BOVINE TUBERCULOSIS: PREVALENCE, ECONOMIC IMPLICATIONS AND DIAGNOSTIC TEST COMPARISON IN SMALLHOLDER DAIRY FARMS IN SELECTED DISTRICTS OF NORTH GONDAR ZONE, AMHARA REGION

By

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As thesis research advisor, I hereby certify that I have read and evaluated this thesis prepared under my guidance, by Fikirte Shewatatek, entitled “Bovine tuberculosis: prevalence, economic implications and diagnostic test alternative in smallholder dairy farms in selected districts of North Gondar zone, Amhara region.” I recommend that it be submitted as fulfilling the thesis requirement.

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Final approval and acceptance of the thesis is contingent upon the submission of the copy of the thesis to the council of Graduate Studies (CGS) through the Departmental Graduate Committee (DGC) of the candidate’s major department.

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Dedication

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ABBREVIATIONS

Ag Antigen

BTB Bovine tuberculosis

CIDT Comparative intradermal tuberculin test

Cm Centimeter

GIS Geographic Information System

GPS Geographic Positioning System

LAM Lipoarabinomannan

Lit Litre

M. bovis *Mycobacterium bovis*

mm milli meter

MTB *Mycobacterium tuberculosis*

MTBC *Mycobacterium tuberculosis complex*

OIE Office Internacional des Epizooties (World Animal Health organization)

POC Point of care

PPD Purified protein derivatives

PSM Propensity score matching

SITT Single intradermal tuberculin test

TB Tuberculosis

ABSTRACT

Bovine tuberculosis (BTB) is a chronic disease of mainly cattle caused by bacteria called Mycobacterium bovis. In Ethiopia, BTB is a prevalent disease in cattle populations. This study was initiated to estimate the prevalence of BTB, to enumerate economic impact of BTB, to compare Lipoarabinomannan antigen test (LAM Ag) with tuberculin skin test (TST), to identify risk factors, to draw BTB distribution map and to assess zoonotic awareness in Gondar town and Gondar Zuria district of smallholder dairy farms. A cross sectional study was carried out from September, 2015 to April, 2016. A total of 109 study dairy herds were selected randomly and 434 eligible animals were sampled. Questionnaire survey and GPS data was taken. [Single intradermal tuberculin test (SITT) was used in Gondar town and Comparative intradermal tuberculin test (CIDT) used in Gondar Zuria]. In smallholder dairy farms, with 95% CI, individual animal prevalence was 8.26% (9/109) in Gondar town and 7.78% (14/180) in Gondar Zuria district. Herd prevalence was 23.53% (8/36) and 18.97% (11/58). Propensity Score Matching (PSM) estimated economical impact of BTB. Accordingly, 3.84 litre milk yield lossper day per cow is due to BTB infection. Compared to TST results, LAM Ag using urine has moderate agreement (54.39%) and slight agreement (23.23%) LAM Ag using milk. Purchased animal (OR=2.33), Male animals (OR=0.31) and crossbreds (OR=2.69) are individual animal risk factors. Feed type (OR=41.9) and dairy farmer heard about BTB (OR=12.64) are risk factors in herd level. The distribution of Gondar town BTB positive herds was higher than Gondar Zuria district. BTB zoonotic awareness is lower 11.48% (7/61) in Gondar Zuria district than in Gondar town 54.16% (26/48). Raw milk consumption is higher 37.7% (23/61) in Gondar Zuria district than Gondar town 11.6% (5/43). A total of 27% dairy farm owners consume unboiled milk. BTB is prevalent and its economical implication has significant loss in dairy farms income. The test agreement between LAM Ag using urine test and TST is better than LAM Ag using milk. Animal and herd level risk factors have important role in BTB prevalence. Cross breed and male animal management should be improved; early culling BTB positive animal has economical and epidemiological benefits. Zoonotic awareness on BTB should be created.

Key words: ***Bovine Tuberculosis, Dairy farm, Economical impact, tuberculin skin test, Smallholder, North Gondar Zone***

1. INTRODUCTION

1.1 Background

Bovine tuberculosis (BTB) is found throughout the world and the disease is more prevalent in most of Africa, parts of Asia and of the Americas (OIE, 2015). BTB is a chronic disease of animals caused by bacteria called *Mycobacterium bovis*, which is closely related to the bacteria that cause human. This disease can affect practically all mammals, causing a general state of illness, coughing and eventual death. The name TB comes from the nodules, called 'tubercles', which form in the lymph nodes of affected animals. Until the 1920s when control measures began in developed countries, it was one of the major diseases of domestic animals throughout the world (Shitaye *et al*., 2007).

The level of testing and control of BTB in Africa is also considerably constrained by the lack of infrastructure, including physical (both veterinary and public health settings) as well as skilled-man power. Transhumance means that cattle herds are not sedentary and may move hundreds of kilometers a year. In many countries in Africa, access to the herds is difficult due to lack of roads or areas that become inaccessible during the rainy season. Another limitation in implementing intervention is the economic consequences and social systems.Even when cows are infected, it is difficult to force culling, because the cattle value is deeply interwoven with the social system and they are the savings of the rural poor (McCrindle and Michel, 2006).

Economic returns from cattle in Africa are rather low and do not provide impetus to control major diseases, which hampers trade with developed countries. In addition, most developed countries impose strict regulations on importing livestock and products from the developing regions to prevent the introduction of disease from foreign countries. The livestock industry in most Sub Saharan Africa is significantly underproductive in comparison to that in South Africa, Asia and Europe. Despite large herd size, comparable to that of Europe and larger than that of the United States, productivity and economic returns remain very low. There are many facets to this underperformance, but it is mainly a result of livestock diseases causing relatively low outputs (Zinsstag *et al.*, 2006).

Ethiopia is among the nation that possesses the largest livestock population in the Africa continent with the total cattle population for the country is estimated to be about 56.71 million. (CSA, 2015). In contrast to the large livestock resources, the livestock productivity is found to be very low. The major biological and socio-economic factors attributing to the low productivity include: the low genetic potential and performance, poor nutrition (in quality and quantity terms), the prevailing of different diseases, traditional way of husbandry systems and inadequate skilled manpower. Ethiopia is one of the African countries where BTB is considered as a major disease burden in animals. BTB is one of the endemic infectious diseases that have long been recorded in Ethiopia. In Ethiopia, BTB is considered to be a prevalent disease in cattle populations where tuberculin skin test survey indicates that the prevalence ranges from 0.8% in extensive rural farming systems that keep Zebu cattle to 50% in intensive husbandry systems (Ameni *et al.*, 2008).

Today BTB remains an important disease of cattle, wild animals, and is a significant zoonosis transmitted from animal to humans and vice versa. Many studies have shown that there are many risk factors responsible for the spread and persistence of BTB in developing countries such as demographic factors, eating habits, living and socio-economic status of families, illiteracy, culture and customs, the existence of HIV/AIDS, and close proximity with animals (Regassa *et al*., 2008)..

