



MEKELLE UNIVERSITY
COLLEGE OF VETERINARY SCIENCE



**BOVINE TUBERCULOSIS PREVALENCE, POTENTIAL RISK FACTORS AND
ITS PUBLIC AWARENESS IN DAIRY AND HUMANS, IN AND AROUND
SHIRE INDASILASE, TIGRAY, NORTHERN ETHIOPIA**

By:

Negasi Arefayne

A Thesis Proposal Submitted to the College of Veterinary Science, Mekelle University, in Partial Fulfillment of the Requirements for the Degree of Master of Zoonosis and Food Safety

Advisor:

Dr. Yishak Tsegay (DVM, MSc): Mekelle University, college of veterinary medicine,
Department of Microbiology and Immunology, Mekelle, Ethiopia.

Co advisors

Dr. Bruk Mekonnen (DVM, MSc): Mekelle University, college of veterinary medicine,
Department of Microbiology and Immunology, Mekelle, Ethiopia.

February, 2024

Mekelle, Ethiopia

Mekelle University
College of Veterinary Science

Department of Zoonosis and Food Safety

As member of the examining board of the final MSc. open defense, we certify that we have read and evaluated the thesis prepared by: **Negasi_Arefayne** Titled: **“Bovine Tuberculosis Prevalence, Potential Risk Factors And Its Public Awareness In Dairy Cattles And Humans, In And Around Shire Indasilase, Tigray, Northern Ethiopia”** and recommended that it be accepted as fulfilling the thesis requirement for the degree of Master in zoonosis and food safety.

BOARD OF EXTERNAL EXAMINERS

Dr. _____	_____	_____
Chairman	Signature	Date
Dr. _____	_____	_____
External Examiner	Signature	Date
Dr. _____	_____	_____
Internal Examiner	Signature	Date
Dr. _____	_____	_____
Major Advisor	Signature	Date
Dr. _____	_____	_____
Co-Advisors	Signature	Date
Dr. _____	_____	_____
Department Chairperson	Signature	Date

DEDICATION

I dedicate this MSc thesis to my father ArefayneTikue; I dreamed a little dream, Once upon a time. I dreamed we'd be together one day, Sweet Father of mine. Sadly that dream was not meant to be, and it's very difficult to know, that now you won't be coming to me. You weren't strong enough to thrive and grow, but I know that you're in soon may your soul rest in peace and that's a very good place to be.

STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an (MSc) degree at Mekelle University, College of Veterinary Science and deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Name: Negasi Arefayne

Signature: _____

Date of Submission:

Advisor:

Dr. Yishak Tsegay (DVM, Msc): Mekelle University, college of veterinary medicine, Department of Microbiology and Immunology, Mekelle, Ethiopia.

Signature: _____-]

Co advisors

Dr. Bruk Mekonnen (DVM, MSc): Mekelle University, college of veterinary medicine, Department of Microbiology and Immunology, Mekelle, Ethiopia.

Signature: _____

1. Contents	pages
DEDICATION.....	i
STATEMENT OF AUTHOR	iii
ACKNOWLEDGMENTS	vii
LIST OF FIGURES	ix
LIST OF TABLES	x
LIST OF ABBREVIATIONS	xii
LIST OF APPENDIXS	xv
ABSTRACT.....	xviii
2. INTRODUCTION.....	1
2.1. Background and Justification	1
2.2. Objective of Study.....	4
2.2.1. General Objective	4
2.2.2. Specific Objectives	4
2.3. Statement of Problem	4
2.4. Scope and Limitation of the Study	5
2.5. Hypotheses of the Study.....	6
3. LITERATURE REVIEW	8
3.1. Taxonomy of Mycobacteria	8
3.1.1. General Characteristics of <i>Mycobacteria</i>	8
3.2. Source of Infection and Mode of Transmission	9
3.3. Pathogenesis of BTB	10
3.4. Clinical Sign of BTB.....	12
3.5. Diagnosis of BTB.....	12
3.5.1. Clinical examination	13
3.5.2. Tuberculin skin test	13
3.6. Prevention and control.....	14

3.7. Risk Factors Conducive to Spreading of <i>Mycobacterium Bovis</i>	15
3.8. Economic Importance of <i>Bovine Tuberculosis</i>	17
3.9. Zoonotic Importance of Bovine Tuberculosis.....	18
3.10. Status of Bovine Tuberculosis in Ethiopia.....	18
4. MATERIALS AND METHODS.....	20
4.1. Description of the Study Area	20
4.2. Study population.....	22
4.3. Sample size determination.....	23
4.4. Study Design and Sampling Method.....	24
4.5. Study Methodology	25
4.6. Questionnaire Survey	29
4.7. Data Analysis	30
4.8. Data Collection and Management	31
4.9. Ethical Clearance.....	31
5. RESULTS.....	32
5.1. Prevalence of Bovine Tuberculosis	32
5.2. Risk Factors Associated with Prevalence of Bovine Tuberculosis	33
5.3. Assessment of Cattle Owners awareness on Bovine Tuberculosis	40
5.4. Assessment characteristics awareness on zoonotic tuberculosis among tuberculosis lymphadenitis patients.....	42
6. DISCUSSION.....	46
7. CONCLUSION AND RECOMMENDATION.....	53
8. REFERENCES	55

ACKNOWLEDGMENTS

I would like praise the almighty God with his mother St. Mary for his endless mercy. My heartfelt gratitude goes to my advisors Dr yisak Tsegay, who was supportive all the way for helping me to get *MTB* PPD kit connected to Dr. Solomon Gebre (DVM, PhD/ARPPIS)NAHDIC coordinator and overall supervision and unlimited support

I like to express my sincere gratitude to my advisor Dr. Bruk Mekonnen for his professional guidance to discuss in the areas of bovine tuberculosis (*M. bovis*) and its risk factors in cattle and also integrity, sincerity, friendliness throughout up until the write up of this thesis where I ended so far and also for his hospitality, generosity and for always being there to help.

It is also my pleasure to acknowledges all staff members of NAHDIC up to all member especially Dr. Solomon Gebre for their genuine and cooperativeness to get the *MTB* PPD kit and other supportive materials to do this task.

I special thanks colleagues and friends of colleague of Veterinary Sciences, Mekelle University for their friendship, hospitality and support during stay for training and supportive field works.

I am very grateful to my family, most of all, to my father(Yaye) heartlessness helping me with his prayer and my sister Raheal Arefayne with her family for their moral support and love.

My sincere gratitude goes to my best friends Dr. Henok Welay ,Dr. Michele Yirdaw, Sisay Kahsay, Aledin Abdu, Dr Tesfay Hailu and other friends who made me feel support and joyous with their unlimited moral support, generosity and unconditional, friendship love and social values while stay in the study area.

I thank Tahtay koraro veterinary clinic, office of agriculture, North West, Tigray, for their supporting my field work through the generous provision of data and coordinating staff throughout the district to cooperate during the fieldwork, and for cooperated day experts' in the field. I thank all the staff who assisted me during the field works, specially, Tadese Aregawe, solomon, Yosef , Dr. Semon Tesfay and

I thank Shire Agricultural College and federal ATVET for giving me the permission to pursue my Msc study, and for support throughout the study time. I thank everyone who has contributed to my Msc study directly or indirectly.

My utmost gratitude goes to my late father, Arefayne Tkue (yaye), who passed away when I was on the verge of completing my study, for his unlimited prayer on me, care, support, unconditional and never ending love. I thank my father very much for making me who I am today.

LIST OF FIGURES

Figure 1: cycle of transmission of M. bovis between cattle and human.....	10
Figure 2: Main bovine tuberculosis risk factors classified into animal, herd and environment levels.	17
Figure 3: Map of the study areas.....	21
Figure 4:Steps of Comparative Intradermal Tuberculosis Test	29
Figure 5: Prevalence rates among CIDT reactors (95% CI) at animal and herd level.....	33
Figure 6:Assessment of behavioral factors and practice of the of farm owners in study area.	42

LIST OF TABLES

Figure 1: cycle of transmission of *M. bovis* between cattle and human **Error! Bookmark not defined.**

Figure 2: Main bovine tuberculosis risk factors classified into animal, herd and environment levels. **Error! Bookmark not defined.**

Table 1: Official cattle population of each Taebia / kebele of study area (2018 G.C) 22

Figure 4: Steps of Comparative Intradermal Tuberculosis Test **Error! Bookmark not defined.**

Table 3: Prevalence of bovine tuberculosis individual animal and herd prevalence of by > 4mm cut-off value. 32

Figure 5: Prevalence rates among CIDT reactors (95% confidence intervals) at animal and herd level 33

Table 5: Univariate logistic regression analysis of the risk factors associated with herds/f arms level having CIDT reactor(s) of BTB) at a cut-off ≥ 4 mm 36

Table 6: Univariate and multivariate logistic regression analyses of risk factors for CIDT test result in individual animal level. 38

Table 7: Knowledge of cattle owners about BTB and its transmission to humans 40

Table 8: Assessment of behavioral factors and practice of the of farm owners in study area. 41

Table 9: Socio-demographic characteristics awareness on zoonotic tuberculosis among tuberculosis lymphadenitis patients 42

Table 10: socio demographic characteristics of awareness on Habit of raw milk and meat 43

LIST OF ABBREVIATIONS

AIDS.....	Acquired Immune-Deficiency Syndrome
BCG.....	Bacilli Calmette Guerin
BTB.....	Bovine Tuberculosis
CI.....	Confidence Interval
CIDT.....	Comparative Intradermal Tuberculin Test
CMI.....	Cell Mediated Immunity
CSA.....	Central Statistics Agency
DNA.....	Deoxyribonucleic Acid
ELISA.....	Enzyme Linked Immuno-Sorbent Assay
FAO.....	Food And Agriculture Organization
GDP.....	Gross Domestic Product
HIV.....	Human Immune Deficiency Virus
IFN.....	Interferonn
IU.....	International Unit
<i>M.bovis</i>	<i>Mycobacterium Bovis</i>
Mm.....	Milimeter
MoA.....	Ministry Of Agriculture
MTBC.....	<i>Mycobacterium Tuberculosis</i> Complex
MTC.....	Mycobacterium Tuberculosis Complex
OR.....	Odds ratio
OIE.....	Office International Des Epizooties
PPD.....	Purified Protein Derivatives
SICCT.....	Single Intradermal Comparative Cervical Tuberculin Test
PTB.....	Pulmonary Tuberculosis lymphadenitis
TB.....	Tuberculosis
TBLA.....	Tuberculosis lymphadenitis
WAHID.....	Worldwide Animal Health Information

WHO

Database

World Health Organization

LIST OF APPENDIXS

- Appendix 1 Questionnaire to interview farmers and dairy farm workers
- Appendix 2 Questionnaire to interview hospital coming in patients
- Appendix 3: CIDT Test Format
- Appendix 4: Description of body condition scores (BCS)
- Appendix 5: Age determination
- Appendix :6 Steps of CIDT test and Result of PPD after 72hr negative (A) and positive

ABSTRACT

A cross-sectional study was carried out from December, 2018 to May, 2019 on 382 heads live cattle selected using systematic random sampling technique recruited in 109 households/farms to investigate prevalence of bovine tuberculosis (BTB) using comparative intra dermal tuberculin test (CIDT) and identifying the risk factors concomitant in cattle as well as to assess the public health awareness through questionnaires towards bovine tuberculosis in and around Shire Indasilase town, Northern Ethiopia. The overall prevalence of bovine tuberculosis animal and herd level in cattle was 39 (10.20%) and 29 (26.61%) with ≥ 4 mm at cut-off in skin thickness increase respectively. Relatively, the occurrence of bovine tuberculosis was had less likely in cattle found in Tahtay korero district (OR =10.020, 95% CI: 513-2.041) than shire Indasilase town. univariate logistic regression analysis identified associated risk factors of age, breed, body condition score, lactation and pregnancy as well as study site, herd size, farm system, management status, type of house, respiratory problem and Wild animal were statistically significant. BTB positivity was cross breed had more sensitive being BTB positivity twice (OR= 2.36, 95% CI: 1.138-4.890) than local breed. While pregnant cow had higher BTB prevalence (OR= 4.33, 95% CI: 1.461-12.857) than non-pregnant cow. Multivariate logistic regression analysis was statically significance associated with Breed, body condition, pregnancy, and lactation. The present study revealed that relatively low prevalence of a diseases in cattle and also poor awareness of the respondents on zoonotic implication of bovine tuberculosis. There should be segregation and practice of boiling of raw milk before consumption at home and awareness creation should be done for the whole society about bovine tuberculosis and its mode of transmission.

Keywords: *Bovine tuberculosis, Comparative intradermal tuberculin test, prevalence, risk factors, Zoonosis,*

2. INTRODUCTION

2.1. Background and Justification

Ethiopia has the largest livestock population in Africa, with 65 million cattle, 40 million sheep, 51 million goats, 8 million camels and 49 million chickens in 2020 (Central Statistics Agency, CSA, 2020a). Livestock is a major source of animal protein, power for crop cultivation, means of transportation, export commodities, manure for farmland and household energy, security in times of crop failure, and means of wealth accumulation. The sector contributed up to 40% of agricultural Gross Domestic Product (GDP), nearly 20% of total GDP, and 20% of national foreign exchange earnings in 2017 (World Bank, 2017). In Tigray region, the total number of livestock population is 4,817,104 cattle, 2,097,619 sheep, 4,838,969 goats (CSA, 2020a).

In contrast to the huge livestock resource, the livestock productivity is very low. The major biological and socio-economic factors attributing to the low productivity includes: the low genetic potential and performance, poor nutrition (in quality and quantity terms), the prevailing of different diseases and traditional way of husbandry systems. However, among the diseases that affected introduction and performance of high yielding exotic dairy breed animals is bovine tuberculosis (Vordermeier, *et al.*, 2012).

Bovine tuberculosis (BTB) has been significantly widely distributed throughout the world and it has been a cause for great economic loss in animal production and the most frequent cause of zoonotic TB in man (Muhammed, *et al.*, 2018).However, it has been controlled successfully in most developed countries through the implementation of test-and-slaughter schemes, meat inspection at abattoirs and pasteurization of milk, BTB remains a problem in some developed countries in the face of extensive control programs .(Guétin-Poirier,*et al.*,2020) and *M. bovis* data is scarce in most developing countries due to lack of active control programmes, the high cost of testing programme, social unrest due to political instability, ethnic wars resulting in displacement of large numbers of people and animals, and a lack of veterinary expertise and communication networks (Thoen, *et al.*, 2009 and Uduak, A., 2015).

In Africa, Prevalence data on BTB infection is scarce. There is, however, sufficient evidence to indicate that it is widely distributed in almost all African countries and even is found at high prevalence in some animal populations (WHO, 1997; Zinsstage *et al.*, 2006). Animal husbandry and management systems contribute to the development and dissemination of BTB. The disease is endemic in various livestock production systems (Radostits *et al.*, 2007; Munyeme *et al.*, 2009, Regassa *et al.*, 2010;).

BTB has been shown to be endemic in cattle in Ethiopia, with the prevalence ranging between 15.6 and 50% have been reported in few studies conducted in dairy farms in the country. However, still there is lack of information about the actual prevalence and distribution of the disease at a national level. Despite this, the economic impacts and zoonotic importance of the BTB infection are either not well studied or documented. In Ethiopia, several prevalence studies have been performed recently that show BTB is endemic in cattle; however, prevalence vary depending on the geographical areas, breeds and husbandry practices. Abattoir and dairy farm studies from central Ethiopia have reported prevalence between 3.5 and 13.5% and locally in peri-urban Addis Ababa up to 50% (Ameni, *et al.*, 2007; Regassa *et al.*, 2010; Firdessa *et al.*, 2012; Zeru, *et al.*, 2014). In contrast, lower prevalence of 0.9% was reported in traditionally kept zebu cattle (Tschopp, 2015).

Bovine tuberculosis is a contagious, chronic, granulomatous *Mycobacterial* infectious disease; it is primarily caused by *Mycobacterium bovis*. *M. bovis* belongs to a group of pathogenic mycobacteria called Mycobacterium tuberculosis complex (MTC) which also includes *M. tuberculosis*, *M. africanum*, *M. microtti*, and *M. canetti* (Müller, *et al.*, 2011). *M. bovis* is intracellular, non-motile, facultative, weakly Gram-positive, acid-fast bacillus (Smith, 2006) and characterized by the formation of granulomatous lesions in tissues and organs, more significantly in the lungs, lymph nodes, intestine and kidney (Carrisoza, *et al.*, 2019). Those are generally regarded as host adapted but with the ability to spill over into other species. *M. bovis* has an exceptionally wide range of mammalian hosts and

affects all age groups of susceptible hosts of domestic, wild animals and human (Smith, 2006). Cattle are the most common maintenance host for *M. bovis* infection from which transmission can occur to wildlife, or people animals (Dejene, *et al.*, 2016)

Livestock population were influence the potential of direct and indirect (via faces and urine) exposure of cattle to the wildlife reservoir when at pasture; stocking regime (set stocking), rotational versus strip grazing, stocking densities, farm habitat types and livestock production intensity (Ghebremariam, *et al.*, 2018). Sharing feed or water between cattle and wildlife when housed or at pasture, housing type and storing manure indoors are associated with the differential risk of transmission between cattle and wildlife. The most frequently identified risks for herd to herd transmission included herd movements and trading, where general trading or purchase from markets or herds in hotspot areas or from infected herds have all been linked with increased risk for the receiving herd (Hristov, *et al.*, 2018).

Human tuberculosis (HTB) of animal origin, particularly *M. bovis* is becoming increasingly important in developing countries, as humans and animals are sharing the same micro-environment, eating habits, living and socio-economic status of families, illiteracy, culture and customs, the existence of diseases, and close proximity with animals (Zeru, *et al.*, 2014 and Hussein, *et al.*, 2024). *M. bovis* transmitted from animals to humans to be mainly through consumption of contaminated or unpasteurized raw milk, inhalation of infected aerosols because of close contact with animals having chronic cough due to tuberculosis (Bekele, *et al.*, 2016; and WHO, 2017) the habit of raw meat and milk consumption and the increasing prevalence of HIV may all increase the potential for transmission of *M. bovis* and other *Mycobacteria* between cattle and humans (Shitaye *et al.*, 2007 and Mbugi, 2012).

