unboiled milk habit				
No	33	47.1	9(12.9)	24(34.3)
Yes	37	52.9	11(15.7)	26(37.1)
Taking uncooked meat habit				
No	43	61.4	15(21.4)	28(40.0)
Yes	27	38.6	5(7.1)	22(31.5)
Sputum culture result				
Negative	20	28.6		
Positive	50	71.4		

Numbers in parenthesis are percentages.

Seventy AFB positive sputum samples were collected in North East and North West Ethiopia from pulmonary tuberculosis patients. Sputum samples were collected from 8 different health institutes which are found in North Gondar and North Wollo zones. All 50 AFB positive samples were *Mycobacterium tuberculosis* based on deletion typing. Further, isolates characterizations were done using spoligotyping by comparison with the International SpoIDB4 database. Of these, ninety

four percent (47/50) showed good interpretable patterns. Forty six point eight percent (22/47) and 53.2% (25/47) were from North Wollo and North Gondar zones, respectively.

As it is presented in **Figure-1**, three lineages namely; lineage 3 (East African-Indian) 57.4% (27/47), 4 (Euro-American-African) 38.3% (18/47) and 7 (Ethiopian) 4.3% (2/47) were recorded in the study areas. Among lineage 3, 5 SITs and 6 new SITs, lineage 4, 9 SITs with 4 new SITs, and lineage 7, 2 SITs were identified in both areas. In general 16 shared international types with 10 new ones were disclosed by the current study. Among the SITs, SIT 25 (7), 26 (3) and 289 (8) were the predominant ones. From the new SITs 4 and 6 were from North Wollo and North Gondar zones, respectively. Based on spoligotyping 8 clusters (with a cluster size ranging from 2-8), 8 unique and 10 new patterns were identified in the study areas. SITs (21, 25, 26, 35, 53, 109, 149 and 289) was found as clusters in both zones. From 3 cluster size/isolates of SIT 53, 66.7% (2/3) and SIT 26 with cluster size of 3, 66.7% (2/3) were from North Wollo zone. SIT 149 with cluster size 2 and SIT 109 with cluster size 2 were noticed from North Wollo zone only. SIT 35 and SIT 21 each with cluster size 2 reported in North Gondar zone, particularly the first one registered in Chilga Woreda. Among SIT 25 with cluster size 7, 57.1% (4/7) and SIT 289 with cluster size 8, 75% (6/8) occurred in Debark Woreda areas and Chilga Woreda of North Gondar zone, respectively (**Table-2**). The rest was identified as unique which may indicate reactivation of an old infection, or imported TB or may result from missing isolates.

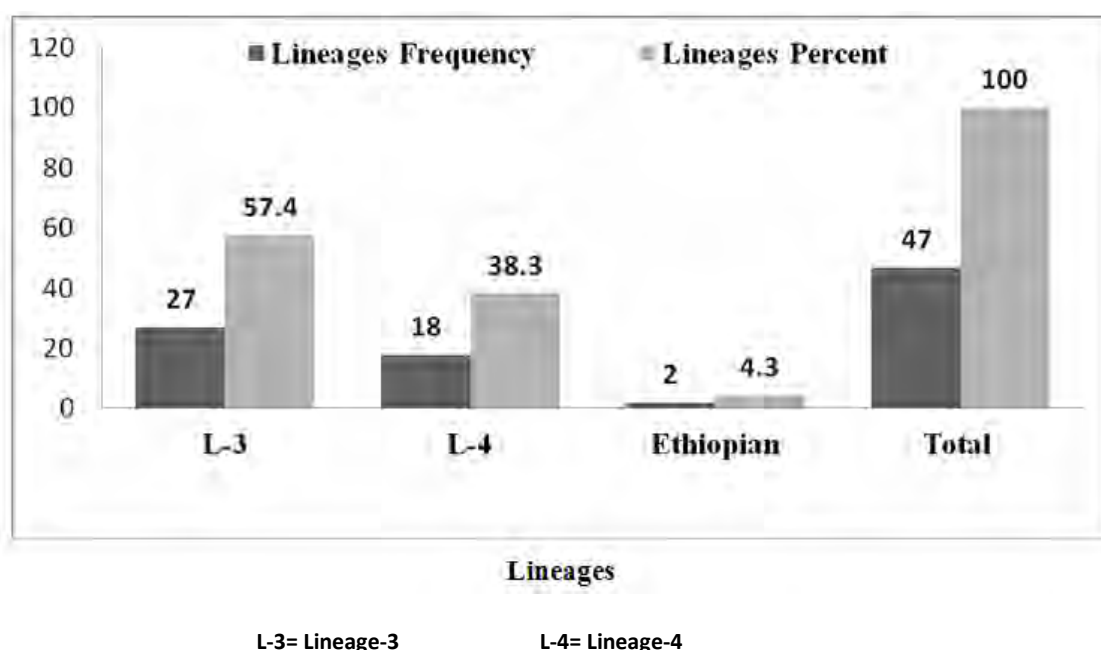


Figure-1: Lineages identified in the Northwest and Northeast part of Ethiopia, 2014

and modern lineages of *Mycobacterium tuberculosis* phylogeny prominently in North Wollo (woldiya) area compared to the current study. The difference in the proportion could be due to sample size in the case of North Wollo zone and geographical situations in the case of central Ethiopia. In Uganda, researchers reported 22% (394/1746) and 11% (187/1746) L- 4 and 3, respectively [41]. The current finding showed relatively higher values compared to the work in Uganda and this might be due to the presence of dominant and common types of prevailing isolates differences in both countries. Lineage East African-Indian showed higher cluster size by SIT 25 (7 cluster size) and 289 (8 cluster size).

Although 3 *Mycobacterium tuberculosis* lineages recorded in this study, failure of isolation of any of *Mycobacterium tuberculosis complex species* from skin test positive cattle should be seen as a limitation of this study, since the aim of the study was to see the possible role of cattle in the epidemiology of human tuberculosis by determining the presence of cross infections with detecting the same strain in the same house.

6 CONCLUSION

It is concluded that 3 *Mycobacterium tuberculosis* lineages, namely; the ancient, intermediate between the modern lineages and modern were identified in the study areas. Among the isolates, 61.7 % (29/47), (including the new isolates to the denominator) were clustered indicating the presence of recent tuberculosis transmission in both areas. Health institutes need to work more to mitigate TB transmission among the community. Early disease detection and treatment of active TB cases timely as well as effectively will definitely curve TB incidence as well as prevalence. The reasons for the restricted occurrence of Lineage 7 in the area, including socio-cultural determinants should be investigated.

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Annex 13.6: Letter of declaration

LETTER OF DECLARATION (Dissertation Work)

I the undersigned declared that this is my original work, has never been presented in this or other university, and that all the resources and materials used for the dissertation, have been fully acknowledged.

Name _____

Signature _____

Date _____

Place _____

Date of submission _____

This dissertation has been submitted for examination with my approval as University advisor.

Name _____

Signature _____

Date _____