Ethiopian milk consumers generally prefer raw milk (as compared to pasteurized milk) because of its taste, availability and lower price. The zoonotic risk of BTB is often associated with consumption (ingestion) of unpasteurized milk and other dairy products infected with *M. bovis*. Also, aerosol transmission from cattle-to-human (or vice versa) should be considered as a potential risk factor (Gebremedhin *et al.*, 2014).

1.2. Statement of the Problem

Bovine Tuberculosis is endemic in cattle population of Ethiopia (Ameni *et al.,* 2008) and is prevalent in North Gondar smallholder dairy farms (Mohammed *et al.,* 2012; Marshet, 2014). The economic losses due to the disease is said to be enormous, where infected animal loses 10 to

25% of its productive efficiency. Direct losses due to the infection become evident by decrease in 10 to 18% milk and 15% reduction in meat production (Zinsstag *et al.*, 2006).

Both intensive and extensive dairy production system are available in Gondar area. Now a day, intensification of dairy farms is increasing which favors the occurrence of disease of intensification like bovine tuberculosis (Ameni, 2008) .Diagnosis of bovine tuberculosis is based on TST and culture which can take 3 days and 6 weeks respectively (OIE, 2015), that makes the diagnosis of BTB economical as well as epidemiological costly. So that, TB rapid tests at small dairy farm level are needed. Apart from lower awareness of most of the communities of our country 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 farmers in North Gondar districts where sharing shelter during the night time 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 human tuberculosis. Therefore, knowing the status of the disease and its possible economic implications in the stipulated study area will contribute its share for the efforts made to prevent and control human tuberculosis. Therefore, this study had the following objectives.

1.3. Objective

General objective: To assess bovine tuberculosis (BTB) status using two diagnostic tests and its economic implications in smallholder dairy farms in selected districts of North Gondar zone, Amhara regional state.

Specific objectives:

- To estimate the prevalence of BTB in dairy farms using tuberculin skin test,
- To enumerate the economical impacts of BTB,
- To compare newly develop Alere TB Lam Ag test with tuberculin skin test,
- To identify risk factors and display spatial distribution of BTB and
- To asses BTB zoonotic awareness among smallholder dairy farm owners.

2. LITERATURE REVIEW

2.1 An overview of Bovine tuberculosis

The name Tuberculosis comes from the nodules, called 'tubercles', which form in the lymph nodes of affected animals. (OIE Manual, 2015). *M.bovis* is a member of the Mycobacterium tuberculosis complex (MTC). The complex encompasses *M. tuberculosis, M. bovis*, *Mycobacterium microti*, *Mycobacterium canetti*, and *Mycobacterium africanum* (Smith *et al*., 2006). Bacteria of the *M.tuberculosis* complex are aerobic, non-motile, non-spore-forming, slow-growing and acid fast bacilli (Thoen *et al.*, 2004).

Bovine tuberculosis has been significantly widely distributed throughout the world and has been a cause for great economic loss in animal production and the most frequent cause of zoonotic TB in man (Tenguria *et al*., 2011). The disease is more prevalent in most of Africa, parts of Asia and of the Americas. Many developed countries have reduced or eliminated bovine TB from their cattle population; however significant pockets of infection remain in wildlife in Canada, the United Kingdom, the United States and New Zealand. Although cattle are considered to be the true hosts of *M. bovis*, the disease has been reported in many other domesticated and non-domesticated animals. Isolations have been made from buffaloes, bison, sheep, goats, equines, camels, pigs, wild boars, deer, antelopes, dogs, cats, foxes, mink, badgers, ferrets, rats, primates, llamas, kudus, elands, tapirs, elks, elephants, sitatungas, oryxes, addaxes, rhinoceroses, possums, ground squirrels, otters, seals, hares, moles, raccoons, coyotes and several predatory felines including lions, tigers, leopards and lynx (OIE, 2015).

The disease is contagious and spread by contact with infected domestic and wild animals. The usual route of infection is by inhaling infected droplets which are expelled from the lungs by coughing. Calves and humans can also become infected by ingesting raw milk from infected cows. Because the course of disease is slow, taking months or years to kill an infected animal, an animal can spread the disease to many other herd mates before it begins to manifest clinical signs. Therefore, movement of undetected infected domestic animals and contact with infected wild animals are the major ways of spreading the disease (OIE Manual, 2015)

2.2. Risk factors

Risk factor of BTB is divided in to animal level risk factor and herd level risk factor. At animal level; Age, sex, breed, body condition, immune status, genetic resistance & susceptibility to BTB, vertical and pseudo vertical transmissions and auto-contamination are considered to be risk factors. Risk factors at herd level are history of BTB outbreak, human antecedent of TB in the house hold, herd size, type of cattle enterprise, management, lack of performance of diagnostic tests, reduced opportunity of detection, introduction of purchased cattle in the herd, movements of animals, other domestic species, contact between animals, wild life and climate influence (Marie *et al*., 2009).

2.3. BTB diagnostic tests

The standard method for detection of TB is the tuberculin test, where a small amount of antigen is injected into the skin, and the immune reaction is measured. Single intradermal tuberculin skin test (SITT) is the test that bovine tuberculin injection can be at the site of hairless area of caudal fold to observe the skin reaction against *M.bovis*. Comparative intradermal tuberculin test (CIDT) is the test that many studies usually used to observe the skin reaction against *M. bovis* and *M. avium.* Definitive diagnosis is made by culturing the bacteria in the laboratory, a process that takes at least eight weeks (OIE, 2015).

Recently, a new rapid diagnostic TB test particularly used in humans, called Determine TB-Lipoarabinomannan antigen (LAM Ag) is developed. This immunchromatographic test is used for qualitative detection of LAM Ag of Mycobacteria in human urine sample. This involves a point of care (POC) assay lateral flow dipstick version of urinary LAM detection method. Following its commercial launch in 2013, Determine TB-LAM remained the focus of ongoing clinical evaluation studies. This simple, low-cost, POC provides a qualitative (yes/no) readout of TB diagnosis within 30 min in clinically infected subjects (Lawn, 2014). Studies largely confirm that the sensitivity of Determine TB-LAM is greatest (range 60–70%) thus this kit could assist to establish a quicker diagnosis in the high risk population (Lawn *et al.*, 2013; Tucci *et al.*, 2014).

2.4. Economic impact of BTB

The main productivity losses in cattle are reduced milk and meat production and increased reproduction efforts. Milk productivity of total livestock is lower compared to that of non infected cows. Losses in meat production are divided into losses in beef processing caused by emergency / illness slaughter and losses in processing caused by normal slaughter and reduction of increment meat production. In totally TB infected livestock meat production is lost because of emergency and illness slaughter. The losses are mainly in cattle and cows over 18 months of age and no differences of growth could be found in young animals in fattening schemes (Zinsstag *et al.,* 2006).

In England, economic impact assessment of BTB report revealed that the monthly loss of dairy farm due to BTB varies considerably. Clearly, there are many factors that account for the range of losses including the type of farm, the scale of operation, restocking policy, area farmed, and number of holdings away from the farmstead, the marketing of livestock and livestock produce and by how much this is restricted. In general, dairy farms tend to accrue the greatest losses during a BTB occurrence (Allan *et al.*, 2010). In Ethiopia, the economic impact of BTB on cattle productivity and related points as well as BTB control programs have not yet been well documented or studied (Shitaye *et al.,* 2007).