In Tigray region, livestock play an important socio-economic role. These include draught power for traction and transport, household cash income from sale of animals and animal products, food such as milk for household consumption and manure to maintain soil fertility (Gebremedhin *et al.*, 2004). On the other hand, productivity obtained from

livestock sub-sector, both qualitatively and quantitatively is below standard level, due to, among other factors a widely prevailing disease BTB being one of them. Thus it need for implementing efficient and sustainable disease control program in the region. However, this requires prior and adequate understanding of the disease epidemiology (Livingstone, *et al.*, 2018).




Despite of the expansion dairy cattle population in northern west zone of Tigray, to determine prevalence the BTB has not been assessed so far. In addition, no attempt was carried out on contaminated risk factors toward BTB in the study area will contribute its share for the efforts made to prevent and control animal and human tuberculosis.. Therefore, this study was formulated with the following objectives:

2.2. Objective of Study

2.2.1. General Objective

To estimate prevalence of bovine tuberculosis with comparative intradermal tuberculin test (CIDT) and the associated risk factors with the occurrence of tuberculosis in dairy cattle and human in and around Shire Indaslassie, Tigray, and Northern Ethiopia

2.2.2. Specific Objectives

-  To estimate the prevalence of BTB in dairy cattle with CIDT test
-  To identify potentially risk factors associated with the occurrence of BTB in dairy cows;
-  To asses awareness of the zoonotic importance of BTB among dairy farm owners.

2.3. Statement of Problem

In Ethiopia, the livelihood of the people is extremely dependent on livestock sector. However, several constraints including feed shortage, poor genetic performance and diseases prevalence are limiting the livestock production and productivity. BTB is one among the major disease problems reported widely in cattle from different regions of the country (Sibhat, B., *et al.*, 2017 and Youya, *et al.*, 2022). Similarly, In Tigray region had

studies were conducted in different agro ecological, breed and the husbandry practices (Romha, *et al.*, 2013, Zeru, *et al.*, 2014 and Hadush, *et al.*, 2015).

The disease in cattle appears both on intensive dairy farms and in extensive pastoral systems (Bekele., *et al.*, 2016 and Habitu, *et al.*, 2019). Similarly, In Tigray region, had studies were conducted in different agro ecological, breed and the husbandry practices (Romha, *et al.*, 2013; Haftu *et al.*,2014; Zeru, *et al.*, 2014; Ataklti, *et al.*, 2015).

While higher prevalence rates have been reported from regions in Tigray, where intensive husbandry systems are more common lack communities' awareness regarding BTB and its routes of transmission (Romha *et al.*, 2013; Zeru, *et al.*, 2014, and Ataklti, *et al.*,2015 and Habitu, *et al.*, 2019).

Apart from lower awareness of most of the communities in study area regarding zoonotic diseases, particularly TB, Bovine TB is a known zoonotic disease causing in most of the cases extra pulmonary TB in humans. However, the extent of its contribution to human TB is not assessed very well. The tradition of raw milk consumption and the life style of small dairy farm in study area districts where sharing shelter is practiced may increase zoonotic importance of BTB through ingestion and inhalation of *M.bovis* (Mohammed *et al.*, 2012). This in turn might alter the dynamics of bovine tuberculosis. The main scope is Identify the prevalence diseases and causative agent of the disease is a key factor for prevention, control and eradication program of the disease in animal and human and development of public awareness related to agent.

2.4. Scope and Limitation of the Study

The study was done to examined individual animal and herd level prevalence of *M. bovis* through comparative intradermal tuberculin skin test (CIDT) as well as to determined public awareness of BTB transmission as an example, animal related potential risk factors was physiological status (sex, age, breed, BCS, pregnancy, parity, lactation of dairy animals) and herd size, farm system and management condition of the farms through prepared questionnaire. This can be achieved prevalence of *M. bovis* through

screened of individual and herd level of animals and farms level respectively. In addition there was determined awareness and knowledge of TBLA humans on BTB transmission between human and animal and its public health importance. Having such information was help to develop cost effective preventive options and avoids the under estimation of the results of different previous findings done by routine CIDT test. And then, all the information found were serve as an input for animal as well as public health diseases control and prevention measures in the study area. All these can serve as an input for animal health and human information. On top of this, the study was limited to animals less than six month, late pregnancy, clinically sick animal exclusive in the study. On other hand, there was a few tabeia didn't involve to test of dairy animals. Because of lack of access dairy farm and logistic problems encountered in the field.

2.5. Hypotheses of the Study

The study tested the following hypotheses:

There is a significant difference on small dairy farms holders and TB positive. Accordingly to assessed the awareness and knowledge of farmers and TB patients towards bovine tuberculosis: prevalence, risk factors and zoonotic implication in smallholder dairy farms and humans TB patients in the farm and hospital respectively, in terms of dairy animals individual and herd level prevalence related to associated risk factors which is physiological status and general animal husbandry system and socio demographic character of (sex, age, educational background,) practice of farm husbandry, access to health management, farm system, participants' farm experience and establishment. Moreover, investigation of awareness of BTB and habit of milk and meat consumption implication with related variables to transmission of BTB animals and zoonotic importance. The respondents have been practiced to environmental dairy farms; they are influenced by different variables. For example, place of birth influences significantly whereby these from rural area are aware of and have favorable Knowledge and awareness issues. Educational back ground influences significantly where by these from illiterate are aware of and have favorable attitude about knowledge of BTB. Class level and age difference also affect in that these who spent more years in dairy farm and older age groups could have better BTB awareness and favorable attitude towards the

problem. This may be due to getting from training diseases and experiences. Similarly, gender also affect individual awareness towards BTB issues to differ between male and female, in which female can have higher awareness and favorable attitude due to pass more time with animal.

3. LITERATURE REVIEW

3.1. Taxonomy of Mycobacteria

Mycobacterium belongs to the Kingdom of Bacteria; Phylum of Actinobacteria; Order of Actinomycetales; Family of *Mycobacteriaceae*. *M. bovis* among a pathogenic species which belongs to the *Mycobacterium tuberculosis* complex (MTBC), a group of genetically closely related mycobacteria (Mostowy, *et al.*, 2005). The MTBC sub-group also comprises *M. tuberculosis*, *M. africanum*, *M. canettii*, *M. pinnipedii*, *M. microti* and *M. caprae* that are generally regarded as host adapted but with the ability to spill over into other species. *M. bovis* is the primary cause of bovine tuberculosis (BTB). *M. tuberculosis*, *M. africanum*, *M. caprae* and *M. canettii* are human pathogens (Bilal *et al.*, 2010). *M. caprae* which causes infection in goats and *M. microti* affects rodents and *M. pinnipedii* have been isolated from seals (Smith *et al.*, 2006).

3.1.1. General Characteristics of *Mycobacteria*

The genus *mycobacterium* is characterized phenotypically as non-motile, non-capsular, non-spore forming, obligate, aerobic, thin rod usually straight or slightly curved having 1-10µm length and 0.2-0.6µm width, facultative intracellular microbe (Quinn *et al.*, 1999). It is a slow-growing, aerobic, acid fast bacterium with a broad host range. The disease is characterized by the formation of granulomas in tissues and organs, mainly in the lungs, lymph nodes, intestines and kidneys (Amanfu, 2006).

Mycobacterium is similar to other bacteria in many aspects except for a unique cell wall that is made up of four parts. The first part is a peptidoglycan layer that is similar to that found on other bacterial species. The second layer contains arabinogalactan, which is made up of branched macromolecules of arabinose and galactose (Abosse, 2018). The third layer, which contributes to the thickness of mycobacterial cell walls, is made of mycolic acids that are long branched chains of fatty acids with differing 50 and 30 carbon atom lengths. The mycolic acids are responsible for the acid fast staining reaction of mycobacteria cells (Anwar, *et al.*, 2017). The fourth, outer, layer consists of a mixture of

lipids and related compounds such as trehalose-containing glycolipids and peptidoglycolipids called mycosides (Abosse, 2018.).

3.2. Source of Infection and Mode of Transmission

The main reservoir of *M. bovis* is cattle, which can transmit the infection to many mammalian species including man (Tsegaye, *et al.*, 2010). Organisms leave the host in respiratory discharges, faces, milk, urine, semen and genital discharges as well as discharges from open peripheral lymph nodes, saliva and discharging lesions (Tschopp *et al.*, 2009). These body excretions may contaminate grazing pasture, drinking water, feed, water and feed troughs or fomites, which may act as source of infection to other animals (Allen, *et al.*, 2021).

The principal route of transmission of *M. bovis* is via inhalation, as this route requires as few as one bacillus to induce infection (Skuce *et al.*, 2012 and Admassu,*et al.*, 2015;). Infection through ingestion of contaminated pasture (feed) and drinking of contaminated water, and milk (calves) are also known routes of *M. bovis* transmission, though they require more (> 107) bacilli as compared to the respiratory route (Radostits, *et al.*, 2007). Less common routes of *M. bovis* infection are congenital (via umbilical vessel to the foetus from infected uterus of the dam), via wound or broken skin, and seldom, via infected genitalia when either male or female is infected, and sometimes when the preputial orifice is contaminated by infected dust (Pal, M, *et al.*, 2022).

M. bovis can infect human primarily by the ingestion of unpasteurized dairy products, inhalation of aerosols and through breaks in the skin. The possibility of transmission of this Mycobacterium to humans from infected animals could be high in areas where there is close contact between human and animals (O'Reilly and Daborn, 1995).

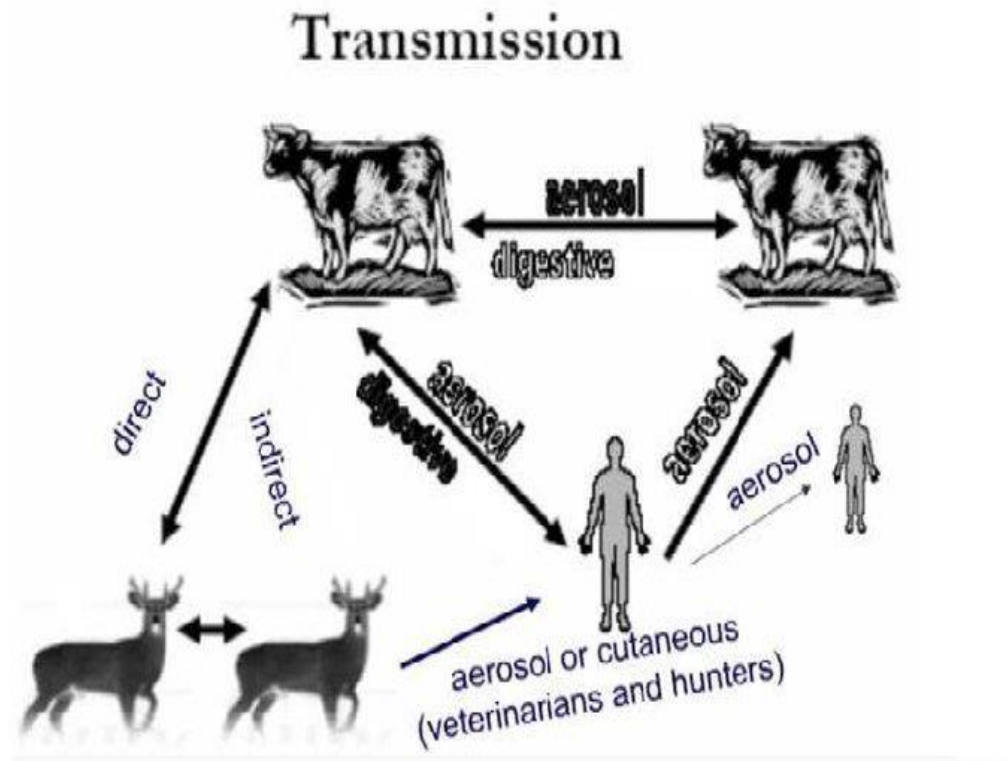


Fig
ure 1: cycle of transmission of *M. bovis* between cattle and human

Source: (Grange and Collins, 1987)

3.3. Pathogenesis of BTB

The route of infection in cattle is significant to the pathogenesis of BTB. When the causative organism is inhaled or ingested, it is drained to the local lymph node and a lesion usually develops at the lymph nodes nearest to the point of entry, after spreading in macrophages via the lymphatic vessels into the local lymph nodes. The lesions are caused by multiplication of the infectious organism in the local lymph nodes. These initial lesions are referred to as the primary complex. The primary lesion develops by calcification and the necrotic focus is surrounded by granulation tissue and lymphocytes forming a tubercle or granuloma. A tubercle is an attempt by the host's immune system to arrest the infection (Palmer, *et al*, 2022).

Granuloma formation within the lung and secondary lymphoid structures of the host is one of the defining features of BTB (Dlamini, *et al.*,2013). Tubercles are typical granulomas containing a central core of caseous necrotic tissue. The tubercles are typically pale orange in colour. The centers of tubercles may later calcify. When bacteria escape from this original focal point, they can spread to other parts of the body via the lymphatic ducts and lymph nodes or the blood stream. (Hamilton, *et al.*, 2016). If many organisms find their way into the bloodstream in this way, general dissemination throughout the body takes place and multiple lesions are formed which can lead to toxemia, debility, weakness and death.(Neill *et al.*, 1994).Granulomas act to control the spread of infection but can also lead to extensive tissue damage and loss of function (Dlamini, *et al.*,2013). They consist of multiple cell types, including infected and uninfected macrophages surrounded by B and T lymphocytes. The interaction of these cells leads to the mounting of an effective immune response (Widdison *et al.*, 2011) Dissemination of the bacteria from the primary complex, in non-self-limiting infection, is variable in rate and route. It may be in the form of acute miliary tuberculosis, discrete nodular lesions in various organs or chronic organ tuberculosis.

The infection may spread to affect a local area as in the lung, or spread via the lymphatic vessels or blood to infect other organs and tissues This process is called post-primary dissemination (Salguero,, 2018).Body organs such as the lungs, bone, kidneys and meninges are referred to as vulnerable sites in body organs that are not vulnerable sites, the infection is controlled by cell mediated immunity(CMI).In the vulnerable sites, CMI only reduces the infection but is not able to eliminate it (Palmer, *et al.*,2022).

The organism survives intracellular in host cells by avoiding being phagocytised by the production of sulfotides and heat shock proteins. Subsequent macrophage and lymphocyte activity contribute to cell death and tissue destruction which causes caseous necrosis Liquefaction and cavity formation may occur due to enzymatic actions on protein and lipids(Palmar, 2022). The end result of all these attempts by the body to fight the infection is the formation of the granuloma. Bursting of a

granuloma may lead to the spread of the infection while in other cases the granuloma may become encapsulated and be well organized by connective tissue (Domingo, *et al.*, 2014).

3.4. Clinical Sign of BTB

Bovine tuberculosis is usually a chronic debilitating disease can remain subclinical for a long period (De la Rúa-Domenech *et al.*, 2006) whereas animals may become infectious even before manifestation of clinical signs and lesions. Some cows with extensive miliary tubercular lesions may seem clinically healthy (Radostits *et al.*, 2007). Clinical signs are loss of appetite, fluctuating temperature (intermittent fever), and rough hair coat, cough that might progress to dyspnea, progressive emaciation and enlargement of lymph nodes. Infected animals may become more docile and sluggish (Radostits *et al.*, 2007; OIE, 2009). Animals with pulmonary involvement usually have a moist cough that is worse in the morning, during cold weather or exercise, and may have dyspnea or tachypnea and also lymph nodes enlarge obstruct blood vessels air ways and in some case rupture and drain (Tschopp, *et al.*, 2011).

In humans, tuberculosis due to *M. bovis* is indistinguishable from that due to *M. tuberculosis* in terms of clinical signs, radiological and pathological features (Ayele *et al.*, 2004). Pulmonary TB may result in cough, dyspnea and respiratory distress. Extra pulmonary tuberculosis may lead to various clinical signs, depending on which organs are affected. Enlarged lymph nodes may obstruct air passages, the alimentary tract or blood vessels. Cervical lymphadenitis (scrofula) is typically found in milk-borne tuberculosis infection in humans and is characterized by visually enlarged lymph nodes of the head and neck, which can sometimes rupture and drain (Davis and Danker, 2000).

3.5. Diagnosis of BTB

Diagnosis of bovine tuberculosis infection in live animals is usually based on delayed hypersensitivity reactions. A presumptive diagnosis of TB in cattle is often made on history, clinical findings, tuberculin skin tests and/or necropsy findings. In-vitro lymphocyte assays, including an interferon gamma assay and enzyme linked immune

sorbent assays have been developed for the detection of the disease in cattle (Sharipour *et al.*, 2014; and Špičić *et al.*, 2012). Infection is often subclinical when present, clinical signs are not specifically distinctive and can include weakness, anorexia, emaciation in the presence of good nutrition, dyspnea, enlargement of lymph nodes and cough, particularly with advanced tuberculosis (OIE, 2009). However, diagnosis based on clinical signs is not conclusive as the signs are not specific to bovine tuberculosis. The most effective methods for diagnosis of bovine tuberculosis are those diagnostic methods carried after death/slaughter of suspected animals. These diagnostic methods include gross postmortem examination (necropsy), histopathology, mycobacteriological culture methods and various molecular techniques for identification of the isolates. *Mycobacterial* culture diagnostic method remains the gold standard method for routine confirmation of infection (OIE, 2009).

3.5.1. Clinical examination

Tuberculosis is usually a chronic debilitating disease, but it can occasionally be acute and rapidly progressive. Early infections are often asymptomatic. In late stages, common symptoms include progressive emaciation, fluctuating fever, weakness and in-appetence. Animals with pulmonary involvement usually have a moist cough that is worse in the morning, during cold weather, exercise and may have dyspnea or tachypnea. In terminal stage, animals may become extremely Emaciated and develop active respiratory distress (Radostits *et al.*, 1994).

3.5.2. Tuberculin skin test

Tuberculin skin tests are the international standard for ante-mortem diagnosis of bovine tuberculosis in cattle herds and individual animals (Smith *et al.*, 2006). It involves the intra-dermal injection of bovine tuberculin purified protein derivative (PPD) and the subsequent detection of swelling (delayed hypersensitivity) at the site of injection 72 hours later. Skin delayed type hypersensitivity is characterized by leukocyte infiltration that is dominated by macrophages and CD4+ and CD8+ T-lymphocytes. It is convenient, cost effective method for assessing cell mediated responses to a variety of antigens and it

is “gold standard” for diagnostic screening for detection of new or asymptomatic *M. tuberculosis* complex infection (Tadesse *et al.*, 2015). This may be performed using bovine tuberculin alone or as a comparative test using avian and bovine tuberculin (Radostits *et al.*, 1994; OIE, 2008).

3.6. Prevention and control

The aim of controlling bovine tuberculosis is to eventually eradicate the disease from a country. However, eradication on a national level is not an easily achievable target and requires a combination of policies and strategies, such as the test and slaughter policy described below accurate detection and removal of infected cattle, using immunodiagnostic tests such as the comparative intra-dermal skin tests, are the basis of a control strategy for BTB (Godfray, *et al.*, 2018). Vaccination of animals against BTB is suggested to be a viable strategy in domesticated animals in developing countries, and in wildlife and feral reservoirs of disease in industrialized countries where test and slaughter programs have failed to achieve elimination of the disease (Palmer, *et al*; 2022)

BTB control strategies that are based on systematic and regular tuberculin skin testing of cattle herds, followed by the compulsory removal of all test positive animals, movement restrictions of infected herds and an effective surveillance system at abattoirs has proven to be successful in eradicating BTB from other regions and/or countries (Ameni *et al.*, 2008). The testing of the animals should be compulsory designated regular intervals and which show suspicious clinical signs and new animals are to be introduced into the population are kept in quarantine, depending on the outcome of the results A compensation scheme for destroyed animal is helpful to get farmers on board. There are several challenges associated with this method paramount amongst them being the huge amount of resources, financial and human, required (Conlan *et al.*, 2015).

In preventing human infection from meat, specifically the viscera or internal organs, the head and meat cuts containing lymph material, it is recommended that cattle should undergo both ante- and post mortem inspection. All suspicious cases

should be confirmed in the laboratory and all positive carcasses should be completely destroyed. In a carcass, the internal organs and meat cuts containing lymph nodes pose a high risk compared to ordinary meat cuts (Conlan *et al.*, 2015).

3.7. Risk Factors Conducive to Spreading of *Mycobacterium Bovis*

M. bovis have been suggested had several risk factors for the spread so far. (Humblet *et al.*, 2011). Risk factors will vary across regions due to factors such as differing farm structures and management practices. They can broadly be separated into genetic and non-genetic (environmental), which act jointly to influence susceptibility of the hosts (Palmer, *et al.*, 2022). Risk factors can vary based on the susceptibility hosts involved.

- a) *Management and Environmental risk factors:* Housed animals are more at risk than those cattle graze on the pasture that is the disease is common in intensive dairy farms than extensive farms. However, even under extensive pastoral condition, aggregation of cattle at watering points may lead to increased transmission of the disease. Calves which are housed with cows are exposed to a constant risk of infection by aero-genous route.

As bovine tuberculosis is a disease of intensification, housing predisposes to the disease, as high stocking intensity and a large number of animals on a farm so that the disease is more common and serious where these forms of husbandry are practiced. The closer the animals are in contact the greater is the chance that the disease will be transmitted. In spite of the low overall incidence in countries where cattle are at pasture all the year round, individual herds with 40-65% morbidity may be encountered (Hussein *et al.*; 2024;).

b) *Agent risk factors:* The ability of the etiological agent (*Mycobacterial* species) is moderately resistant to heat, desiccation, and to many disinfectants. It is readily destroyed by direct sunlight unless it is in a moist environment. In warm, moist, protected positions, it may remain viable for weeks (Corner, *et al.*, 2011). It can survive for long periods in environment under favorable conditions. *M.bovis* can survive for four weeks in non-sterile dry and moist soil under shed. Persistence of *M.bovis* in the carcass and this may

act as source of infection for scavengers and cattle grazing around the site of decomposition (McCormick, 2023).

c) Host risk factors: In cattle, likelihood *M. bovis* infection had various risk factors as animal and human population (Salguero, 2018). Several past and recent studies have shown that susceptibility to bovine TB in animal and human risk factors identified in our country are discussed as follows.

Animal level: Animal level risk factors susceptibility of the disease are depend on age, sex, breeds, body condition score (BCS), genetic resistance, stress, concurrent infection, immune status and physiological state of the animal (Gumi *et al.*, 2011 and Tamiru *et al.*, 2013 and Muhammed, *et al.*, 2018).

Herd level: According to several studies indicated several risk factors at herd level were herd size, types of farming practice and housing of cattle, geographical origin, history of bovine TB in the herd and human antecedent of tuberculosis in the household, contact between animals and with wildlife reservoirs, introduction of cattle in a herd, herd movements and trading, lack of performance of diagnostic tests, the use of hired/shared bulls, manure and environmental persistence of *M. bovis*. (Tschopp *et al.*; 2010; Mekonnen, *et al.*, 2019).

Risk factor for human: The main risk factors which contribute to the acquisition *M. bovis* infections in both urban and rural human populations are poverty, malnutrition, HIV infection, illiteracy, the consumption of raw milk (unpasteurised milk), uncooked or poorly cooked meat, work condition and close contact to livestock and using cow dung for plastering wall or floor. (Kemal *et al.*; 2019)

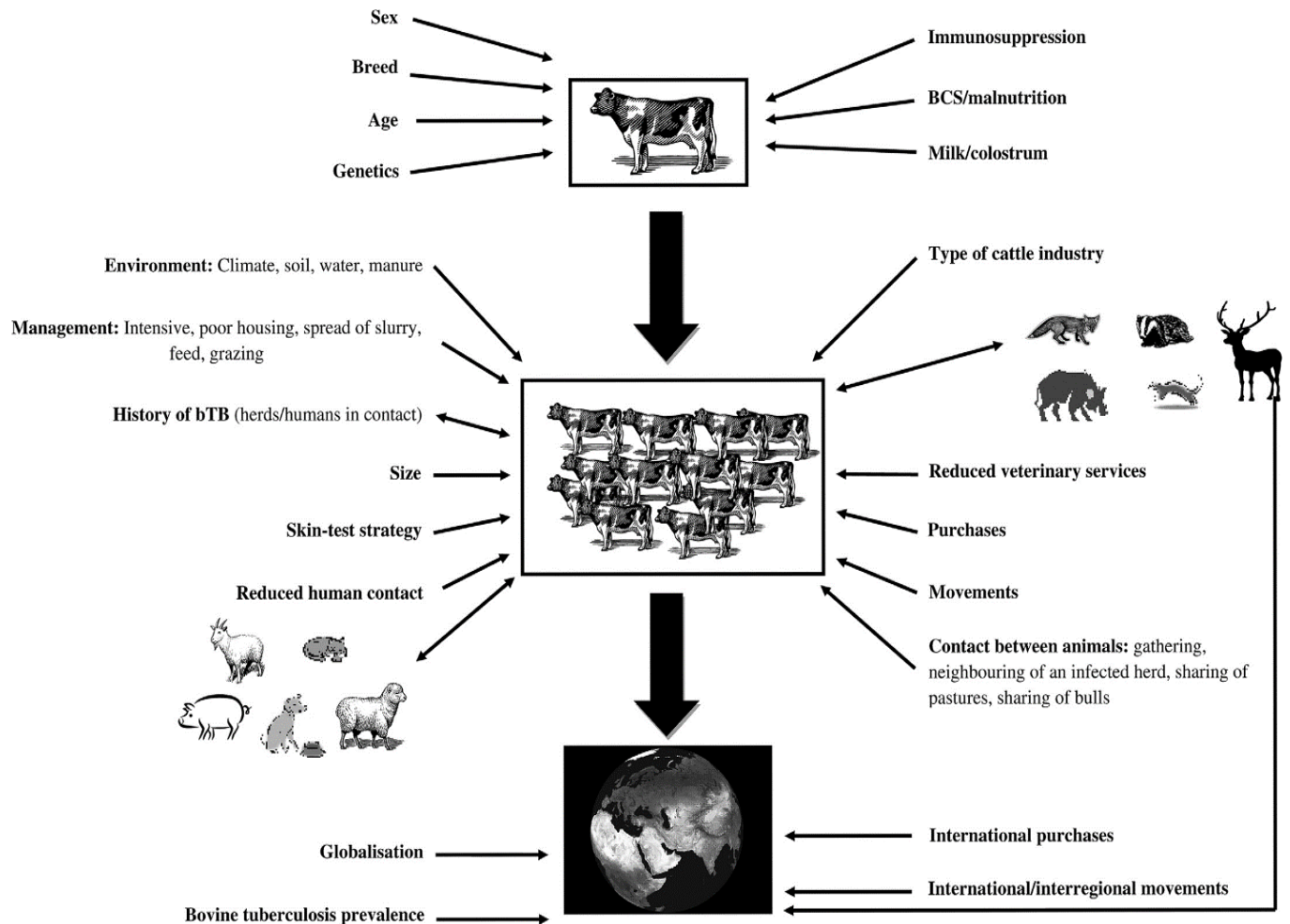


Figure 2: Main bovine tuberculosis risk factors classified into animal, herd and environment levels.

Source: Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. (Humblett, *et al*; 2009).

3.8. Economic Importance of *Bovine Tuberculosis*

Bovine Tuberculosis has been widely distributed throughout the world and it represents a very significant economic and public health problem in numerous countries in both developed and developing country (Tenguria *et al.*, 2011). Consequently, most developed nations have embarked on campaigns to eradicate *M. bovis* from the cattle population or at least to control the spread of the infection (Mohamed, A., 2020) In developed

countries, although tuberculosis is eliminated in cattle, the disease still has a major economic impact, mainly due to the existence of a permanent wildlife reservoir that reduces the efficiency of control strategies. Although the disease has zoonotic threat, economic and financial burden to society, its cost has rarely been assessed and is largely unknown for Africa (Youya, *et al.*, 2022).

Bovine tuberculosis is a disease of high economic relevance within the context of livestock farming as it directly affects animal productivity. The disease considerably reduces milk and meat production of infected animal and affect animal reproduction as well as it reduce pulling power in traditional farming system (Mulatu, *et al.*, 2023 and Muhammed, *et al.*, 2018). Milk productivity of total livestock is lower compared to that of non-infected cows. In totally TB infected livestock meat production is lost because of emergency and illness slaughter. (Mekonnen ,*et al.*;2021)

3.9. Zoonotic Importance of Bovine Tuberculosis

Human tuberculosis is usually under estimated or under diagnosed because of no clinical, radiological and histopathological differential of tuberculosis caused by *M. tuberculosis* and *M. bovis* (da Silva Rabello, *et al.*, 2017).*M. bovis* is not the major cause of human tuberculosis but infect human too by either consuming raw milk, meat, their product from infected animals or by inhaling infective droplet. (Kanchora, *et a.*;l 2020)

This disease is one of the seven most neglected endemic diseases in the world, particularly in developing countries, and is a significant zoonosis that can spread to humans through aerosols, by the consumption of unpasteurized milk and dairy products and through meat from infected cows Veterinarians, farmers, and abattoir workers were most considered at the risk of this disease. The disease induces high animal morbidity and mortality that eventually reduces the financial capital and increases production costs (Kanchora, *et al.*, 2020, and Hemecho, 2017)

3.10. Status of Bovine Tuberculosis in Ethiopia

Ethiopia is one of the African countries where BTB is considered as protruding disease in animals. Detection of BTB in Ethiopia is carried out most commonly on the basis of

tuberculin skin testing, abattoir meat inspection and rarely on bacteriological techniques. However, the current status on the actual prevalence of BTB at a national level is yet unknown. In Ethiopia, Bovine TB has been shown to be endemic, with the prevalence ranging between 7.9 and 49% (Ameni, *et al.*, 2003 and Regassa, *et al.*, 2010)

4. MATERIALS AND METHODS

4.1. Description of the Study Area

The study was conducted from December 2018 to May 2019 in Shire Indaslassie town and Tahtay koraro District, North West zone of Tigray region, Northern Ethiopia.

The study was conducted from December 2018 to May 2019 in Shire Indaselassie town and Tahtay koraro District, North West zone of Tigray region, Northern Ethiopia.

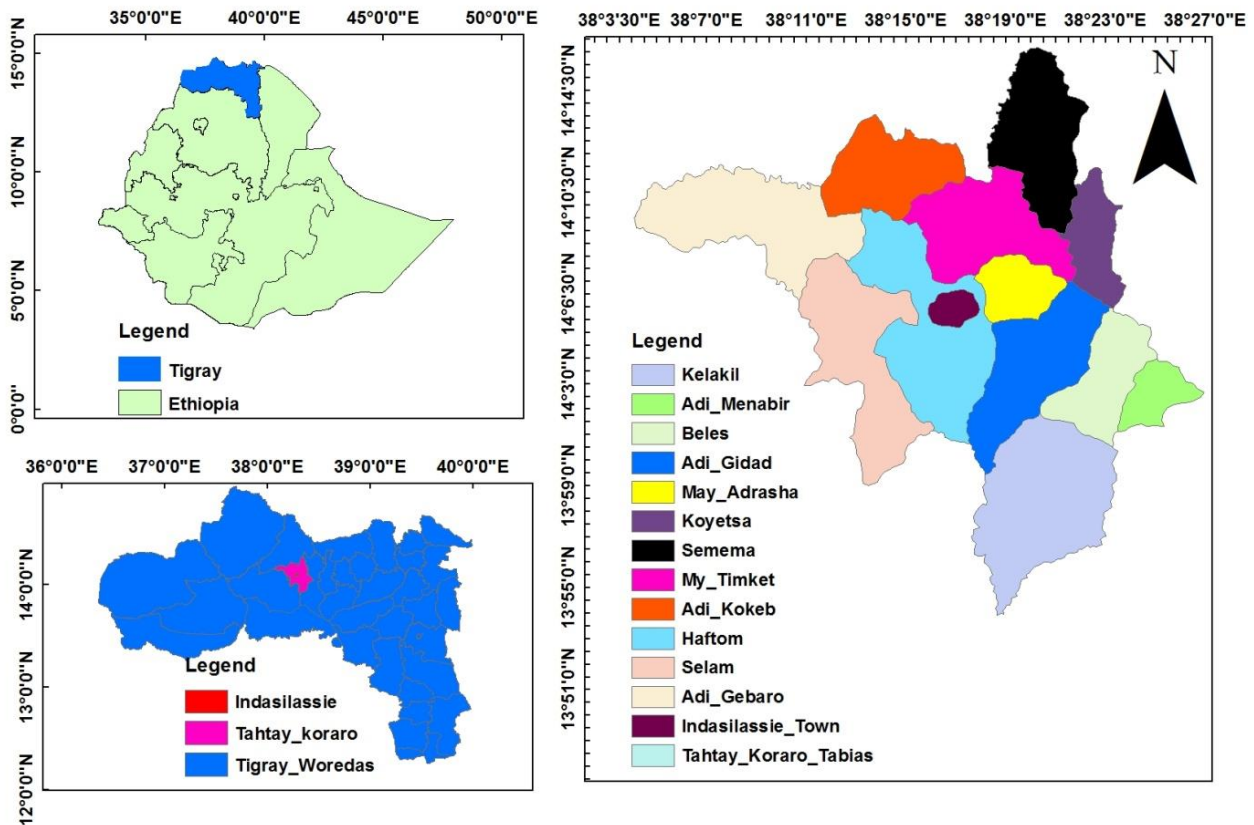
Shire Indaselassie is a zonal administrative city of Northwestern Tigray, Northern Ethiopia. The city is located about 1084 kilometers away from Addis Ababa, the capital city of Ethiopia, at latitude and longitude of 14.1057° N, 38.2849° E geographical coordinates, with an elevation of 1600-2200 above sea levels, the annual rainfall gets in summer season 700-1135 mm (905 mm), the temperature ranges 18- 34.6°C. It has divided into five kebele/Tabeia administratively, namely: Dedebit (01), Suhul (02), Hibret (03), Adikentybay (04) and Lekatit (05) (Figure 3). The town totally situated in Woina-dega agro (subtropical) climatic zone. The livestock population of the town has a total head of 3226 of cattle, 404 sheep, and 585 of goats. The livestock production is characterized by intensive and semi intensive production. The town is totally situated in woina dega agro (subtropical) climatic zone. (Source: Tahtay Koraro agricultural and developmental office, 2018).

Tahtay Koraro is one of the 36 districts in the Tigray Region of Ethiopia part of the North Western Zone. Tahtay Koraro is bordered in the south west by Asigede Tsimbela, in the north by Lailay Adiyabo, and in the south east by Medebay Zana. It has divided into Tabeia administratively, namely: Tahtay-adi gebaro, Laelay adi gebaro, Selam,Haftom, Adikokeb,May-timket, Semema town, Semema Rural, Seqela-Qoyetsa, May-adrasha,,Adigdad, Beles,Adi-menabr and Kelakil (Figure 3).The altitude range from 1800 meter to 2398 meter amsl and a longitude is 1064 to 2464 amsl. The climate is generally sub-tropical with an extended dry period of nine to ten months and a maximum effective rainy season of 50 to 60 days. The rainfall pattern is predominantly uni-modal (June to early September). with its minimum and maximum annual temperature 12 °C and 28 °C

respectively and total annual rainfall ranging 800 mm to 1100 mm. Tahtay Koraro woreda is covered by sub-tropical (locally called, Woina Dega) 73% tropical (locally called, Kolla)

23 % and 2% temperate (locally named, Dega). The Total population of livestock in this district is 79477 cattle, 34763 sheep, and 66800 goats. (Source: TahtayKoraro Agricultural and developmental office, 2018). In Tahetay koraro district was practiced mostly mixed crop-livestock production system and it's hadn't well-constructed dairy house. Some of the

dairy farms where they lived share a house with their livestock. However, a few Tabeias



of the dairy farm followed intensive production system namely; Semema and Belese. *

Figure 3: Map of the study areas

Source: Ethio-GIS and (CSA 2007)

4.2. Study population

The study animals were cattle of household/ farmers and the human population of the study areas. The cattle Populations of those small herd size dairy farms available in Shire Indaselassie town and Tahiti korero district was 3,236 and 126,124 respectively (Table 1). However, in both study areas, the village/Tabias were selected purposely based on the availability of dairy farms (both local and crossbred types) and ease of their accessibility. This study was executed on 382 dairy cattle belonging to 109 smallholders' dairy farms in the study areas depending on the willingness of the cattle owners. The dairy cattle eligible for this study (excluding calves aged below six months and late pregnancy (>7 month) cows) were exposed to tuberculin testing using Purified Protein Derivatives (PPD).