2.5. Zoonotic Importance

M. bovis is not the major cause of human tuberculosis, but humans remain susceptible to BTB. Humans can be infected primarily by ingesting the agent by drinking raw milk containing the infective bacilli, secondly, by inhaling infective droplets when there is close contact between the owner and his/her cattle, especially at night since in some cases they share shelters with their animals. In some countries, it is estimated that up to 10% of human tuberculosis are due to BTB (Gebremedhin *et al.,* 2014; OIE, 2015).

2.6. Prevention and Control

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

The standard control measure applied to BTB is test and slaughter. Disease eradication programs consisting of postmortem meat inspection, intensive surveillance including on-farm visits, systematic individual testing of cattle and removal of infected and in contact animals as well as movement controls have been very successful in reducing or eliminating the disease. Pasteurization of milk of infected animals to a temperature sufficient to kill the bacteria has prevented the spread of disease in humans. Treatment of infected animals is rarely attempted because of the high cost, lengthy time and the larger goal of eliminating the disease. Vaccination is practiced in human medicine, but it is not widely used as a preventive measure in animals: the efficacy of existing animal vaccines is variable and it interferes with testing to eliminate the disease. A number of new candidate vaccines are currently being tested (OIE, 2015).

3. MATERIAL AND METHODS

3.1 Study area description

The study was conducted in two woredas purposefully selected based on LIVES project working sites; i.e, Gondar town and Gondar Zuria, which are found in North Gondar Zone, Amhara region.

Gondar town, which is the capital city of North Gondar zone is located 747 km North West of Addis Ababa and 180km North East of Bahir Dar. It is located at 120 30' North and 370 20' East. The town limits of Gondar enclose an area of 48.27 km2 and standard altitude is 1966m above sea level. Gondar zuria district is located at 37°24'24"E-37°45'43"E and 12o7'23"N-12°39'24"N and its total area is 1286.76 km2. It is part of the North Gondar Zone, which is bordered South by South Gondar Zone, Southwest by Lake Tana, West by Dembia, North by Lay Armachiho, Northeast by Wogera, and to the southeast by Belessa (CSA, 2015).

3.2 Study population

The source population was those small holder dairy herds in the two districts, i.e Gondar Zuria district and Gondar town. Three kebeles of Gondar zuria district were involved in the study, namely; Tsion seguaj, Bahri ginib and Chira manterno. In Gondar town, 10 Kebeles were included in the study (Kebele1, 2, 3, 6, 10, 14, 15, 16, 18 and 19). In both study areas Kebeles were selected based on the availability of dairy farms.

A total of 109 smallholder dairy farm herds were used as the study population. A total of 433 eligible heads of cattle were tested using the two antigens (PPD-Avian & bovine type and TB LAM).

For the purpose of this study, smallholder dairy farms were defined as dairy farms that hold≤10 dairy animals. Medium scale dairy farms are farms that hold >10 and < 50 dairy animals. Number of sampled Smallholder dairy farm in Gondar town and Gondar zuria were 34 and 58,

respectively. Medium scale dairy farms, 14 and 3 medium scale dairy farms were included in Gondar town and Gondar Zuria, respectively. A total of 92 smallholder dairy farms and 17 medium scale dairy farms were included in the study.

3.3 Study design

The study was a cross sectional and deployed from September, 2015 to April, 2016. Test using the two antigens was carried out from February 2016 to March 2016. A structured pretested questionnaire was administered to 109 smallholder dairy farmers. GPS coordinates of the dairy farms were taken in both districts to produce BTB distribution map.

3.4 Sampling method

All smallholder dairy farmers found in both study sites were registered. Study herds were selected randomly based on the registry. On the sampling frame, in the randomly selected herds, all eligible animals were sampled except aggressive animals, those less than 6 months of age, pregnant cattle near to term, recently delivered cows or added to the herd were excluded from the test. Some of these are justifiable in that animals lose sensitivity to tuberculin shortly before and after calving (Radostits *etal*. 2007).

Data regarding age using dentition, (Radostits *etal*.2007), body condition (using body score method; poor, fair, good), parity, milk yield per day by lit., breed, feed type, source, (using data sheet by asking owners) of the tested animal within the herd, as well as other related variables were taken while testing the cattle and the study follow up.

3.5. Sample size determination

The sample size was determined using recommendations as published in Thursfield (2005). For this purpose, a herd prevalence of 10% recorded in Ambagiorgies town smallholder dairy farms (Marshet, 2014) with 95% confidence interval and 5% absolute precision were considered to calculate the required sample size.

$$N = \frac{1.96^2 \times Pexp(1-Pexp)}{d^2}$$

Where, 1.96 is the alpha value of 95% CI; Pexp = expected prevalence; d= absolute precision; N =sample size.

Based on the assumption, sample sizes of 138 dairy herds were targeted. Due to resource limitation 109 dairy herds were sampled in the two study districts, with in the North Gondar zone.

3.6 Data collection methods

The materials used for this study were digital vernier caliper, insulin syringe, blades and scalpel handle, detergents, Determine LAM Ag strip, sample bottle, dropper, alcohol, cotton, gloves and rope and bovine (*M.bovis*, strain AN-5 25.000 IU/ml & avian PPDs (*M. avium*, strain D4 ER 25.000 IU/ml).

3.6.1. Single Intradermal Tuberculin Test (SITT)

In Gondar town, 48 smallholder dairy herds of 10 kebeles were included. A total of 247 cattle were tested using single intradermal tuberculin test. At the caudal fold, the injection site was cleaned. The skin fold thickness was measured using caliper and recorded before injection. Then after, 2000 IU dose or 0.1ml of bovine tuberculin was injected. Pea like small nodule was

palpated on injection site. After 72 hours the thickness of the skin at the injection site was measured. To avoid errors, the same person who measured the skin before injection measured 72 hours after injection. The result was interpreted as <2mm = negative, >2mm and <4mm = doubtful and $\geq$4mm = positive (OIE, 2009).

3.6.2. Comparative Intradermal tuberculin Test (CIDT)

In three kebeles of Gondar zuria district, CIDT injection was given for 187 smallholder dairy cattle within 61 smallholder dairy herds. Prior to injection, the two injection sites around the middle neck region were cleaned and shaved. A fold of skin thickness within shaved area was measured with calipers and recorded (OIE, 2009).

A 0.1 ml needle, graduated syringe charged with tuberculin was inserted obliquely into the deeper layers of the skin. The dose of tuberculin injected was 2000 International Units (IU) or 0.1 ml of bovine and avian tuberculin on two different sites on one animal. The distance between the two injections was approximately 12 cm apart. Correct injection was checked by palpating a pea like small nodule on the injection site. The skin-fold thickness of each injection site was re-measured. To avoid errors, the same person who measured the skin before injection measured 72 hours after injection. The result was interpreted as Negative <2mm, >2mm and <4mm as doubtful and $\geq$4mm as positive (OIE, 2009).