Table 1: Official cattle population of each Taebia / kebele of study area (2018 G.C)

Tabia/kebeles	Cattle	Sheep	Goat
Tahtay-adi gebaro	6121	2921	5747
Laelay adi gebaro	12018	1947	10667
Selam	8562	3408	1126
Haftom	19946	736	3376
Adikokeb	7842	7356	16662
May-timket	10665	6555	11525
Semema town	9180	3801	3290
Semema Rural	1020	519	448
Seqela-Qoyetsa	4595	988	876

May-adrasha	3601	1135	2606
Adigheid	13552	4496	8669
Beles	13959	2856	10861
Adi-menabr	2607	565	9682
Kelakil	12456	6492	2181
Shire town	3226	204	658
Total	129350	43979	88374

Source: (Tahtay-Koraro Office of Agriculture, 2018, unpublished)

4.3. Sample size determination

The total sample size was calculated for tuberculin testing based on the pre-definition of the following parameters: 95% level of confidence, 5% desired level of precision and 11.7 % with expected prevalence of bovine tuberculosis based on previous study (Ataklti, *et al.*, 2015). It was calculated to obtain the sample size according to (Thrusfield, 2007).

$$N = \frac{z^2 * P \text{ exp} (1 - P \text{ exp})}{D^2}$$

N= required sample size

P exp = expected prevalence

Z= 1.96 at 95% confidence interval

D= Desired absolute precision of 5%

Accordingly, the minimum sample size required to estimate the prevalence of BTB was 109 cattle. However, considering the small sample size, the design effect (to account for clustering of cases in a herd), and to increase level of precision and resources available to carried out the study, the sample size was re-calculated by choosing the population survey option. Assuming design effect of 2.4 times the sample size required from the selected 109 clusters (households/farms) would be 382 cattle. The questionnaire survey

was administered to 109 selected individuals depending on their willingness of farmers and small and micro enterprises. For human case, a total of 29 acid fast positive patients were interviewed about their association with cattle, habit of consumption of meat and milk and other relevant information related to tuberculosis and the questionnaire is indicated at appendix 3.

4.4. Study Design and Sampling Method

A cross-sectional study was used to operate a comparative intradermal tuberculin (CIDT) test on 382 heads of dairy cattle in and around shire Indaselassie town, Northern west Tigray. It was performed to estimate the prevalence of Bovine Tuberculosis (BTB) and assess potential risk factors related with BTB prevalence in dairy cattle. In addition, a questionnaire survey was conducted by using structured format to assess the awareness of cattle holders on bTB and its zoonotic impacts as well as the courses of transmission of the disease

List of dairy farms, and field cattle herd sites in each area were registered obtained from Tahiti korero agricultural and rural development office. Two-stage cluster sampling technique was used, households/farms were considered as primary units; while individual animals were secondary units. Simple random sampling method was used to select the farms and cattle herd sites and then individual animals were then used systematic random sampling technique to select to detect for BTB. Select two districts from Northern west Tigray and 10 Kebeles/ Tabeia of them were included in the study based on inclusion criteria (logistic, accessibility of animals, willingness of owner).

Physical examinations of animals and a questionnaire was carried out before the cattle were injected with PPD. For this study, cattle were grouped into herd size and were characterized into three groups. These were: small herds (1–5 cattle), medium herds (6–10 cattle), and large herds (>10 cattle). The management practice of studied farms was categorized as extensive, semi-intensive and intensive. Cattle were grouped into age groups by looking at an eruption of one or more teeth and Information obtained from animal owners or farm records and were classified in this study as (Torrell, 1998). Instead of a breeds group, cattle were classified as local and cross breeds. The body condition

score of the cattle was recorded using the guide line established by (Nicholson and Butterworth; 1986) aggregated as poor, medium and good). Instead of lactation status; classified as lactating and non-lactating (included heifers) through asking the owner and observation of mammary gland, but instead of reproductive status classified as pregnant and non-pregnant (included heifers) through history of conception date of the cattle from the owners' and through rectal palpation of the uterus to diagnose pregnancy before test was performed and registered on the format of record sheet.

4.5. Study Methodology

The comparative intradermal tuberculin test was conducted on 540 cattle using both avian and bovine purified protein derivates (PPD) supplied by the NAHDIC. Intradermal injections of 0.1 ml (2500 IU ml⁻¹) bovine PPD and 0.1 ml (2500 IU ml⁻¹) avian PPD were administered in two shaved sites, 12 cm apart from each other in the middle neck region, after having recorded initial skin thickness with a caliper and recorded as A1 for avian PPD site and B1 for bovine. One site was injected with an aliquot of 0.1ml of 2,500 IU/ml PPD bovine toward the head and the other site was similarly injected with 0.1ml of 2,500 IU/ml PPD avian in dermis toward the shoulder of the animal. Final skin thickness was measured at both injection sites after 72 hr and recorded as A2 avian PPD and B2. The results were interpreted according to the recommendation of the Office of International des Epizooties (OIE,2010).When the change in skin thickness was greater at the avian PPD injection site, the animal was considered as positive for Mycobacterium species other than the mammalian type (*M. tuberculosis* and *M. Bovis*). When increase observed at both sides of injection the difference was considered between the two sites of injections thus if the increase in skin thickness at the injection site for bovine PPD (B) was greater than the increase in skin thickness at the injection site of avian PPD (A), the manipulation of the change was done as this manner by subtracting A from B. When the change is recorded as less than 2mm it should be considered as negative, between 2mm and 4mm suspected and equal to 4mm and/or above positive for bovine tuberculosis (OIE, 2010).The same person conducted the entire process of tuberculin testing and reading of the result to avoid bias related to injection and reading technique (Ameni,2008)

Cattle data and coding of the variables

In addition to the CIDT result, the following information was collected for each tested animal: herd level, herd size sex, breed, age, body condition score, pregnancy status lactation status, herd size, farm system and farm management were addressed and also Names, descriptions and coding of the variables and their categories included in the study as potential risk factors of BTB considered for data collection during tuberculin testing/PPD injection in cattle of the study area

Table 2: Description of code Variable

Variable	Description	Coding of the variable
Educational level	Farmers' educational level	Illiterate(can/not read and write), read and write, primary school &secondary school and above
Age respondent	Age of the farmer in years (yrs.	18-28 yrs., 29-39yrs., 40-50 and >50 yrs.
Sex	Sex of cattle available in the farm	Male, female
Age (cattle)	Age of cattle available in the farm	<2, 2<x<4, 4<x<7, >7 (year)
BCS	The animal available in the farm coverage body muscle and fat	Poor, medium ,good
Breed	Breed of cattle available in the farm	Local, cross breed
Herd size	Total number of cattle in the farm categorized	Small(1-5),medium.6-10 & large >10,
Presence of livestock animals	Other livestock of animals available in the farm in addition to the farm cattle	Yes, Not available,
Building status	The floor types in the dairy houses, whether the dairy farms have concrete floors or other types of floor(example:	With concrete floors, without concrete floors

	sand, soil)	
Ventilation status	The status of the houses in terms of air supply and removal by natural means through windows and doors	Poor, medium & Good
Cattle contact with	With which livestock of animals do your animals get contact	No contact, yes contact
Wild animals present	Wild animals noticed in the farm or around the farm premises	No, yes
Signs of respiratory diseases?	Any clinical signs observed in relation to respiratory Diseases	No, Yes
Housing type	Dairy house types in which either the animals are kept tied (stanchioned) in the house/shade or kept at a liberty to move freely Stanchion(tied) system, other	No, Yes
Housing system	System of housing that either keeps the animals all the time inside, or allows the animals to stay both inside or outside, or outside all the time	Yes, Housed indoors, No, all the time housed indoors
Knowledge of BTB zoonotic	Knowledge about BTB of the respondents Transfer between human and animals	Yes, No
Awareness of BTB	Knowledge about BTB of the respondents	Yes, aware No, don't aware

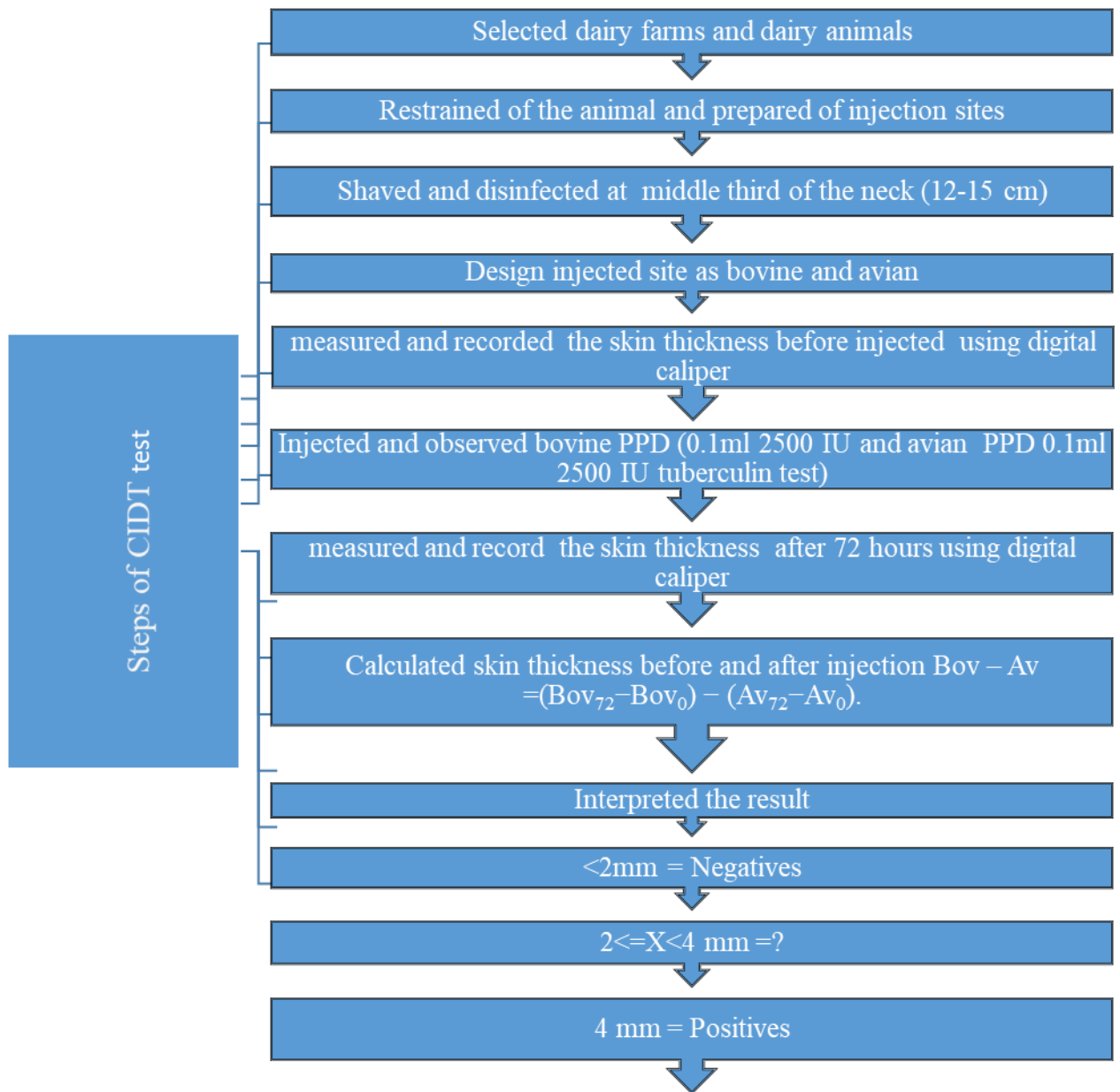


Figure 4:Steps of Comparative Intradermal Tuberculosis Test

4.6. Questionnaire Survey

A structured questionnaire was designed and administered to cattle owners and TB patients in English language and then translated in to Tigrigna languages. A total of one hundred nine (N= 109) individual animal owners and (N=29) TB patients were interviewed from the study area. Interviews were conducted concerning sex, age, education status and habit of consumption of raw foods of animal origin such as milk and meat and also management and health status of their farms. All sampled respondents were interviewed in order to gather necessary information related to the variables of interest of the study and to identify potential risk factors related with the occurrence of and transmission of a diseases and zoonotic implications.

4.7. Data Analysis

Prevalence was calculated as the proportion of suspected CIDT positive animals from the total number of animals sampled (Thrusfield, 2007). Data related with skin thickness, ID, age, sex, breed, BCS, herd size, herd level, management system, farm type of each animal were recorded on a data sheet during PPD injected. The skin thickness for each “bovine” and “avian” injection sites was measured and recorded again 72 hours after injected of tuberculin. The recorded data was entered, stored, classified, coded and filtered using Microsoft Excel computer program and was exported and analyzed by STATA version 14.

Descriptive statistical analysis was used for qualitative data collected using questionnaires. The association of different risk factors with prevalence of BTB was analyzed logistic regression test. Ordinary logistic regression model to investigate relationship between the potential risk factors and outcomes. Univariate logistic regression analysis was used to assess the strength of association between the outcome variable (prevalence) and the various explanatory variables like, ID, age, sex, breed, BCS ,herd size, herd level, management system, farm type and skin thickness . The outcomes of all statistical analyses were individual animal and herd level binary outcomes. Secondly, multivariate logistic regression was applied to estimate the measures of associations between the potential risk factors (species, age, and sex of the tested animals) and the outcome were tested resulting in odds ratios (OR). A herd was

considered positive if it had at least one skin test positive reactor using the ≥ 4 mm cut-off. A confidence level of 95% and the level of significance at p-value <0.05 was used in all statistical analysis.

4.8. Data Collection and Management

Data obtained from the farms were first recorded on data collecting sheets. Structured questionnaires were administered to the livestock owners to generate data physiological status of animal and general management condition of the farm. Questionnaires were delivered to the farmers by the local languages (Tigrinya) and translated back to English upon filling the forms. Putative exposure variables were given specific codes and dataset established in an Excel spreadsheet for appropriate storage. Age of an animal was determined based on dentition characteristics combined with owner's recall information on birth date. Body condition score (BCS) was recorded as poor, moderate and good based on guidelines described in the manual for body condition scoring of cattle (Nicolson, 1986)

4.9. Ethical Clearance

Ethical approval to implement the research was granted by colleague of the research committee veterinary science Mekelle University. This study was supported by NAHDIC to get MTB PPD kit and other supportive materials to do this task. Public awareness of human subjects also obtained consent from Sehul Hospital Shire Indaslassie. Skin testing was based on the international standards (OIE, 2009), and all skin testing and data collections were reliant on the willingness of herd owners and/ or managers following elaboration of the study purpose, adverse effect and benefits of the research. The owners has were informed, when their animals were found BTB positive to take some measures of disease prevention and control and also advised owners to seek further advice from the local veterinary clinic for close follow up and further medication of their sick animals.

5. RESULTS

5.1. Prevalence of Bovine Tuberculosis

Individual animal and herd level prevalence

In the present study, a total of 382 head of cattle out of 109 herd/farms were tested using CIDT to determine prevalence of BTB. The overall prevalence of BTB animal and herd level was 10.20 % (95% CI: 0.568-3.479) (n=39) and 26.6 % (95% CI: 0.122- 0 .729) (n=29) was found BTB positivity at cut-off ≥ 4 mm. respectively. Similarly, prevalence of BTB positive at individual animal and herd level in Shire town was (14.89 %, 21/141) and (39.58 %, 19/48) respectively while in Tahtay koraro district was (7.48%, 18/241) and (16.40%, 10/61) respectively, described below in (Table 3).

Table 3: Individual animal and herd level prevalence of BTB using CIDT test based on ≥ 4 mm cut-off value.

Study	Animal level prevalence			χ^2	P	–	Herd level prevalence		χ^2	P-value
	No. tested	Positive	(95% CI)				No. tested	Positive		
Shire	141	21(14.89)		5.35	0.021*		48	19(39.58)	7.40	0.007*
T/koraro	241	18(7.48)					61	10(16.40)		
Total	382	39(10.20)					109	29(26.61)		

*=Statistically significance CI = Confidence Interval X^2 = chi square

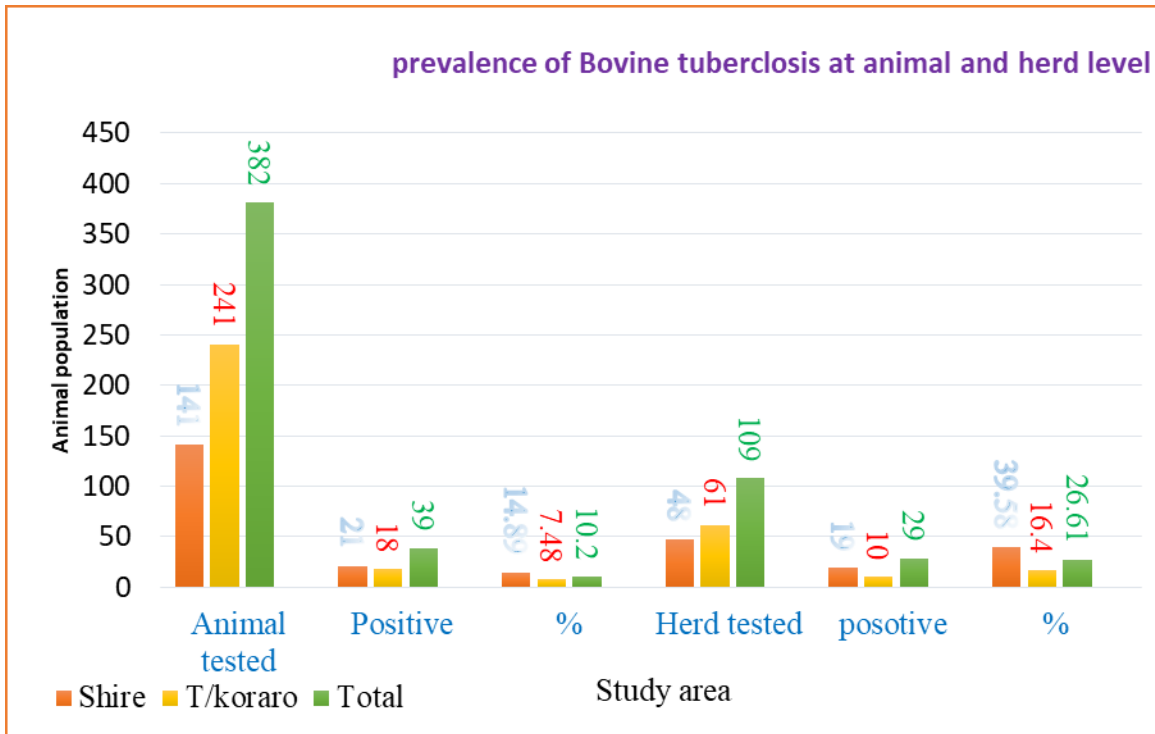


Figure 5: Prevalence rates among CIDT reactors (95% CI) at animal and herd level

5.2. Risk Factors Associated with Prevalence of Bovine Tuberculosis

Animal level prevalence and associated risk factors

Upon detailed CIDT test of dairy cattle presented univariate logistic regression analysis was determined the relationship between risk factors and cattle tuberculin reactivity with respect to individual animal prevalence indicated that there were a statistical significant association across age, breed, BCS, pregnancy, lactation, herd size, farm system and management status at ($p < 0.05$). Cross breed had more sensitive being BTB positivity twice (OR= 2.36, 95% CI: 1.138-4.890) than local breed while pregnant cow had higher BTB prevalence (OR= 4.33, 95% CI: 1.461-12.857) than non-pregnant cow. Likewise, good BCS lower tuberculin positive than medium (OR=9.98) and poor (OR=23.08) BCS. On the other hand, there wasn't a significant difference bovine tuberculin reactivity in study area, sex, and parity class, and age ($2 < x < 4$ or 10 years or $4 < x \leq 7$ years old). (Table 4).

Table 4: Univariate logistic analysis of association of animal level risk factors with bovine tuberculin reactivity based on ≥ 4 mm cut-off value.

Risk factors	Categories	No. of cattle		OR (95%)	P-value
		Tested	Positive (%)		
Study area	Shire	139	14(10.7)	Ref	
	T/keraa	243	25(10.28)	10.02(0.513-2.041)	0.946
Sex	Male	123	9(7.32)	Ref	
	Female	259	30(10.58)	1.659s(0.762-3.612)	0.202
Age	<2	97	9(9.27)	Ref	
	2<x<4	124	7(5.65)	0.58 (0.209-1.631)	0.306
	4<x≤7	132	14(10.61)	1.16 (0.480-2.801)	0.741
	>7	29	9(31.03)	4.4 (1.549-12.495)	0.005*
Breed	Local	176	11(6.25)	Ref	
	Cross	206	28(13.60)	2.36(1.138-4.890)	0.021*
BCS	Good	89	1(1.12)	Ref	
	Poor	77	16(20.78)	23.08(2.981-178.672)	0.003*
	Medium	216	22(10.19)	9.98(1.324-75.211)	0.026*
Pregnancy	Not Pregnant	99	4(4.04)	Ref	
	Pregnant	162	25(15.43)	4.33(1.461-12.857)	0.008*
Lactation	Not Lactating	151	11(7.28)	Ref	
	Lactating	110	18(16.36)	2.49(1.125-5.514))	0.024*
Parity class	Heifer	60	4(6.67)	Ref	
	Parity 1-3	136	15(11.03)	1.74(0.550-5.467)	0.346
	Parity>=4	64	9(14.06)	2.29(0.666-7.878)	0.188
Herd size	1 -5	107	3(2.80)	Ref	
	6-10	120	19(7.5)	6.52(1.872-22.717)	0.003*

	>10	155	17(10.97)	4.27(1.219-14.957)	0.023*
Farm system	Extensive	120	2(1.67)		
	Intensive	206	31(32.98)	10.45(2.454-44.504)	0.002*
	Semi extensive	56	6(10.71)	7.08(1.381-36.284)	0.019*
Management status	Good	89	1(1.12)	Ref	
	Poor	77	16(20.78)	6.09(1.335-27.734)	0.020*
	Medium	216	22(10.19)	6.56(1.514-28.404)	0.012*

* = statistically significant, BCS = Body condition score χ^2 =chi square Ref = Reference
 No = Number

Herd level prevalence and associated risk factors

In the Univariate logistic regression analysis indicated that farm management, herd size, farm system, type of house, respiratory problem, sanitations and cattle mixing with wild animal were statistically predictor ($p < 0.05$) on tuberculin reactivity among herd level. Thus, the result showed that poor managerial inputs had 6.58 times the odds of being bovine tuberculin reactor compared to good managerial condition practice and herds size had with >10 heads (OR= 5.80, 1.19-28.20, P=0.029) and 6-10 heads (OR=10.35, 95% CI: 2.136-50.216, P=0.004) of cattle were more likely to become tuberculin positive compared to those herds with 1-5 heads of cattle. Herds maintained in closed confinement were at a greater risk of becoming tuberculosis positive 3.75(1.189-11.824; 0.024) than those maintained in open-air barn. Poor hygienic condition of animal barn appeared to be more sensitive exposed to infection linked to high risk of positive reactivity to skin test 3.60 (1.135-11.411; 0.030). The odds of cattle mix with wild animal was at a greater risk of becoming tuberculosis positive (OR=4.60 95% CI=1.86-11.31, P=0.001) than those doesn't mix with wild animal. Moreover, in the present study, intensive farm system (OR=18, 95% CI: 2.30-14, 0.611, P=0.006) was eighteen times more reactive to bovine tuberculin positivity as compared to extensive farming practice. On the other hand, the rests assessed as risk factors in this study were no statistically significant difference between/among them of ventilation, Mix their cattle with other cattle and medium sanitation with tuberculin reactive test.

Table 5: Univariate logistic regression analysis of the association of animal level risk factors with prevalence of bovine tuberculin positivity at a cut-off ≥ 4 mm

Risk factor	Categories	No. of herd		COR	P-value
		Tested	Positive (%)		
Study Site	Shire	48	19(39.58)	Ref	
	T/keraro	61	10(16.39)	0.30(0.122-0.729)	0.008*
Herd size	1 -5	31	2(6.45)	Ref	
	6-10	36	15(41.66)	10.35(2.136-50.216)	0.004*

	>10	42	12(28.57)	5.80(1.192-28.202)	0.029*
Farm system	Extensive	31	1(3.22)	Ref	
	Intensive	64	24(37.5)	18(2.304-140.611)	0.006*
	Semi extensive	14	4(28.57)	12(1.196-120.316)	0.035*
Management	Good	27	2(7.40)	Ref	
status	Poor	29	10(34.48)	6.58(1.288-33.617)	0.024*
	Medium	53	17(32.07)	5.90(1.251-27.851)	0.025*
Type of house	Open	34	4(11.76)	Ref	
	Closed	75	25(33.33)	3.75(1.189-11.824)	0.024*
Mix their cattle	No	78	24(30.76)	Ref	
with	Yes	31	5(16.12)	0.43(0.148-1.262)	0.125
other cattle					
Respiratory	No	58	10(17.24)	Ref	
problem	Yes	51	19(37.25)	2.85(1.174-6.917)	0.021*
Ventilation	Good	26	6(23.07)	Ref	
	Medium	48	7(14.58)	0.56(0.168-1.916)	0.363
	Poor	35	16(45.71)	2.80(0.907-8.680)	0.073
Sanitations	good	27	5(18.51)	Ref	
	Medium	42	6(14.29)	0.73(0.199-2.690)	0.640
	Poor	40	18(45)	3.60(1.135-11.411)	0.030*
Share the same	No	76	21(27.63)		
house					
with their					
animals	Yes	33	8(24.24)	0.83(0.356-2.148)	0.713
Wild animal	No	70	11(15.71)		
	Yes	39	18(46.15)	4.60(1.868-11.312)	0.001*

Multivariate logistic regression analysis was used to assess the relationship between risk factors and CITD test reactors in the study area. Among the risk factors assessed breeds, BCS, pregnancy and lactation, herd size (> 10 cattle) and management condition demonstrated statistical significance ($p < 0.05$). From breed composition of cattle, cross breeds (adjusted OR=18.41, 95% CI: 1.91-195.83) was eighteen times more reactive to bovine tuberculin positivity compared to local breeds (zebu). In addition to pregnant (adjusted OR = 14.86, 95% CI: 2.341-94.37) becoming BTB positive fourteen times more sensitive than non-pregnant cow. While lactating cows had higher BTB prevalence five times (adjusted OR = 5.20 95% CI=1.451-18.626, P=0.011) compared to non-lactating cows. Likewise risk factors showed statistically significant tuberculin reactivity higher in medium BCS (OR=23.08) and followed by the poor BCS (OR=84.62) as compared to the good BCS. However, the rests assessed as risk factors in this study were no statistically significant difference between them Study site, sex, age, parity class, herd size with tuberculin reactive test shown in Table 6.

Table 6: Univariate and multivariate logistic regression analyses of risk factors affecting reactivity of cattle to bovine tuberculin reactivity.

Risk factor	Categories	No. animals	COR		P-value	AOR	P-value
			Examined	Positive			
Study Site	Shire	139	14(10.7)				
	T/keraro	243	25(10.28)	10.02(0.513-2.041)	0.946	0.73(0.172-3.092)	0.671
Sex	Male	123	9(7.32)	Ref	Ref	-	-
	Female	259	30(10.58)	1.659(0.762-3.612)	0.202	-	-
Age	<2	97	9(9.27)	Ref			
	2<x<4	124	75.(65)	0.58(0.209-1.631)	0.306	0.23(0.033-1.606)	0.139
	4<x≤7	132	14(10.61)	1.16(0.480-	0.741	0.67(0.107-4.159)	0.667

	>7	29	9(31.03)	2.801) 4.4(1.549- 12.495)	0.005*	0.88(0.083-9.231)	0.915
Breed	Local	176	11(6.25)	Ref			
	Cross	206	28(13.60)	2.36(1.138- 4.890)	0.021*	18.41(1.912- 195.833)	0.002*
BCS	Good	89	1(1.12)	Ref			
	Poor	77	16(20.78)	23.08(2.981 -178.672)	0.003*	84.62(5.941- 1205.416)	0.001*
	Medium	216	22(10.19)	9.98(1.324- 75.211)	0.026*	23.06(2.250- 236.332)	0.008*
Pregnancy	Not Pregnant	99	4(4.04)	Ref			
	Pregnant	162	25(15.43)	4.33(1.461- 12.857)	0.008*	14.86(2.341- 94.370)	0.004*
Lactation	Not Lactating	151	11(7.28)	Ref			
	Lactating	110	18(16.36)	2.49(1.125- 5.514))	0.024*	5.20(1.451- 18.626)	0.011*
Parity class	Heifer	60	4(6.67)	Ref			
	Pairty 1-3	136	15(11.03)	1.74(0.550- 5.467)	0.346	1.53(0.244-9.576)	0.648
	Pairty>=4	64	9(14.06)	2.29(0.666- 7.878)	0.188	1.89(0.224- 15.854)	0.559
Herd size	1 -5	107	3(2.80)	Ref			
	6-10	120	19(7.5)	6.52(1.872- 22.717)	0.003*	7.51(0.578- 97.680)	0.123
	>10	155	17(10.97)	4.27(1.219- 14.957)	0.023*	24.52(1.837- 327.411)	0.016*

Farm system	Extensive	120	2(1.67)				
	Intensive	206	31(32.98)	10.45(2.454-44.504)	0.002*	16.17(0.286-916.176)	0.026
	Semi extensive	56	6(10.71)	7.08(1.381-36.284)	0.019*	19.46(1.164-325.246)	0.039*
Management status	Good	88	2(2.27)	Ref			
	Poor	94	13(13.83)	6.09(1.335-27.734)	0.020*	20.639(1.488-286.103)	0.024*
	Medium	161	24(14.91)	6.56(1.514-28.404)	0.120	19.37(1.905-196.833)	0.012*

BCS=body condition square *=statistically significance COR= **Crude Odds Ratio**

AOR= Adjusted Odds Ratio

5.3. Assessment of Cattle Owners awareness on Bovine Tuberculosis

109 cattle owners or members of households were questioned to assess awareness of participants on the public health impact of BTB. About 23.85% (26/109) reported that they had the knowledge of or 19.27% (21/109) and had heard about BTB. Similarly, 10 (23.25%) respondents were aware of the zoonotic importance of BTB. Out of the total interviewed households, 22 (20.18 %) farm workers/attendants had TB cases that had transmitted with direct contact with the animals (Table7).

Table 7: Knowledge of cattle owners about BTB and its transmission to humans

Knowledge assessed	Category of the Responders	
	Owner of farm (N=109)	positive (%))
Heard of about bovine TB	26	23.85
Had respiratory problems in their cattle	35	32.11
Knew BTB can affect animals	21	19.27
Knew BTB is zoonotic	19	17.43

Consumption raw milk is source of infection for TB	21	19.27
Consumption raw meat is source of infection for TB	12	11.00
Knew that cattle transmit bovine TB to humans	11	10.09
Knew symptom of bovine TB	8	7.34
Knew symptom of human TB	29	26.60
Know close contact with cattle can facilitate BTB transmission	22	20.18

Table 8: Assessment of behavioral factors and practice of the of farm owners in study area.

Habit of respondents	No. of interviewed	Percentage (%)
Habit of drink raw milk	51	46.79
Habit of eat raw meat	46	42.20
Share the same house with their animals	33	30.28
Mix their cattle with other animals	31	28.44

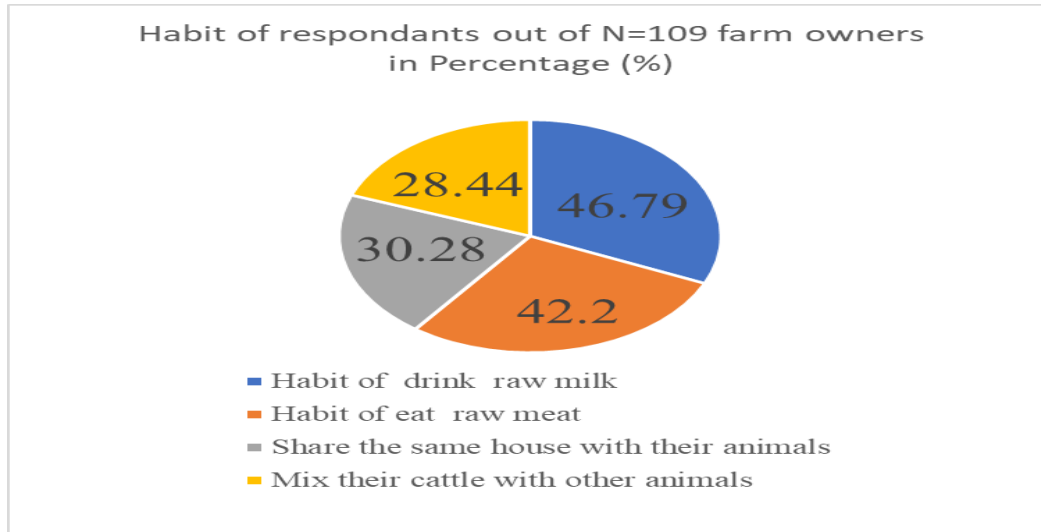


Figure 6: Assessment of behavioral factors and practice of the of farm owners in study area.

5.4. Assessment characteristics awareness on zoonotic tuberculosis among tuberculosis lymphadenitis patients

In the study area, 35 individuals were available in patient medication (direct observation treatment) and outside hospital. Among the available patient six non-respondents, these questionnaires were incomplete and excluded from final analysis. On the other hand twenty nine (82.86%) individuals who completed the questionnaire had got appropriate information. Among the interviewed 29 human TBLN patients regarding of Socio demographic characteristics Respondent’s in such a way that individuals between secondary and above highly awarded of age (AOR 0.06, 95% CI 0.01–0.53) and elderly, 50+ years (AOR 0.16, 95% CI 0.03–0.78) were less likely to be aware of BTB compared to the 18–29 year olds (Table 9). Moreover, TB positive humans’ patients were also interviewed regarding their habit of raw milk drinking and raw meat eating (Table 9).