3.6.3. Determine TB Lipoarabinomannan Antigen Test (LAM Ag test)

TB LAM Ag is a production of Alere Production Company of USA, as rapid TB diagnostic kit. It is commercially launched in 2013, this is a simple technique that provides qualitative binomial (yes /no) readout of TB diagnosis which labeled with positive and control groups with purple and gray colors band (Lawn, 2014).

LAM point of care diagnostic tool is more applicable in human than animals. This study aimed to evaluate the test in dairy animals of Ethiopia. Using urine and milk samples as the test has easy procedure, low cost and fast in early diagnosis of tuberculosis infection in smallholder dairy

farms. In both study areas, the test strip was used for TB diagnosis using milk and urine samples within 30 minutes at the field. LAM Ag positive samples had purple/gray bars appear in both control window (labeled control) and patient window (patient window) of the strip. In LAM Ag positive samples, the color intensity of the patient bar could be lighter, equal or stronger than of the control window color intensity. In LAM Ag negative samples, only one colored bar on control window but no purple or grey bar appears in patient window.

A. Milk samples

In Gondar zuria district, 99 milk samples were collected. The udder of the cow was cleaned and disinfected using diluted Savlon. Milk was collected from dry and clean teats using clean milk container. The collected sample was tested on-site at the field. The test card protection foil cover was removed and a drop of milk was put on the sample pad of the rapid test card tip. The sample strip was visualized after 25 minutes for result. [The results were read within 35 minutes]

B.Urine samples

In Gondar town and in Gondar zuria district, 60 and 115 urine samples were collected, respectively. The urogenital area was cleaned and disinfected using diluted Savlon and the midstream fresh urine was collected using urine collection container. After removal of the protection foil of the test card, a drop of urine sample was applied on the sample pad, under the shed. The card was visualized for reading the result after 25 minutes.

3.6.4. Questionnaire survey

Questionnaire survey was applied for 109 smallholder dairy farm owners and farm attendants. Participants from Gondar zuria and from Gondar town were 61 and 48 respondents, respectively. The questionnaire was focused on issues related to production system, housing, feeding and

watering frequency, BTB history of the herd, awareness about BTB, milk selling, raw milk consumption and human TB history (Appendix).

3.6.5. Global Positioning System (GPS)

To develop bovine tuberculosis map for Gondar town and Gondar zuria districts, longitudinal, latitude and elevation points were taken from satellite using GPS. To identify spatial distribution of bovine TB in the study area herds, 45 and 68 coordinate points were taken from smallholder dairy farms of Gondar and Gondar zuria district, respectively. The GPS coordinate data entered to excel spread sheet and imported in to the map using QGIS . The coordinates were incorporated in Gondar town and Gondar zuria shape files. Kebeles were labeled based on the BTB prevalence in both study area dairy herds.

3.6.6 Propensity Score Matching (PSM)

An economical implication of bovine tuberculosis in the dairy animal was measured using Propensity Score Matching (PSM). This method enables to capture the effects of different observed covariates on milk production between test positive and test negative individual. Then, outcomes of BTB positive and BTB negative individuals with similar propensity scores are compared to obtain milk yield value. Individuals smaller and larger from the common support area were dropped for which no match is found and because no basis exists for comparison (Shahidur *et al.,* 2010). Only 339 female animals were included in the model; the male animals and heifers are excluded.Age, breed, body condition and source of the animal are common risk factors for both BTB positive and negative individual animals to estimate impact of BTB on milk yield.

3.7 Data collection & Statistical Analysis

3.7.1. Data collection

During the TST, urine and milk sample collection related to each animal; name of the animal, age, sex, parity, milk yield, and source and body condition data was collected using the data collection sheet.

3.7.2. Statistical analysis

Data from skin test, LAM Ag, questionnaire and GPS *Stata* version 12 was used for different Statistical analysis of variables. Initially, a descriptive analysis of mean, frequencies and percentages were used to describe the results while tables, figures and graphs used to present the findings. A univariate analysis using Ch-square was done to determine the herd size and association with BTB at individual animal and also herd levels. In addition, Propensity Score Matching (PSM), Logstic regression model to determine the significance of proposed risk factors and Kappa test to validate and compare agreement among diagnostic methods was used. For entering GPS coordinates in to map, *QGIS* software was used.

3.7.2. Logistic regression Model

Univariate and multivariate logistic regression model were used to identify potential risk factors from different independent variables data which are collected using questionnaire and data collection sheet. Individual animal and herd level risk factors for prevalence of BTB were analyzed using Odds ratio.

3.7.3. Kappa test

Since there is no gold standard test used here, Kappa test was applied for evaluation of test agreement between CIDT and LAM Ag by Urine, CIDT and LAM Ag by milk, SITT and LAM Ag by Urine samples in diagnosis of TB positive and TB negative samples. The Kappa tests strength of agreement was interpreted as 0= no better than chance, 0.01- 0.2= slight, 0.21-0.40= fair, 0.41-0.60=moderate, 0.61-0.80=substantial, 0.81-0.99=almost perfect and 1.00=perfect.

4. RESULT

4.1. Prevalence of BTB

4.1.1. Bovine TB prevalence in Gondar town and Gondar Zuria District

Table1. Individual animal and herd level prevalence of BTB in Gondar town.

Gondar Town

Variable	Total no. examined	No. (%) of positive	P value
Animal prevalence			
Herd size<10	109	9 (8.26)	
Herd size>10	138	20(14.49)	0.131
Herd prevalence			
Herd size<10	34	8 (23.5)	
Herd size>10	14	8 (57.14)	0.025*
Age (years)	247	29 (11.74)	
≥1≤4	146	21 (14.38)	
>4≤7	99	7 (7.07)	
>7	2	1 (50)	0.053
Sex			
Male	5	0	
Female	242	29(11.98)	
Breed			
Cross	246	29 (11.78)	
Local	1	0	0.715
Body condition			
Poor	102	17 (16.66)	
Fair	131	12 (9.16)	
Good	14	0	0.078
Source			
Home	215	23 (10.6)	
Purchased	32	6 (18.75)	0.187

In Gondar town, individual animal level prevalence in small holder dairy farms was 8.26% that is 9/109 individuals were positive and have lower prevalence than that of medium scale dairy farms which has 20/138 positive animals with 14.49% prevalence. The herd level prevalence in smallholder dairy farms, 8/36 was positive herds with prevalence of 23.53% and in medium scale dairy farms the herd prevalence was 57.14%, which have 8/14 positive dairy herds. The prevalence is higher than the smallholder dairy farms herd prevalence.

Table 2. Individual animal and herd level prevalence of BTB in Gondar Zuria district.