Table 9: Socio-demographic characteristics awareness on zoonotic tuberculosis among tuberculosis lymphadenitis patients

Demography factors	Categories	No. of respondents	of aware of BTB	of COR (95% CI)	AOR (95%CI)
--------------------	------------	--------------------	-----------------	-----------------	-------------

Age (year)	<18	8	3(28.57)	Ref	Ref
	18-28	8	3(37.5)	1(0.130-7.570)	4.81(0.358-64.770)
	29-50	12	3(14.29)	0.55(0.799-3.858)	0.31(0.012-7.914)
	>=50	1	-	-	1.14(0.088-14.924)
Sex	Male	6	2(20)	Ref	Ref
	Female	23	7(15.7)	0.87(0.128-5.942)	1.49(0.158-14.168)
Educational status	Illiterate	8	2		
	Read and write only	6	2(11.1)	1.5(0.145-15.460)	0.48(0.041-5.734)
	Primary(1-6)	6	2(16.66)	1.5(0.145-15.460)	0.19(0.009-4.146)
	Secondary and above	9	3(33.33)	0.5(0.180-12.455)	0.31(0.022-4.301)

BTB=Bovine tuberculosis COR= Crude odds ratio AOR= Adjusted odds ratio Ref= Reference

Table 10: socio demographic characteristics of awareness on Habit of raw milk and meat

Demography factors	Categories	Number of respondents	Habit of raw milk (%)	p-value	Habit of raw meat (%)	p-value
Age (year)	18-28	8	6(75)	0.091	3(37.5)	0.840
	29-39	8	5(62.5)		2(25)	
	40-50	12	3(25)		3(25)	
	>=50	1	-		-	
Sex	Male	6	4(66.6)	0.311	1(16.66)	0.502
	Female	23	10(43.47)		7 (30.43)	
Educational status	Illiterate	8	3(66.6)	0.423	4(50)	0.210
	Read and write only	6	4(66.6)		-	
	Primary(1-6)	6	4(66.6)		2(33.3)	
	Secondary and above	9	3(33.3)		2(22.22)	

6. DISCUSSION

Prevalence of BTB

In the current study, the prevalence of BTB was determined among dairy farms located North West Tigray, Namely shire Indaselassie and Tahtay koraro district. A total of 109 herds comprising 382 dairy cattle were sampled from these study centers, and tested for BTB using the CIDT test. The results demonstrated that the overall animal and herd level prevalence was 10.2% (95%CI: 0.568-3.479) and 26.60% (95%CI: 0.122-0.729) respectively. This result individual animal level prevalence was comparable agreed on reported previous findings in different parts of the Ethiopia; 11% in Afar (Mamo *et al.*, 2013); 11.3% in Mekelle town (Zeru *et al.*, 2014), 11.7% in Adigrat town (Hadush *et al.*, 2015); 11% in Adama town central Ethiopia (Ameni and Erkihun, 2007), 14.2% in southern Ethiopia (Ameni *et al.*, 2001) and 11.6% in Hawassa town and its surroundings (Regassa *et al.*, 2010). However, this result was lower than results previously reported, 20.3% (Kemal *et al.* 2019) and 24.3%, 27.3% and 27.8% at Holleta, Ziway and Ambo dairy farms (Ameni *et al.*, 2003b) respectively.

Similarly, herd level prevalence agreed with reported 29% (Ameni *et al.*, 2001), 20% in Mekelle (Zeru *et al.*, 2014) and 23.53% in Gondar town (Fikirte *et al.*, 2015). However, Our finding contrary in herd level prevalence Comparative BTB prevalence was reported 48.7%, 43.4% and 53.6% were in central Ethiopia (Regassa *et al.* 2008), in Addis Ababa (Elias, 2008) and in Addis Ababa (Tsegaye *et al.*, 2010) respectively. This could be due to the variation of in tested animal, herd size and production systems. In the present studies, the majority of the tested herds were smallholders found in and around urban areas with smaller herd size and with similar herd compositions. In addition to this, in rural areas animals were kept in the open air in contrast to previous studies. BTB prevalence variations among different researchers finding could be due to geographical location, socio economic aspect of the areas, production systems, herd size, and breed type, production system and others

According to univariate regression analysis in current study found that statistically significance ($p=0.05$) with risk factors of age, breed, BCS, pregnancy, lactation status, herd size, farm system and management status with PPD reactivity intradermal skin test.

prevalence of BTB had significant association with aged >7 years old (OR = 4.4) had a higher odds for tuberculin reactivity when compared to younger animals, which was this finding was in agreement with previous reports by others (OIE, 2009; Mekonnen *et al.*, 2019)

Most of cattle included in the study was cross breed and bovine tuberculosis prevalence was strong association compare to local breed at ≥ 4 mm cut-off value and this is shown in Table (4) and figure (2). Several studies have shown a variation in susceptibility to bTB among cattle breeds. These findings agreed with (Bermingham *et al.*, 2009, Nuru *et al.*, 2015; Admasu *et al.*, 2014; Romha *et al.*, 2014; Zeru *et al.*, 2014). The probable reason could be the fact that genetically improved cattle suffer more severely from poor housing, under-and malnutrition and kept under intensive and semi-intensive production systems subsequently become more susceptible to infection.

The current finding indicated that correlation between body condition and BTB as shown in table (4) and figure (2), body condition score (BCS) was statistically associated ($P=0.003$) with positive skin reactivity animals having different body condition scores. Animals having poor BCS (20.78) were associated to the increased risk of bovine tuberculin positivity as compared to with medium BCS and good BCS of cattle. This result is in agreement with the previous studies (Nemomsa, 2014, Zeru, *et al.*, 2014, Romha, *et al.*, 2013, and Hadush, *et al.*, 2015). This is in line with study reported by (Radostits *et al.*, 1994, Radostatits *et al.*, 2007) were poor BCS had weak protective immune response, wasting of the body condition as well as the chronic nature of the disease This parameter should be analyzed carefully, since while a poor BCS might be a cause of disease, it is also extremely influenced by the seasonal climatic changes (rain or dry season) and the consequently more or less availability of pasturage and/or prevalence of intestinal parasites in the small scale small holder farming. These conditions could lead to a decline in the cell mediated immune responses of the animal making them less sensitive (anergic state) to tuberculin test (dela Rua-Domenech *et al.*, 2006).

The prevalence of bovine tuberculosis in this study indicated that pregnancy (0.008) and lactation ($P = 0.024$) were significantly linked with reactivity in CIDT. Prevalence of BTB among skin test reactive lactation and pregnancy cows was 16.36% (18/110) and 7.28% (11/151) of lactating and non-lactating cows and 15.43(25/162) and 4.04 % (4/99) for pregnant and non-pregnant cows respectively. The odds of lactating cows (OR = 4.33) was four times more sensitivity had being bovine TB positivity as compared to non-lactating cows. In comparison with our finding, (Elias *et al.*, 2008, Gebremariam *et al.*, 2014) reported that among physiological status group, lactating animals had the highest prevalence of BTB reported lactating female animals had higher BTB prevalence than non-lactating animals. Similarly, (Kazwala *et al.*, 2001) found significantly more (14.6%) PPD reacted lactating cattle in the skin test than did non-lactating cows (12.0%). pregnant cows were most at risk four times (OR=4.33) to show positive reactions in the skin test compared to non-pregnant cow. These results are in agreement with a similar study in (Elias *et al.*, 2008) that reported that among ‘physiological status’ group’s pregnant lactating animals had the highest prevalence of BTB (Kazwala *et al.*, 2001) also reported high reactivity in CIDT test in lactating cows. This significant difference could possibly be due to greater production stress experienced by dairy cows and also gathering of cows during milking which promotes the risk of transmission of the disease. In contrast to the current study (Gebremedhin, *et al.*, 2014), Zeru, *et al.*, 2014, Hadush, *et al.*, 2015) and (Dejene, *et al.*, 2016) found insignificant association between lactating and non-lactating cattle with the prevalence of BTB in their study.

Herd/farm management conditions suggesting that poor managerial inputs increase the risk of BTB. Previous studies similarly had reported higher infection rates in farms under poor management conditions compare to good management condition (Radostits, 2007; Zeru, *et al.*, 2014; Romha, *et al.*, 2014; Hadush, *et al.*, 2015 and Habitu, *et al.*, 2017). It can therefore be generalized that the status of BTB could be improved by adopting sanitary measures that improve hygiene conditions on farms. Moreover, in the present study, the odds of cross breeds (OR=2.36) was twice times more reactive to bovine tuberculin positivity as compared to local (Zebu) breeds. Our finding comparable to which is similar with previous finding reports Previous studies (Romha, *et al.*, 2013,

Gebrezgabiher, & Ameni, 2014; Sibhat, *et al.*, 2017) also indicated the importance of breed difference for increased risk of BTB in cattle. The probable reason could be the fact that cross breed cattle suffer more severely from poor genetic resistance, poor housing, under-and malnutrition and when they kept in more hot and overcrowded environment. Subsequently become more susceptible to infection (O'Reilly, 1995). In contrary to this finding (Romha *et al.*, 2013) reported less likely prevalence of BTB in cross breed comparable to local breed (Zebu) this is may be due to the differences of the study sites, free grazing, and less confinement of cattle who had conducted the study in Western part of Tigray had few number of cross breed relative to local breed.

According to univariate regression analysis of risk factors in current study found that statistically significance ($p=0.05$) with factors of study site, breed, BCS, lactation status, pregnancy, herd size, farm system, type of house and management conditions of the farms with PPD reactivity intradermal skin test. The result of this study statistical significant ($P=0.008$) between study site and PPD reactivity cattle. Prevalence of the BTB was 39.58% (19/48) and 10.39% (10/61) in shire Indaslassie town and Tahetay koraro district respectively. In Tahetay koraro herd level (OR = 0.30, 95% CI=0.122-0.729) were less sensitive to BTB skin reaction as compared to shire Indaslassie town. This difference in which relatively low prevalence reported as compared to previous studies in Debre Zeit area could be due to the variation in recent improvement in management system of the dairy farms This statistically significant infection variation between the two districts might probably be due to agro-ecological, farm system and type house differences among the farms and also in addition to agro ecological feature differences in between the study areas, the dairy housing system of the sites also differs that cattle kept under indoor system and house with poor ventilation may facilitate transmission of the pathogen between cattle.

The study finding indicated that herd tuberculin from univariate analysis of the association between herd level exposure factors and herd positive reactivity to tuberculin test. The results showed that the positive tuberculin test in the study population showed a statistically significant association with herd management conditions suggesting that poor

managerial inputs increase the risk of BTB. Previous studies similarly have reported higher infection rates in farms under poor management conditions

In the present study, the herd tuberculin test finding showed a statistically significant association between large and medium herd size were four (OR = 4.27%) and six times more reactive and (OR = 6.52%) than compare to small herd size respectively. This result is in consistent with earlier finding (Ameni and Erkihun, 2007; Tsegaye *et al.*, 2010; Zeru, *et al.*, 2014 and Hadush, *et al.*,2015). This could be due to the fact that the present study was conducted in smallholder farms with smaller herds, in which some animals were kept in the open air in contrast to previous studies, which were conducted on relatively large herd-sized farms The transmission of BTB from cattle to cattle is largely influenced by herd size; the larger the herd size the greater the chance of transmission (O'Reilly, *et al.*, 1995). However, compatible with the result of the present study, low prevalence of BTB have been reported in smallholder dairy farms (Radostits, *et al.*, 2006) and in cattle of rural livestock production systems (Tschopp, *et al.*, 2010). When larger proportion of the study animals was grazing in the field, the level of confinement is reduced to a certain degree, which in turn minimizes the rate of infection in the herd (OIE, 1996). Moreover, this study revealed that intensive production system (OR = 10.45) had strong association with prevalence of bovine tuberculin positivity which was in line with the previous reports of (Ayele ,*et al.*, 2004) and (Elias *et al.*, 2008). This could be due to the fact that intensive farming system promotes close contact between animals, thereby favoring the spread of the infection. Similarly, herd tuberculin results showed a statistically significant association with herd management conditions, signifying that poor managerial inputs increase the risk of tuberculosis (Griffin *et al.*, 1993). Previous studies (Ameni *et al.*, 2003; Elias *et al.*, 2008) had similarly documented.

In this study, Assessment on the level of awareness of cattle owners about BTB showed that 19.27% (21/109) of the respondents know cattle can be infected by tuberculosis, and 17.43% (19/109) recognized that BTB is zoonotic. Only 19.7 % and 11% of them aware about the transmission of BTB through consumption of raw milk and raw meat respectively. (Ameni *et al.*; 2007) have indicated that lack of understanding regarding the zoonotic importance of BTB, food consumption habit and poor sanitary measures is the

potential risk of BTB to public health. The proportion of BTB contributes to total tuberculosis cases in humans depends on the prevalence of the disease in cattle, consumer habits, socio-economic conditions, level of food hygiene and medical prophylaxis measures in practice (Tigre *et al.*, 2011). In the present study, 60% of the respondents consume unpasteurized or raw milk. This report is higher than the previous findings (52.1%) by Ameni *et al.*, (2003) and 45 % by (Regassa *et al.*, 2010) stated that cattle owners who consumed raw milk were at greater risk of having active tuberculosis than those who consumed boiled milk. However, the current report on proportion of respondents.

Assessment on the level of awareness of cattle owners about BTB showed that 21/109 19.27% of the respondents know cattle can be infected by tuberculosis, and 17.43% (19/109) recognized that BTB is zoonotic. High number of respondents had therefore, no detailed and accurate knowledge about tuberculosis and its zoonotic importance. (Ameni *et al.*, 2008) and (Radostits *et al.*,2007) indicated that lack of understanding regarding the zoonotic effect of BTB, food consumption behavior and poor sanitary measures are among the potential risk factors of BTB to public health. Only (19.27 %) and (11%) of the interviewed farm owners and/or farm attendants know milk and meat could be vehicles for zoonotic transmission of BTB, respectively. The interview indicated 20.18% of the respondents' knowledge close contact with cattle can facilitate BTB transmission. Transmission of the disease tuberculosis may be cyclical i.e. cow-to-man-to- cow (Cosivi, *et al.*, 1998), underlying the existence of risk of dissemination of the pathogen among human and cattle population. Humans attain the infection mainly by ingesting the causative agent in raw milk and its products as well as by inhaling the pathogen during close physical contact between the owner and his/her cattle, particularly at night time since in some cases they share shelters with their animals (Anderson, *et al.*, 1997).Our assessment of the knowledge of the society on BTB is in line with the findings of (Mohammed *et al.*, 2012. Akililu *et al.*, 2014 and zeru *et al.*, 2014).

7. CONCLUSION AND RECOMMENDATION

The output of present study has revealed that an overall BTB prevalence in dairy cattle in and around Shire Indaslassie town was moderately high in both animal and herd levels, 10.2%.and 26.6% respectively. The prevalence of BTB positive at animal and herd level was higher in Shire town (14.89 %) and (39.58 %) compared to Tahtay koraro district (7.48%) and (16.40 %) respectively.

The presence of cross-breed cattle, poor body condition, large herd size, pregnant cows, and lactating cows were significant predictors of positive tuberculin test results in dairy cattle. At the herd level, the potential risk factors of positive tuberculin test results included closed barns, intensive farms, poor management, and large herd size.

The result of this study also revealed that the awareness level of farm owners/attendants towards the bovine TB (19.27%) and its zoonosis (23.25%) or transmission from farm animals to humans was generally low. Large portion of the respondents had a habit of consuming raw milk and meat, and sharing a house with their cattle implying the possible potential of acquiring BTB. Though the causative agents were not identified, 22(20.18%) individuals who were raising cattle had TB cases.

Based on the above conclusions the following recommendations are forwarded:

- Public education and creation of the awareness campaigns should be carried out on BTB to raise communities' knowledge about the public health risk of BTB and its mode of transmission for effective implementation of TB control measures.
- Crossbreds should have better management since they are more exposed to BTB Infection as compare to local breed
- In dairy farms appropriate stocking of herd size and hygiene should be followed.
- Pasteurization of milk and milk products should be done as routine practice most notably in rural communities.

- Animal husbandry practices, particularly grazing lands, should be properly managed in order to reduce/prevent transmission of *M. bovis* between domestic cattle and wildlife
- Dairy cattle owners/ attendants should be screened for human TB.
- Further in-depth epidemiological studies of BTB should be a top priority to establish a strong epidemiological control program: to verify a direct transmission route from cattle to owner and link between human TB and bovine TB through molecular techniques.

8. REFERENCES

- Acha, P.N. and Szyfres, B. (1987): Zoonotic tuberculosis, in Zoonoses and communicable diseases common to man and animals, 2nd edition, Pan American Health Organization/World Health Organization, Scientific publication No **503**, Washington.
- Admassu, B., Kebede, E. and Shite, A. (2015): Review on bovine tuberculosis. Pal, M., Tolawak, D. and Bikila, U., 2022. Zoonotic Importance of Bovine Tuberculosis in Ethiopia: An Overview. *Research in Veterinary Science and Medicine*, 2.
- Admasu, P., Berihun, W. and Niguse, A. (2014): Prevalence of bovine tuberculosis in dairy cattle of Yeki District, Southern Ethiopia. *African Journal Basic Applied Science*, **6**(5):135-40.
- Allen, A.R., Ford, T. and Skuce, R.A. (2021). Does *Mycobacterium tuberculosis* var. *bovis* survival in the environment confound bovine tuberculosis control and eradication? A literature review. *Veterinary medicine international*, 2021.
- Amanfu, W. (2006): The situation of tuberculosis and tuberculosis control in animals of economic interest, *Tuberculosis* **86**: 330-335.
<http://dx.doi.org/10.1016/j.tube.2006.01.007>
- Ameni, G. and Erkihun, A. (2007): Bovine tuberculosis on small-scale dairy farms in Adama Town, central Ethiopia, and farmer awareness of the disease. *Revue Scientifique et Technique-Office International des Epizooties*, **26**(3):711-720.
- Ameni, G. and Erkihun, A. (2007): Bovine tuberculosis on small-scale dairy farms in Adama Town, central Ethiopia, and farmer awareness of the disease. *Revue Scientifique et Technique-Office International des Epizooties*, **26**(3), pp.711-720.
- Ameni, G., Amenu, K. and Tibbo, M. (2003): Bovine tuberculosis: prevalence and risk factor assessment in cattle and cattle owners in Wuchale-Jida district, Central Ethiopia. *Journal of applied research in veterinary medicine*.
- Ameni, G., Hewinson, G., Aseffa, A., Young, D. and Vordermeier, M. (2008): Appraisal of interpretation criteria for the comparative intradermal tuberculin test for

diagnosis of tuberculosis in cattle in central Ethiopia. *Clinical and Vaccine Immunology*, **15**(8):1272-1276.

Ameni, G., Regassa, A., Kassa, T. and Medhin, G. (2001): Survey on bovine tuberculosis and its public implications to cattle raising families in Wolaita Soddo, Southern Ethiopia. *Ethiopia journal of animal production*, **1**: 55-62.

Anwar Nuru, A.N., Aboma Zewude, A.Z., Temesgen Mohammed, T.M., Biniam Wondale, B.W., Laikemariam Teshome, L.T., Muluwork Getahun, M.G., Gezahegne Mamo, G.M., Girmay Medhin, G.M., Rembert Pieper, R.P. and Gobena Ameni, G.A.(2017): Nontuberculosis mycobacteria are the major causes of tuberculosis like lesions in cattle slaughtered at Bahir Dar Abattoir, northwestern Ethiopia.

Ayele, W. Y., Neill, S. D., Zinsstag, J., Weiss, M. G. and Pavlik, I. (2004): Bovine tuberculosis: an old disease but a new threat to Africa. *International Journal of Tuberculosis Lung Diseases*, **8**(8): 924-937.

Bekele, M., Mamo, G., Mulat, S., Ameni, G., Beyene, G. and Tekeba, E. (2016): Epidemiology of bovine tuberculosis and its public health significance in Debre-Zeit intensive dairy farms, Ethiopia. *J Biomed Nurs*, **2**(2), pp.8-18..

Bermingham, M.L., More, S.J., Good, M., Cromie, A.R., Higgins, I.M., Brother stone, S. and Berry, D.P. (2009): Genetics of tuberculosis in Irish Holstein-Friesian dairy herds. *Journal of dairy science*, **92**(7), pp.3447-3456.

Bilal, S., Iqbal, M., Murphy, P. and Power, J. (2010): Human bovine tuberculosis—remains in the differential. *Journal of Medical Microbiology*, **59**(11):1379-1382.

Carrisoza-Urbina, J., Morales-Salinas, E., Bedolla-Alva, M.A., Hernández-Pando, R. and Gutiérrez-Pabello, J.A. (2019): Atypical granuloma formation in Mycobacterium bovis-infected calves. *PLoS One*, **14**(7), p.e0218547.

Conlan, A.J., Brooks Pollock, E., McKinley, T.J., Mitchell, A.P., Jones, G.J., Vordermeier, M. and Wood, J.L. (2015): Potential benefits of cattle vaccination as a supplementary control for bovine tuberculosis. *PLoS computational biology*, **11**(2), p.e1004038.

- Corner, L.A.L., Murphy, D. and Gormley, E. (2011): Mycobacterium bovis infection in the Eurasian badger (*Meles meles*): the disease, pathogenesis, epidemiology and control. *Journal of comparative pathology*, **144**(1), pp.1-24.
- Cosivi, O., Grange, J.M., Daborn, C.J., Raviglione, M.C., Fujikura, T., Cousins, D., Robinson, R.A., Huchzermeyer, H.F., de Kantor, I. and Meslin, F.X. (1998): Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. *Emerging infectious diseases*, **4**(1):59.
- CSA. 2020b. Agriculture sample survey 2019/2020(2013 E.C.) (September - January 2019/2020), Volume VII. Report on crop and livestock product utilization (Private peasant holdings, Meher Season). Central Statistical Agency (CSA): Addis Ababa, Ethiopia.
- Dankner, W.M. and Davis, C.E. (2000): *Mycobacterium bovis* as a significant cause of tuberculosis in children residing along the United States–Mexico border in the Baja California region. *Pediatrics*, **105**(6), pp.e79-e79.
- De la Rúa-Domenech, R., Goodchild, A. T., Vordermeier, H. M., Hewinson, R. G., Christiansen, K. H., & Clifton-Hadley, R. S. (2006): Ante mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. *Research in Veterinary Science*, **81**(2):190-210. <https://doi.org/10.1016/j.rvsc.2005.11.005>.
- Dejene, S.W., Heitkönig, I.M., Prins, H.H., Lemma, F.A., Mekonnen, D.A., Alemu, Z.E., Kelkay, T.Z. and de Boer, W.F. (2016): Risk factors for bovine tuberculosis (bTB) in cattle in Ethiopia. *PLoS One*, **11**(7), p.e0159083.
- Dejene, S.W., Heitkönig, I.M., Prins, H.H., Lemma, F.A., Mekonnen, D.A., Alemu, Z.E., Kelkay, T.Z. and de Boer, W.F. (2016): Risk factors for bovine tuberculosis (bTB) in cattle in Ethiopia. *PLoS One*, **11**(7), p.e0159083.
- Dlamini, M.E.M. (2013): *The prevalence of bovine tuberculosis and associated risk factors for humans in Swaziland* (Doctoral dissertation, University of Pretoria).
- Domingo, M., Vidal, E.N.R.I.C. and Marco, A. (2014.): Pathology of bovine tuberculosis. *Research in veterinary science*, **97**, pp.S20-S29.

- Elias, K., Hussein, D., Asseged, B., Wondwossen, T. and Gebeyehu, M. (2008): Status of bovine tuberculosis in Addis Ababa dairy farms. *Rev. sci. tech. Off. int. Epiz.* 27
- Firdessa, R., Tschopp, R., Wubete, A., Sombo, M., Hailu, E., Erenso, G., Kiros, T., Yamuah, L., Vordermeier, M., Hewinson, R.G. and Young, D. (2012): High prevalence of bovine tuberculosis in dairy cattle in central Ethiopia: implications for the dairy industry and public health. *PLoS one*, 7(12): 52851.
- Gebremedhin, B., Pender, J., & Tesfay, G. (2004): Collective action for grazing land management in crop–livestock mixed systems in the highlands of northern Ethiopia. *Agricultural Systems*, 82(3): 273-290. doi:<https://doi.org/10.1016/j.agsy.2004.07.004>
- Gebrezgabiher, G., Romha, G. and Ameni, G. (2014): Prevalence study of bovine tuberculosis and genus typing of its causative agents in cattle slaughtered at Dilla Municipal abattoir, Southern Ethiopia. *Afr. J. Basic Appl. Sci*, 6(4), pp.103-109.
- Ghebremariam, M.K., Michel, A.L., Vernooij, J.C.M., Nielen, M. and Rutten, V.P. (2018): Prevalence of bovine tuberculosis in cattle, goats, and camels of traditional livestock raising communities in Eritrea. *BMC veterinary research*, 14(1), pp.1-13.
- Godfray, H.C.J., Donnelly, C., Hewinson, G., Winter, M. and Wood, J., (2018): Bovine TB strategy review.
- Griffin J.M. and Dolan L.A. (1995): The role of cattle-to-cattle transmission of *Mycobacterium bovis* in the epidemiology of tuberculosis in cattle in the Republic of Ireland: A review. *Irish Veterinary Journal*, 48: 228-234.
- Griffin, L.J.(1993): Narrative, event-structure analysis, and causal interpretation in historical sociology. *American journal of Sociology*, 98(5), pp.1094-1133.
- Guétin-Poirier, V., Crozet, G., Gardon, S., Dufour, B. and Rivière, J. (2020): Integrating data of veterinarians' practices in assessing the cost effectiveness of three components of the bovine tuberculosis surveillance system by intradermal tuberculin testing in French cattle farms through a scenario-tree approach. *Research in veterinary science*, 128, pp.242-260.

- Habitu, T., Areda, D., Muwonge, A., Tessema, G.T., Skjerve, E. and Gebrehiwot, T. (2019): Prevalence and risk factors analysis of bovine tuberculosis in cattle raised in mixed crop-livestock farming system in Tigray region, Ethiopia. *Transboundary and emerging diseases*, **66**(1), pp.488-496.
- Hadush, A., 2015. Assessment of bovine tuberculosis in dairy farms and its public health importance in and around Adigrat District (Doctoral dissertation, Mekelle Univeristy).
- Hamilton, C.A. (2016): Neonatal vaccination: role for innate immune cell interactions in BCG vaccination.
- HEMECHO, G.S. (2017): Bovine tuberculosis in crossbred dairy cattle in wolaita sodo town, south-western ethiopia: prevalence, associated risk factors and its effect on milk production (Doctoral dissertation). Jaleta, M., Gebremedhin, B. and Hoekstra, D., 2009. Smallholder commercialization: Processes, determinants and impact. *ILRI Discussion Paper*.
- Hristov, A.N., Degaetano, A.T., Rotz, C.A., Hoberg, E., Skinner, R.H., Felix, T., Li, H., Patterson, P.H., Roth, G., Hall, M. and Ott, T.L. (2018): Climate change effects on livestock in the Northeast US and strategies for adaptation. *Climatic Change*, **146**, pp.33-45.
- Humblet, M.F., Boschioli, M.L. and Saegerman, C. (2009): Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. *Veterinary research*, **40**(5).
- Humblet, M.F., Walravens, K., Salandre, O., Boschioli, M.L., Gilbert, M., Berkvens, D., Fauville-Dufaux, M., Godfroid, J., Dufey, J., Raskin, A. and Vanholme, L. (2011) Monitoring of the intra-dermal tuberculosis skin test performed by Belgian field practitioners. *Research in veterinary science*, **91**(2), pp.199-207.
- Hussein, H.A., Ahmed, J.M., Musse, A.H. and Gizaw, Y. (2024): Prevalence and risk factors of bovine tuberculosis in cattle in selected districts of Fafan pastoral settings, Eastern Ethiopia. *Heliyon*, **10**(2).

- Inangolet FO, Demelash B, Oloya J, Opuda- Asibo J, Skjerve E. (2008): A cross-sectional study of bovine tuberculosis in the transhumant and agro-pastoral cattle herds in the border areas of Katakwi and Moroto districts, Uganda. *Tropical Animal Health Production*, **40**: 501-508.
- Kanchora, G., Jiso, R., Tsegaye, M. and Bisrat, A. (2020): Epidemiological Study on Bovine Tuberculosis in Boran Breeds Cattle at Yabello District, Southern, Ethiopia. *American Journal of Bioscience and Bioengineering*, **8**(6), pp.105-115..
- Kazwala, R.R., Kambarage, D.M., Daborn, C.J., Nyange, J., Jiwa, S.F.H. and Sharp, J.M. (2001): Risk factors associated with the occurrence of bovine tuberculosis in cattle in the Southern Highlands of Tanzania. *Veterinary research communications*, **25**, pp.609-614.
- Kemal, J., Sibhat, B., Abraham, A., Terefe, Y., Tulu, K.T., Welay, K. and Getahun, N. (2019): Bovine tuberculosis in eastern Ethiopia: prevalence, risk factors and its public health importance. *BMC Infectious Diseases*, **19**(1), pp.1-9.
- Livingstone, P. and Hancox, N. (2018): Managing bovine tuberculosis: successes and issues. In *Bovine tuberculosis* (pp. 225-247). Wallingford UK: CAB International.
- Mbugi, E.V., Katale, B.Z., Kendall, S., Good, L., Kibiki, G.S., Keyyu, J.D., Godfrey-Faussett, P., Van Helden, P. and Matee, M.I. (2012): Tuberculosis cross-species transmission in Tanzania: Towards a One-Health concept. *Onderstepoort Journal of Veterinary Research*, **79**(2): 01-06.
- McCormick, B.A. (2023): *Transmission of Chronic Wasting Disease Associated With Livestock Mineral Licks* (Doctoral dissertation, University of Wyoming).
- Mekonnen, G.A., Conlan, A.J., Berg, S., Ayele, B.T., Alemu, A., Guta, S., Lakew, M., Tadesse, B., Gebre, S., Wood, J.L. and Ameni, G. (2019): Prevalence of bovine tuberculosis and its associated risk factors in the emerging dairy belts of regional cities in Ethiopia. *Preventive veterinary medicine*, **168**, pp.81-89.
- Mekonnen, G.A., Conlan, A.J.K., Berg, S., Ayele, B.T., Mihret, A., Olani, A., Asgedom, H., Wood, J.L.N. and Ameni, G. (2021): Dynamics and risk of transmission of

bovine tuberculosis in the emerging dairy regions of Ethiopia. *Epidemiology & Infection*, 149.

Mohamed, A. (2020): Bovine tuberculosis at the human–livestock–wildlife interface and its control through one health approach in the Ethiopian Somali pastoralists: A review. *One Health*, **9**, p.100113.

Mohammed, N., Hailu, M. and Gebreyesus, M. (2012): Prevalence and zoonotic implications of bovine tuberculosis in Northwest Ethiopia Andassa Livestock Research Center, Bahir Dar, Ethiopia. *International Journal of Medicine and Medical Science*, **2**(9): 188-192.

Mostowy, S. and Behr, M.A. (2005): The origin and evolution of *Mycobacterium tuberculosis*. *Clinics in chest medicine*, **26**(2): 207-216.

Muhammed, C., Seboka, F. and Tibesso, G. (2018): Review on epidemiological features of *Mycobacterium bovis* at the human, cattle and wildlife interface in Ethiopia. *Biom Biostat Int J*, **7**(5), pp.458-468.

Mulatu, H. and Mohamed, S. (2023): Epidemiological Interface of Tuberculosis at Human Livestock Wildlife in Ethiopia. *Appl Microbiol Open Access*, **9**, p.248.

Müller, B., de Klerk-Lorist, L.M., Henton, M.M., Lane, E., Parsons, S., van Pittius, N.C.G., Kotze, A., Van Helden, P.D. and Tanner, M. (2011): Mixed infections of *Corynebacterium pseudotuberculosis* and non-tuberculous mycobacteria in South African antelopes presenting with tuberculosis-like lesions. *Veterinary microbiology*, **147**(3-4), pp.340-345.

Munyeme, M., Muma, J.B., Samui, K.L., Skjerve, E., Nambota, A.M., Phiri, I.G.K., Rigouts, L. and Tryland, M. (2009): Prevalence of bovine tuberculosis and animal level risk factors for indigenous cattle under different grazing strategies in the livestock/wildlife interface areas of Zambia. *Tropical Animal Health and Production*, **41**: 345-352.

Neill, S., Pollock, J., Bryson, D. and Hanna, J. (1994): Pathogenesis of *Mycobacterium bovis* infection in cattle. *Veterinary Microbiology*, **40**: 41-52.

- Nemomsa, B., Gebrezgabiher, G., Birhanu, T., Tadelle, H., Tadesse, G. and Getachew, B. (2014): Epidemiology of bovine tuberculosis in Butajira, Southern Ethiopia: A cross-sectional abattoir-based study. *African Journal of Microbiology Research*, **8**(33), pp.3112-3117.
- Nicholson, M. J. and Butterworth, M. H. (1986): A guide to condition scoring of Zebu cattle. International Livestock Center for Africa (ILCA), Addis Ababa, Ethiopia, pp. 1-29.
- Nuru, A., Mamo, G., Worku, A., Admasu, A., Medhin, G., Pieper, R. and Ameni, G. (2015): Genetic diversity of Mycobacterium tuberculosis complex isolated from tuberculosis patients in Bahir Dar City and its surroundings, Northwest Ethiopia. *BioMed research international*, 2015.
- O'Reilly, L.M. and Daborn, C.J. (1995): The epidemiology of *Mycobacterium bovis* infections in animals and man. A review: *Tuberculosis Lung Diseases*, **76**: 1-46.
- Office International des Epizooties OIE, (2009): Bovine tuberculosis. Terrestrial manual: Office International des-Epizootie.
- Office International des Epizooties OIE. Bovine tuberculosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Part 2, Section 2.4. Chapter 2.4.7. World Organization for Animal Health; 2010; pp. 683– 698.
- Palmer, S., Williams, G.A., Brady, C., Ryan, E., Malczewska, K., Bull, T.J., Hogarth, P.J. and Sawyer, J. (2022): Assessment of the frequency of Mycobacterium bovis shedding in the faeces of naturally and experimentally TB infected cattle. *Journal of applied microbiology*, **133** (3), pp.1832-1842.
- Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R. (1999): *Mycobacterium* species. In: clinical veterinary microbiology. London Philadelphia: 157-170.
- Radostits, O.M., Blood, D.C. and Gay, C.C. (1994): Diseases caused by *mycobacterium*. In: veterinary medicine, a text book of disease of cattle, sheep, pigs, goats and horses, **8**: 748-785.

- Radostits, O.M., Gay, C., Hinchcliff, K.W. and Constable, P.D. eds.(2006):. *Veterinary Medicine E-Book: A textbook of the diseases of cattle, horses, sheep, pigs and goats*. Elsevier Health Sciences.
- Radostits, O.M., Gay, C.C., Hinchelift, K.W. and Constabel, P.D. (2007): *Veterinary Medicine, A text book of the disease of cattle, sheep, pig, goat and horses*. Elsevier, London, **10**: 1007-1040..
- Regassa, A., Tassew, A., Amenu, K., Megersa, B., Abunna, F., Mekibib, B., Macrotty, T. and Ameni, G. (2010): A cross-sectional study on bovine tuberculosis in Hawassa town and its surroundings, Southern Ethiopia. *Tropical Animal Health and Production*, **42**: 915-920.
- Romha, G., Ameni, G., Berhe, G. and Mamo, G. (2013): Epidemiology of mycobacterial infections in cattle in two districts of Western Tigray Zone, northern Ethiopia. *African Journal of Microbiology Research*, **7**(31): 4031-4038..
- Salguero, F.J. (2018). The pathology and pathogenesis of Mycobacterium bovis infection. In *Bovine tuberculosis* (pp. 122-139). Wallingford UK: CAB International. da Silva Rabello, M.C., Santos, F.C.F., de Albuquerque Montenegro, R., Guedes, G.D.M.R., Montenegro, L.M.L. and Schindler, H.C., 2017. TUBERCULOSIS: Epidemiological, Molecular, Immunological and Clinical Characteristics. *Tropical Diseases: An Overview of Major Diseases Occurring in the Americas*, p.373.
- Sharifipour, E., Nasiri, M.J., Farnia, P. Mozafari, M. Irani, S. and Velayati, A.A. (2014): Evaluation of Molecular Diversity of *Mycobacterium tuberculosis* Strains by Polymorphisms in RD Regions. *Journal of Mycobacterial Diseases*, **4**: 3.
- Shitaye, J.E., Tsegaye, W. and Pavlik, I. (2007): Bovine tuberculosis infection in animal and human populations in Ethiopia: a review. *Veterinarni Medicina*, **52**(8): 317.
- Sibhat, B., Asmare, K., Demissie, K., Ayelet, G., Mamo, G. and Ameni, G. (2017): Bovine tuberculosis in Ethiopia: a systematic review and meta-analysis. *Preventive veterinary medicine*, **147**, pp.149-157.