Gondar Zuria

Variable	Total no. examined	No. (%) of positive	P value
Animal prevalence			
Herd size<10	180	14(7.78) and 1doubtful (8.33)	
Herd size>10	7	1 (14.49)	0.81
Herd prevalence			
Herd size<10	58	11(18.96)	
Herd size>10	3	1 (33.33)	0.542
Age			
≥1≤4	57	5(8.77)	
>4≤7	90	7(7.77)	
>7	40	3(7.5)	0.887
Sex			
Male	51	9 (17.64)	
Female	136	6 **(16.66)**	0.01*
Breed			
Cross	23	3 (13.04)	
Local	165	12 (18.46)	0.6
Body condition			
Poor	119	6 (5.04)	
Fair	61	8 (13.11)	
Good	7	1(14.28)	0.34
Source			
Home	151	10 (6.62)	
Purchased	36	6 (16.66)	0.09

In Gondar zuria district, 14/180 individual animals were positive and the prevalence is 7.78 % in smallholder dairy farms and one positive animal (1/7) were found giving 14.29% prevalence in the medium scale dairy farm. One animal was doubtful which skin reaction measures between 2mm and 4mm, 2.3mm. This animal may or may not be positive. If this animal is included as positive the individual animal prevalence will be increased to 8.33%. If it is negative the prevalence will remain the same, 7.78%. Smallholder dairy farms herd prevalence of Gondar zuria is 18.97% (11/58). In medium scale dairy farms, 1/3 of the herds were positive and the prevalence is 33.33%.

4.2. Estimation of economical impacts of BTB

Estimation of propensity scores for observations in the individual animal was the first step in Propensity score matching (PSM). A logit model was used to estimate propensity scores for each observation in BTB positive and BTB negative animals.

The dependent variable in the logit model was BTB infection (using PPD), which took the value of 1 if an animal is BTB positive and 0 if the animal is BTB negative. And the independent variables were different risk factors which were theoretically supported to affect the animal to be BTB positive or negative. Here breed were important factor (OR= 1.4) in BTB infection of individual animal that indicates crossbred animals are 1.4 times more likely to be tuberculin positive. Milk yield was taken as an outcome variable for the impact evaluation of BTB in the dairy farm, at individual level.

Table 3. Logistic regression model estimation of individual animal (Here Male animals are excluded).

BTB infection	Coef.	Std. Err.	z	P>z	[95% Conf.	Interval]
Source	0.605435	0.475237	1.27	0.203	-0.32601	1.536883
Age	-0.04168	0.148813	-0.28	0.779	-0.33335	0.249983
Body condition	-0.68936	0.371415	-1.86	0.063	-1.41732	0.038602
Breed	**1.413738**	0.630968	2.24	**0.025***	0.177064	2.650412

4.2.1. Matching of BTB positive and BTB negative animals

The main aim on estimation of economical impact of BTB was to calculate the average BTB effect on milk yield of positive animals. To identify the common support region, basically based on comparing the minima and the maxima of propensity score in both groups, which was the basic criterion to delete the smaller than the minima and the larger than the maxima of common support area in positive and negative group of animals matching. Therefore the common support area took the maximum of the minima and the minimum of the maxima to have the intersection point common for both groups.

Table 4. Distribution of estimated propensity scores in two districts, 2016

Group	Observation	Mean	Std. Dev.	Min	Max
Positive animals	30	.1239552	.0628274	**.0172687**	**.2421078**
Negative animals	309	.0850529	.0520935	.0080487	.2506207
Total	339	.0884956	.0541662	.0080487	.2506207

4.2.2. Choosing best algorism

After the common support is defined, the individual animals outside the region will be dropped and there will not be room for estimation of the BTB effect in these individuals. The three matching estimators namely, nearest neighbor matching, caliper matching and kernel matching nearest neighbor matching. Up on these estimators, nearest neighbor matching with 333 observations was preferred

Table 5. Performance of matching estimators under the three criteria, 2016

Matching Estimator	**Performance criteria**		
	Balancing test*	Pseudo R2	Matched sample size
Neighbor matching			
1 neighbor	2	0.0590	60
2 neighbor	**2**	**0.0590**	**333**
3 neighbor	**2**	**0.0590**	**333**
4 neighbor	**2**	**0.0590**	**333**
Radius Caliper matching			
With 0.01 band width	2	0.0590	57
With 0.1 band width	2	0.0590	59
With 0.25 band width	2	0.0590	60
With 0.5 band width	2	0.0590	60
Kernel Matching			
With 0.01 band width	2	0.0590	326
With 0.1 band width	**2**	**0.0590**	**333**
With 0.25 band width	**2**	**0.0590**	**333**
With 0.5 band width	**2**	**0.0590**	**333**

4.2.3 BTB effect on positive animals

The final step in the PSM model was evaluation of impact, whether BTB brought about the difference in the indicators of impact which the difference revealed on milk yield of BTB positive animals loss 3.38 liter per day per cow.

In this study, average milk yield of dairy cows is 8.843 Liter, the average milk yield of BTB infected cow have 5 Liter per day. Therefore, BTB infected cow produces 3.843Litre lower milk yield per day than healthy cow.

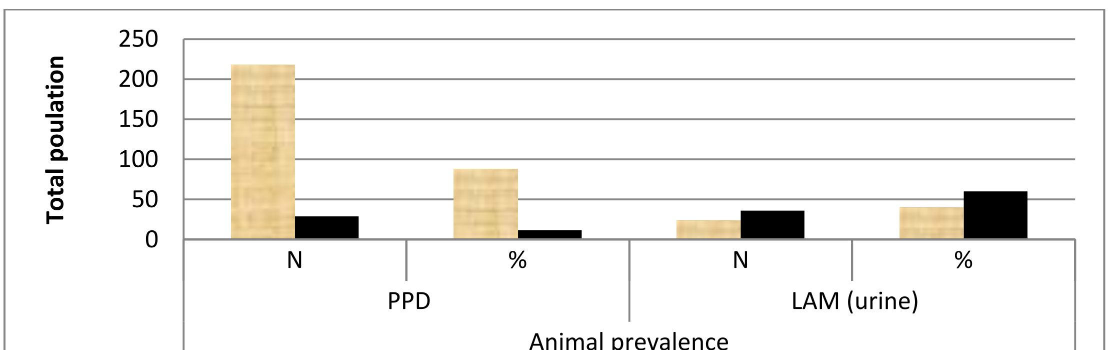
Thus, dairy farm loses, 3.843 lit/day x 13birr = **49.96** birr/ day/cow due to BTB infection.

Table 6 .Impact of BTB using PSM in two districts, 2016

Variable	Positive animals	Negatives animals	ATT	S.E.	T-stat
Average milk yield/day/cow	5	8.84283087	-3.84283087	.471110517	-8.16

4.3. Comparison of diagnostic tests

BTB prevalence in Gondar town using tuberculin test (SITT) was 8.26% and using LAM Ag test by urine sample revealed 60% prevalence of BTB.



	N (PPD)	% (PPD)	N (LAM (urine))	% (LAM (urine))
Negative	218	88.26	24	40
Positive	29	11.74	36	60

Figure 1. Comparison of animal prevalence using PPD and LAM Ag (urine) test in Gondar town.

In Gondar zuria woreda PPD/CIDT result showed that 8.06% (inclusive of doubtful results) of animal prevalence but the LAM Ag test using urine and milk sample showed higher prevalence of BTB than PPD, 44.35% and 78.79%, respectively.

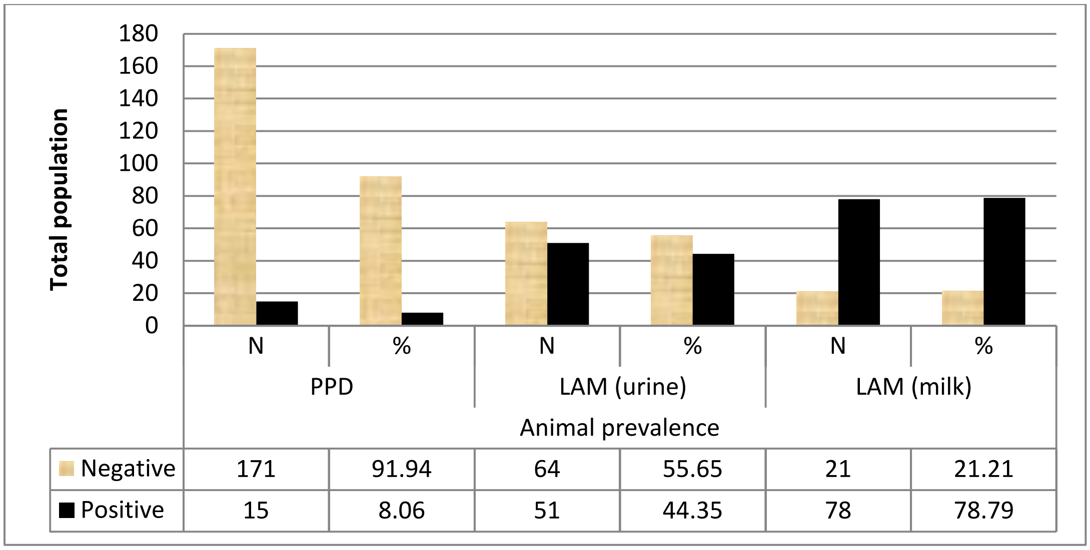


Figure 2. Comparison of individual animal prevalence in Gondar zuria district using PPD, LAM (urine) and LAM (milk) tests.

Table 7. Kappa test, test agreement between PPD, LAM (urine) and LAM (milk) in Gondar town and Gondar zuria district, 2016.

Comparisons	Agreement	Expected Agreement	Kappa	Std. Err.	Z	Prob>Z
PPD to LAM Ag urine	54.39%	50.65%	0.0757	0.0519	1.46	0.0723
PPD to LAM Ag milk	23.23%	22.38%	0.011	0.0149	0.74	0.2292

4.4. Spatial distribution of BTB

4.4.1 Gondar town dairy farms

As the map shown the distribution of BTB in Gondar town sampled dairy farms in different kebeles, BTB positive and negative dairy farm places are displayed.

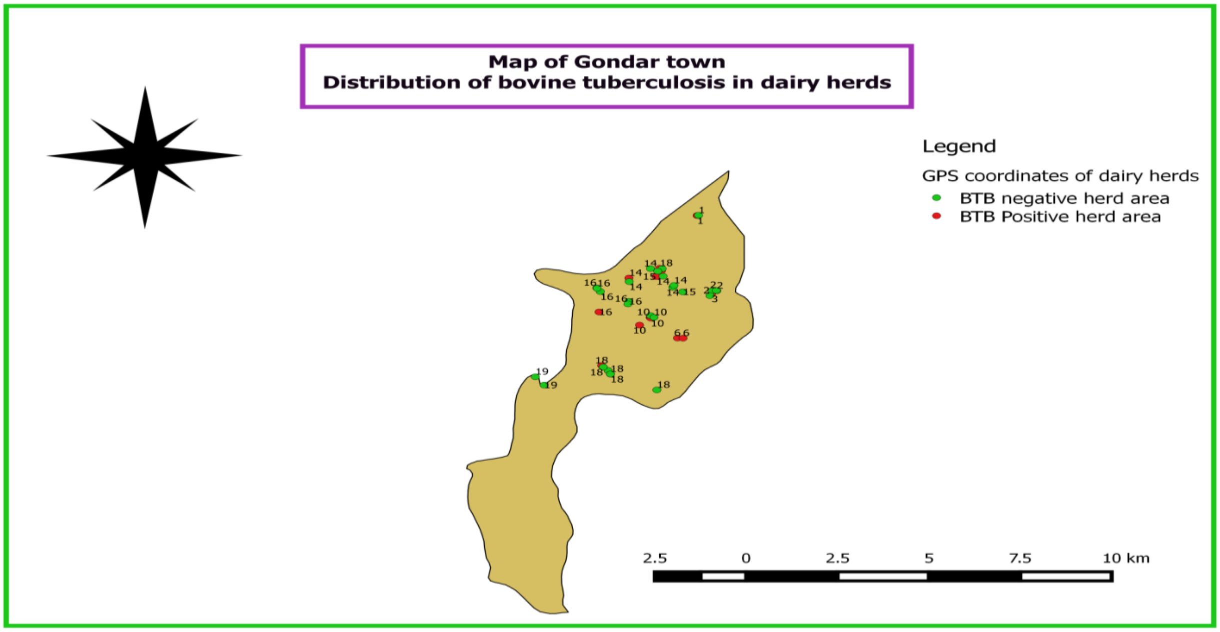


Figure 3. Map of Gondar town with spatial distribution of tuberculosis disease in different kebeles.

4.4.2 Gondar zuria district dairy farms

The distribution of bovine tuberculosis in dairy herds of Gondar zuria displayed as the positive herds mostly concentrated to Tsion seguaj kebele with red spot on the area.