- Sibhat, B., Asmare, K., Demissie, K., Ayelet, G., Mamo, G. and Ameni, G. (2017): Bovine tuberculosis in Ethiopia: a systematic review and meta-analysis. *Preventive veterinary medicine*, **147**, pp.149-157.
- Skuce RA, Allen AR, McDowell S.W. (2012): Herd-level risk factors for bovine tuberculosis: a literature review.
- Smith, N.H., Gordon, S.V., de la Rua-Domenech, R., Clifton-Hadley, R.S. and Hewinson, R.G. (2006): Bottle necks and broomsticks: the molecular evolution of *Mycobacterium bovis*. *NRM*, **4**: 670-681.
- Špičić, S., Pate, M., Duvnjak, S., Katalinić-Janković, V., Obrovac, M., Deždek, D., Kompes, G., Habrun, B., Ocepek, M. and Cvetnić, Ž. (2012): Molecular epidemiology of *Mycobacterium tuberculosis* transmission between cattle and man-a case report. *Veterinarski arhiv*. **82**: 303-310.
- Tadesse, B., Ebisa, M., Eyasu, E. and Ayele, G. (2015): Review on Diagnostic Techniques of Bovine Tuberculosis in Ethiopia. *Rep Opinion* **7**(1):7-14.
- Tadesse, Y.(2019): Socioeconomic characteristics of the community and importance of camel and other livestock species in Tahitay-Adiyabo District, Tigray Region in the Northern Periphery of Ethiopia. *Open Journal of Animal Sciences*, **9**(02), p.217.
- Tenguria, R.K., Khan, F.N., Quereshi, S. and Pandey, A. (2011): Epidemiological study of zoonotic tuberculosis complex (Ztbc). *World Journal of Science and Technology*, **1**(3), pp.31-56.
- Thoen CO, Lobue PA, Enarson DA, *et al.*, (2009): Tuberculosis: a re-emerging disease in animals and humans. *Veterinaria Italiana*, **45**(1):135–181.
- Thrusfield M. (2007): *Veterinary Epidemiology*. 2nd ed. London: Blackwell Science Ltd.;p. 178-198.
- Tigre, W., Wakwoya, A., Regassa, F. and Tolosa, T. (2011): Prevalence and associated risk factors of bovine mastitis in local and cross breed dairy cows in and around Jimma town. *Bulletin of animal health and production in Africa*, **59**(2): 129-135.

- Torrell, R. (1998). Methods of determining age in cattle. Cattle Producer's Library: CL712. University of Nevada, Reno, NV.
- Tschopp, R., Bekele, S., Moti, T., Young, D. and Aseffa, A. (2015): Brucellosis and bovine tuberculosis prevalence in livestock from pastoralist communities adjacent to Awash National Park, Ethiopia. *Preventive Veterinary Medicine*, **120**(2): 187-194.
- Tschopp, R., Berg, S., Argaw, K., Gadisa, E., Habtamu, M., Schelling, E., Young, D., Aseffa, A. and Zinsstag, J. (2010): Bovine tuberculosis in Ethiopian wildlife. *Journal Wildlife Diseases*, **46**:753- 762.
- Tschopp, R., Bobosha, K., Aseffa, A., Schelling, E., Habtamu, M., Iwnetu, R., *et al.* (2011): Bovine tuberculosis at a cattle-small ruminant-human interface in Meskan, Gurage region, Central Ethiopia. *BMC Infectious Diseases*, **11**: 318.
- Tschopp, R., Schelling, E., Hattendorf, J., Aseffa, A. and Zinsstag, J. (2009): Risk factors of bovine tuberculosis in cattle in rural livestock production systems of Ethiopia. *Preventive Veterinary Med*, **89**(3-4): 205-211.
- Tsegaye, W., Aseffa, A., Mache, A., Mengistu, Y., Stefan, B. and Ameni, G. (2010): Conventional and Molecular Epidemiology of Bovine Tuberculosis in Dairy Farms in Addis Ababa City, the Capital of Ethiopia. *The Journal of Applied Research in Veterinary Medicine*, **8**(2), p.143. (3), 915-923.
- Tsegaye, W., Aseffa, A., Mache, A., Mengistu, Y., Stefan, B. and Ameni, G. (2010): Conventional and Molecular Epidemiology of Bovine Tuberculosis in Dairy Farms in Addis Ababa City, the Capital of Ethiopia. *The Journal of Applied Research in Veterinary Medicine*, **8**(2), p.143.
- Uduak, A., (2015): A review on Bovine Tuberculosis. *J Vet Adv*, **5**(3), pp.841-7.
- Vordermeier, M., Ameni, G., Berg, S., Bishop, R., Robertson, B.D., Aseffa, A., Hewinson, R.G. and Young, D.B. (2012): The influence of cattle breed on susceptibility to bovine tuberculosis in Ethiopia. *Comparative immunology, microbiology and infectious diseases*, **35**(3), pp.227-232.
- WHO, O., 2017. One health. *World Health Organization*, 736.

- WHO, O., 2017. One health. *World Health Organization*, 736.
- Widdison, A.L. (1994): Microbiology and sources of pancreatic pathogens in acute pancreatitis. In *Facing the Pancreatic Dilemma: Update of Medical and Surgical Pancreatology* (pp. 291-300). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Widdison, S., Watson, M. and Coffey, T.J. (2011): Early response of bovine alveolar macrophages to infection with live and heat-killed *Mycobacterium bovis*. *Developmental & Comparative Immunology*, **35**(5), pp.580-591.
- World Health Organization (WHO). (1997): Report of the WHO working group on zoonotic tuberculosis (*M. bovis*) with the participation of FAO, 14 June 1997, Mainz, Germany *WHO/CDS/VPH/97*, 137.
- Youya, M.A., Mengistu, S. And Girma, S. (2022): Prevalence And Associated Risk Factors Of Bovine Tuberculosis And Its Zoonotic Potential In Selected Dairy Farms Of Harar City, Ethiopia (Doctoral dissertation, Haramaya, Ethiopia)
- Zeru F., Romha. G., Berhe. G., *et al.* (2004): Prevalence of bovine tuberculosis and assessment of Cattle owners' awareness on its public health implication in and around Mekelle, Northern Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **6**:160–167.
- Zinsstag, J., Schelling, E., Roth, F., Kazwala, R., Thoen, C.O. and Steele, J.H. (2006): Economics of bovine tuberculosis. *Mycobacterium bovis infection in animals and humans*, **2**: 68-83.

8. APPENDICES

Appendix 1: Questionnaire to interview farmers and dairy farm workers

Title: assessment of bovine tuberculosis and associate risk factors in cattle and human in and around shire Indaslassie. (Please tick and write correct answer where applicable)

A. General information

1. Name of the farm or farm owner _____

2. Address of the farm: _____ woreda _____ PA _____

3. Educational qualification: a. Illiterate b. Basic writing & reading C. primary education d. secondary education e. Diploma and above

4. When was the farm established? A. 1-5 year's b. 6-10 years c. >10 years

5. Type of farm: a. Intensive b. semi-intensive c. extensive

6. No. of cattle in the farm (herd size)? a. <5 cattle b. 6-9 c. >10

7. Breed type? A. Local B. Cross C. Exotic

8. Sex A. No of Male animal ----- B. No of Female animal -----

9. Cattle housing

A. Open area B. Separate animal houses C. Enclosures D. Share houses with humans

10. Have you construct a house for the dairy herd? A. Yes B. No

11. If the answer for Q. no. 10 is yes, what are the materials used?

A. Wood B. Grass c. Brick D. Metal sheet E. Others (Specify) -----

12. What is the material used for the house floor? A. Concrete B. Soil C. Sand D. stone E. Others (Specify) -----

13. The purpose of the Dairy farm:

A. To produce dairy products for home consumption

B. To produce dairy products for market

- C. To produce dairy products for market and home consumption
14. Did the house have good ventilation, i.e. window or mesh structure on the bottom of the wall? A. Poor B. Medium C. good
15. Hygiene condition of the barn/house based on odors, waste drainage, cleanness of floor and animals, light source, and animal stocking: A. Poor B. Medium C. good
16. Does the herd have a feeding and water trough? A. Yes B. No
17. Are animals in your farm mixed with animals from other farms?
A. Yes B. No
18. What will do if you have purchased new dairy animal?
A. Isolation from herd for few days B. Introducing to the herd immediately C. Checking health status by veterinarian
19. Do you have a trend isolation of sick animals? A. Yes B. No
20. Do you have a trend treat sick animals? A. Yes B. No
21. What was the response for the treatment? A. Good B. Poor
22. If the answer is “No” for Q. no 20 why? A. Lack of awareness B. Lack of vet service C. The clinic is far from here D. Others Specify -----
23. Have you aware about Bovine tuberculosis? A. Yes B. No
24. Does it affect cattle A.? Yes B. No C. I don't know
25. Does it affect cattle A.? Yes B. No C. I don't know
26. Do you aware that Bovine TB is transfer between animal and humans (zoonosis)?
A. Yes B. No C. I don't know
27. How can bovine TB transmit to human
A. By drinking raw/under boiled milk B. By eating raw/under cooked meat C. Inhalation D. By direct contact E. Other specify _____
28. What symptoms of bovine TB in animals
A. Emaciation B. Low grade C. fever D. Cough E. Lymph node enlargement F. Lethargy G. other specify _____
29. Do you have noticed BTB any effect on the dairy production? A. Yes B. No
30. If you said “yes” for Q. No. 29, which production loss you noticed?

A. Milk yield reduction **B.** Infertility **C.** Long calving interval **D.** Abortion **E.** Others specify -----

31. Which age group of the dairy herd is more affected with BTB than others?

A. < 6 months **B.** > 6months < 1 year **C.** > 1 yrs < 2 yrs **E.**> 3 yrs **D.** >2 yrs < 3 yrs

32. Which sex type is more affected with BTB? **A.** Male **B.** Female **C.** Equally affected

33. In the dairy herd which breed is more infected with BTB? **A.** Local **B.** Cross **C.** Exotic **D.** All breeds are equally infected

34. Do you have any experience of BTB vaccination? **A.** Yes **B.** No

35. Do you have a habit of drinking milk **a.** raw **b.** Boiled **c.** Mixed

36. If the answer is boiling, Reason for boiling? **a.** Fear of milk born disease **b.** Culture **c.** others _____

37. Do you know that bovine tuberculosis can be transmitted to man through raw milk/milk products consumption obtained from bovine tuberculosis infected cattle? 1. Yes 2. No

38. Do you have a Habit of eating meat? **a.** Cooked **b.** Raw **c.** Mixed

39. Do you know that bovine tuberculosis can be transmitted to man through raw meat consumption obtained from bovine tuberculosis infected cattle? 1. Yes 2. No

40. Do you have a habit of consumption of yoghurt? **A.** Yes **B.** No

41. Do you share the same house with your animals? **a.** Yes **b.** No

42. If the answer is “yes” for Q. no 41 **A.** the whole family **B.** farm worker **C.** other

43. Do you have tuberculosis patient in your family or farm worker?

a. Yes **b.** No

44. If you have TB patient in your family or farm worker, indicate the type of TB?

a. Pulmonary **b.** extra pulmonary **c.** Do not known

45. Do the dairy animals have mixed with other domestic animals?

Yes **B.** No

46. Do you sell milk and milk products? **A.** Yes **B.** No

47. If you say “yes” for Q. No 46, who is your customer? **a.** Local people **b.** Milk collection unit **c.** restaurant/cafeteria **d.** Others

(specify _____)

48. If the answer is "No" for Q. no. 46, what will you do with milk?

- A.** For home consumption **B.** For calves **C.** It will be discarded **D.** Others

specify -----

49. Is there any wildlife contact with the herd? **A.** yes **B.** No

50. Do you have any comment? _____

Appendix 2. Questionnaire to interview hospital coming in patients

Name _____

Age _____ Sex _____ Region _____ Zone _____ Wereda _____ Village _____

1. Educational status:

a. Illiterate b. Basic writing & reading c. Primary (Grade 1 to 6) d. Junior secondary (grade 7 to 8) e. Secondary (Grade 9 to 12) f. Diploma g. Degree and above

2. Species of animals commonly slaughtered in the abattoir a. Cattle b. Sheep c. Goat 3. Which breed of cattle commonly slaughtered a. Local b. Cross c. Exotic d.all

4. Source of cattle to be slaughtered?

5. Number of cattle slaughtered in a day.....

6. Did the technician perform ante mortem examination? a. Yes b. No

7. Do you know bovine tuberculosis? a. Yes b. No

8. What type of lesion you has been encountered?

9. In which organ of the animal body the lesion commonly found? a. Liver b. Lung c. Intestine d. Lymph nodes e. Others (specify).....

10. In which group of the cattle are mostly you found the lesion?

a. <2years b. 2-4 years c. 4-7 years d. > 7 years

11. What do you recommend if you get TB lesion on the meat of slaughtered animal?

12. In which body condition group of animal do you found the TB lesion?

a. Emaciated b. Thin c. Medium d. Fat

13. Do you know that tuberculosis can be transmitted between cattle and human?
12. Is there any contact of your cattle with wild animals? a. yes b. No If yes which type, in order of priority? 1..... 2 3.....
13. Do you use the same watering point with animals? a. Yes b. No
14. Do you share the same house with your animals? a. Yes b. No
15. Do you know that tuberculosis can be transmitted from cattle to man and vice versa?
a. Yes b. No
16. If yes, would you please indicate some ways?
17. Habit of drinking milk a. raw b. Boiled c. Mixed
18. Do you boil milk? a. Yes b. No
19. Reason for boiling? a. Fear of milk born disease b. Culture c. others_____
20. Habit of eating meat? a. Cooked b. Raw c. Mixed
21. Habit of eating blood? a. Cooked b. Raw c. Mixed
22. If you sell milk/milk products, who buys it? a. Local people b. Milk collection unit c. restaurant/cafeteria d. Others (specify).....
23. Do you have tuberculosis patient in your family or farm worker? a. Yes b. No 24. If yes how many? -----
25. If you have TB patient in your family or farm worker, indicate the type of TB? a. Pulmonary b. extra pulmonary c. Do not known
26. How long have you been sick? a. Less than a year b. More than a year c. Others
27. Have you ever taken any treatment? a. yes b. No
28. If yes, a. Traditional b. Drugs

Thank you!

Name of interviewer_____ Signature_____ Date_____

Appendix 3: CIDT Test Format

Address: Region_____Zone_____Wereda_____kebelle _____Village_____

Time	Herd level	ID	Age	Breed	BCS	Sex	Lactation status		Pregnancy status		Parity class	B1	A1	B2	A2	B2-B1	A2-A1	ΔB-ΔA	Result
							yes	No	Yes	No									

BSC=Body Condition Score ID=Identification Number

A1=Avian before injection A2=Avian after injection B1=Bovine before injection B2=Bovine after injection ΔA=A2-A1, ΔB=B2-B1

Appendix 4: Description of body condition scores (BCS)

Poor

1. Clearly defined bone structure of shoulder, ribs, back, hooks and pins easily visible.
2. Little muscle tissue or fat present.
3. Small amount of muscling in the hindquarters. Fat is present, but not abundant.
4. Space between spinous process is easily seen.
5. Fat begins to cover loin, back and fore ribs, Upper skeletal structures visible and Spinous process is easily identified.

Medium/ Borderline (Optimum)





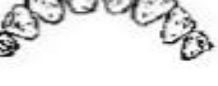
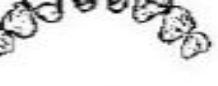
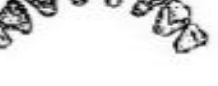
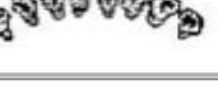
1. Fore ribs becoming less noticeable. The transverse spinous process can be identified by palpation. Fat and muscle tissue not abundant, but increasing in fullness.
2. Ribs are visible only when the animal has been shrunk, Processes not visible.
Each side of the tail head is filled, but not mounded.
3. Ribs not noticeable to the eye. Muscling in hind quarters plump and full. Fat around tail head and covering the fore ribs

Good/ fat

1. Spinous process can only be felt with firm pressure. Fat cover in abundance on either side of tail head.
2. Animal smooth and blocky appearance; bone structure difficult to identify. Fat cover is abundant.
3. Structures difficult to identify. Fat cover is excessive and mobility may be impaired.

Appendix 5: Age determination

Diagram 1. Handy guide to determining the age of cattle by the teeth

	At birth to 1 month	Two or more of the temporary incisor teeth present. Within first month, entire 8 temporary incisors appear.
	2 years	As a long-yearling, the central pair of temporary incisor teeth or pinchers is replaced by the permanent pinchers. At 2 years, the central permanent incisors attain full development.
	2-1/2 years	Permanent first intermediates, one on each side of the pinchers, are cut. Usually these are fully developed at 3 years.
	3-1/2 years	The second intermediates or laterals are cut. They are on a level with the first intermediates and begin to wear at 4 years.
	4-1/2 years	The corner teeth are replaced. At 5 years the animal usually has the full complement of incisors with the corners fully developed.
	5 to 6 years	The permanent pinchers are leveled, both pairs of intermediates are partially leveled, and the corner incisors show wear.
	7 to 10 years	At 7 or 8 years the pinchers show noticeable wear; at 8 or 9 years the middle pairs show noticeable wear; and at 10 years, the corner teeth show noticeable wear.
	12 years	After the animal passed the 6th year, the arch gradually loses its rounded contour and becomes nearly straight by the 12th year. In the meantime, the teeth gradually become triangular in shape, distinctly separated, and show progressive wearing to stubs. These conditions become more marked with increasing age.

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

- Selected dairy farms and dairy animals



- Preparation of material Restrained of the animal and prepared of injection site



- Disinfected and Shaved at middle neck (12-15 cm) Measured shaved skin thickness before injection



Read and observed recorded the skin thickness before and after injected using digital caliper



- Interpreted the result
- Positives

Negatives



-