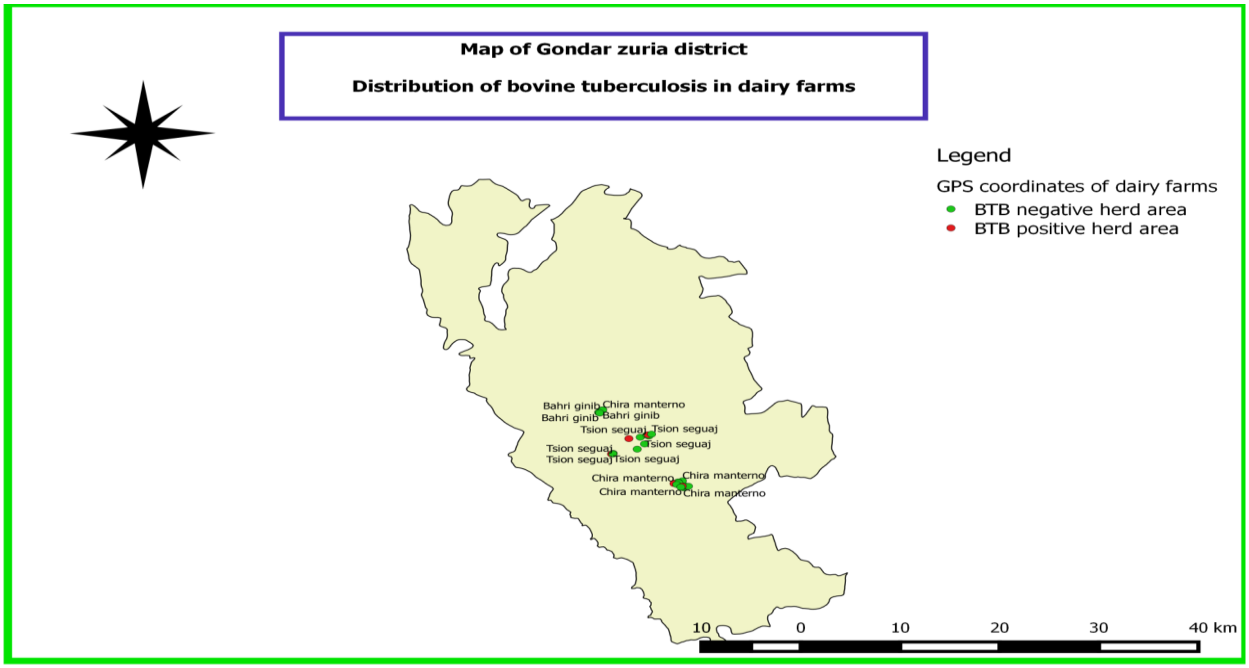


Figure 4. Map of Gondar zuria district with spatial distribution of tuberculosis in different kebeles

4.5 Risk factor

4.5.1 Animal level risk factor

Table 8 Animal level risk factors for BTB infection in Gondar town and Gondar zuria district.

Risk factors	OR	95% CI
Animal level		
Source		
Home	1	
Purchased	2.33*	0.104291-1.590954
Sex		
Male	1	
Female	0.31*	-2.10336 - -0.21804
Body condition		
Poor	1	
Fair	0.78	-0.90786 - 0.415469
Good	0.28	-3.36852- 0.858875
Breed		
Zebu	1	
Cross	2.69*	0.120133-1.863516

Up on risk factors of individual animals listed, source of an animal that are purchased had an exposure to BTB infection more likely 2.33 times (OR= 2.33) than home sourced. Sex Female has an OR= 0.31 which means females are more likely to not be infected than male animals. In breed category, crossbred were an OR=2.69, exposed 2.69 times more likely than the local ones. Age and body condition have no significant (P>0.05) importance for BTB occurrence on individual animals in dairy farms.

4.5.2 Herd level risk factor

Table 9 Herd level risk factors for BTB infection in Gondar town and Gondar zuria district.

Risk factors	OR	95% CI
Herd level		
Production system		
Intensive	1	
Semi-intensive	0.06	0.00235 – 1.77566
Owners awareness		
No	1	
Yes	12.64	2.686049 – 59.53528

Semi intensive production system has protective factor (OR=0.06) against BTB than intensive production system. The dairy farm owners who heard about BTB have 12.64 more likely to have the infection in their dairy farm than who did not. This result was due to the farmers have their animals tested for BTB tuberculin skin test previously.

4.6. Zoonotic awareness of dairy farmers

4.6.1. Dairy farmers BTB zoonotic awareness

Gondar town dairy farmers 28/48 are familiar to BTB, and only 5/61 respondents of Gondar zuria district are familiar to the word BTB. The zoonotic awareness of Gondar town and Gondar zuria is 54.16% and 11.48% respectively.

Table 9. Gondar town and Gondar zuria district dairy farm owners awareness of BTB

	familiar to word BTB (N=109)				Zoonotic awareness(N=109)			
	Gondar town		Gondar zuria district		Gondar town		Gondar zuria district	
	N	%	N	%	N	%	N	%
No	20	41.67	56	91.8	22	45.84	54	88.52
Yes	28	**58.33**	5	**8.2**	26	**54.16**	7	**11.48**
Total	48	100	61	100	48	100	61	100

4.6.2. Raw milk consumption

Raw milk consumption is practiced in the study area. Raw milk consumption habit is higher in Gondar zuria (23/61) than in Gondar town (5/43) dairy farm owners. Out of the total (109) interviewed dairy farm owners 28 (nearly 27%) used to take unboiled milk.

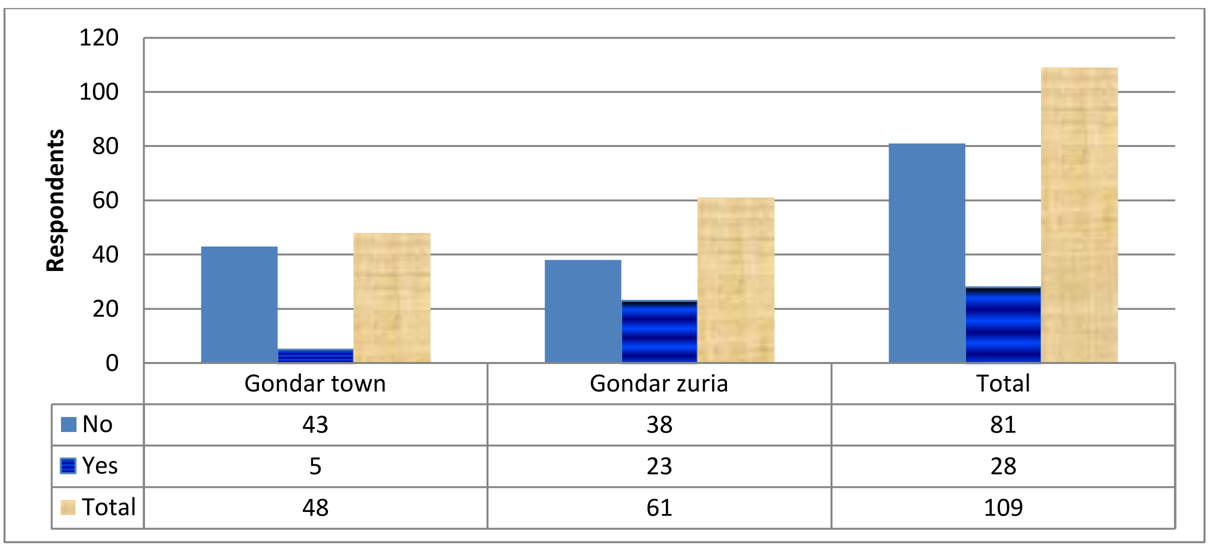


Figure 5. Raw milk consumptions habit in households of Gondar town & Gondar zuria district, 2016

5. DISCUSSION

The individual animal level prevalence in small holder dairy farms in Gondar town was 8.26% and in Gondar zuria district 7.8%. This findings were in agreement with studies done in Gondar town (Mohammed *et al*, 2012) reported a prevalence of 7.2 %. Contrary to this it was lower compared to that of the medium scale dairy farms 14.5%. This might be justified by differences in herd size and possibly the breed composition and feeding/grazing practices. Higher individual animal bovine tuberculosis prevalence was reported in dairy herd size having >10 and those medium herds had an increased risk to be test positive compared to the smaller ones (Zeru *et al* , 2014; Firdessa *et al*. 2012).

A herd prevalence of 23.53% and 18.97% in Gondar town and in Gondar zuria district, respectively were reported in the current study. Mengistu and colleagues reported similar herd prevalence (23.6%) of bovine tuberculosis (Mengistu *et al.*, 2015). But the prevalence is higher than Marshet finding (10%), in Ambagiorgis (Marshet, 2014). The variation with the findings made from Ambagiorgis might be due to the herd composition, where much more crossed cattle involved in the current study. This could be strengthened by the findings of Gondar town herd prevalence (Almost all are crosses), which accounted higher compared to Gondar zuria. This breed difference in susceptibility is reasoned out by different research findings (Ameni *et al.*, 2007).

The prevalence of Gondar zuria dairy animals using tuberculin skin test, LAM urine and LAM milk is 8.06%, 44.35% and 78.79%, respectively. The prevalence is higher in LAM lateral flow milk, followed by LAM lateral flow urine and the least by PPD. The higher prevalence of LAM using milk sample might be due to the nature of the strip where it is principally prepared for the detection of the disease using urine samples not for Milk samples (Alere manual, 2013). The microorganism in milk may cross react with LAM point care and inflating the test result. In general the higher test results using LAM Ag lateral flow test might be due to the fact that interpretation to say positive has three categories, namely; the strong positives (bold color change), weak positives (low color change), and intermediate positives (slight color change) while it is one category for the negative judgments.

The test agreement using Kappa test indicated a percentage of 54.39% with a Kappa value of 0.0757 using urine sample. Therefore, there was a slight agreement between PPD and LAM Ag test (Landis & Koch, 1977) and hence giving possible direction to use this test material for bovine tuberculosis diagnosis or screening. The test agreement between PPD and LAM using milk samples was, 23.23%, with 0.0149 kappa value, showing also slight agreement (Landis & Koch, 1977).

Estimation of propensity scores for observations in the individual animal was the first step in Propensity score matching (PSM). A logit model was used to estimate propensity scores for each observation in BTB positive and BTB negative animals.

Here the fundamental step was checking the overlap and the region of common support between BTB positive animals and the control group (Table 3). Bryson et al. (2002) indicated that when proportion of dropped individual is small the analysis possess few problems. But if the excluded number is too large, the estimated BTB effect in the common support region included individuals representativeness will be under concern (Smith and todd, 2005). Up on these estimators, nearest neighbor matching was preferred based on the advantage on large number of observation matched, bias avoidance and representativeness of estimators. Therefore, 333 animals were included in the common support region and 6 animals were dropped outside the common support region. Then the difference between simple mean of milk yield for BTB positive and BTB negative animals (Table 5). Which the difference revealed on milk yield of BTB positive animals produces per day 3.83 Liter lower than healthy cow.

In Ethiopia the dairy cow has average life span of 11 years (Kefena *etal*., 2013) and the average milk yield is 10lit/day, 200 milking days/year, age at first calving 4.5 years, average calving interval is 2 years, average lactation period 200 days per year (G/egziabiher, 2010, Kefena *etal*., 2013).With the consideration of the above figures, Ethiopian dairy farmers loss ETB 9,992 annually due to bovine tuberculosis.

Using the two antigens as a test material, the prevalence using PPD was 11.7%, whereas it was 60% for the LAM Ag using urine as a sample in Gondar town dairy farms. The author couldn’t find works using LAM Ag test for animal purpose. The higher prevalence due to LAM Ag using

urine sample could be due to the limitation of LAM Ag to differentiate between various *Mycobacterium species* (Alere, 2013) and the possible cross reactivity of the LAM Ag with non-tuberculous mycobacterial antigen that could increase false positive results, since it encompasses a large family of related molecules, which are expressed by *mycobacterial species* and so cross-reactivity between the assay antibodies and non-tuberculous mycobacterial antigen is possible (Lawn *et al.,* 2013).

Source of the animal (OR= 2.3), sex (OR=0.31) and breed (OR=2.69) were seen as a risk factor for the occurrence of bovine tuberculosis compared to their counter parts and the contribution was significant (P<0.05) in each categories. Mengistu and colleagues reported regarding sources of the animal indicated that those purchased had higher odds ratio (OR= 1.6) than home bred ones (Mengistu *et al.,* 2014). Higher risk (OR=6.6) in cross breeds was recorded in different parts of the country too (Zeru *et al.,* 2014). The present study showed that females are in lower risk than male cattle in the dairy farm.This is in disagreement with findings (Birru *et al* 2014; Zeru *et al.*, 2014). This might be attributed to the fact that male animals might be given less attention than females since the motto of the majority is to produce milk in the dairy farms.

At the herd level risk factor Semi intensive production system has protective factor than intensive production system this result support that BTB is a disease of intensification (Ameni, 2008). The dairy farm owners that have heard about BTB are 12 times more likely to have BTB infection than the respondents who never heard about. But the variation might be due to the higher prevalence of crossbreeds in Gondar town where the dairy farm owners are mostly aware about BTB.

Spatial distribution showed that bovine tuberculosis in Gondar town has dispersed almost through the dairy herds in different kebeles. Dispersed occurrence of BTB has epidemiological importance in the transmission of the disease with the neighboring herds through different ways. The dispersion of BTB positive herds are confined mostly in two kebeles that are semi urban areas following the main road of Gondar zuria. Spatially confined infected herds have low probability to transmit to BTB free dairy herds than the dispersed ones in Gondar town.

Gondar town dairy farmers 28/48 were familiar to BTB, and only 5/61 respondents of Gondar zuria district were familiar to the word BTB. This might be due to having access to information

as well as media, where Gondar dairy farmers have better access than Gondar zuria ones since it is urban. Of the respondents 30.1% (33 /109) knew that BTB is zoonotic disease and 25.7% (28/109) households had consumption of raw milk habits. In Gondar town 10.4% (5/48) respondents consumed raw milk where it is nearly 38% (23/61) for Gondar zuria. The variation is obviously visible and this might be due to information access gap as mentioned earlier.

The limitation of this research paper was small number of household are included in the study and some in consistency on diagnostic tests regarding to districts, e.g LAM using milk is done only for Gondar zuria , this is due to some logistics and resource shortage.LAM Ag rapid test false positives due to cross reaction with *M.paratuberculosis* and *M.avium*. With all this imitation the author agreed.

6. CONCLUSION & RECCOMENDATIONS

The individual animal level prevalence in small holder dairy farms in Gondar town was 8.26% and in Gondar zuria district 7.7%. A herd prevalence of 23.53% and 18.97% in Gondar town and in Gondar zuria district recorded, respectively. The PSM estimates had a difference between BTB positive and BTB negative animals, on milk yield of BTB positive animal loss 3.84 lit per day per cow and the dairy farm owner loses 9,992 birr annually due to BTB infection. The prevalence of Gondar zuria dairy animals using PPD, LAM urine and LAM milk is 8.06%, 44.35% and 78.79%, respectively. The prevalence is higher in LAM lateral flow milk, followed by LAM lateral flow urine and the least by PPD. The kappa test agreement is moderate between PPD and LAM Ag test and slight between PPD and LAM using milk samples. Source of the animal, sex and breed were found to be individual animal level risk factor whereas, Feed type and the dairy farm owner familiarity to BTB found to be herd level risk factors. Gondar town has higher dispersal of BTB infected herds in different kebeles than Gondar zuria. The zoonotic awareness among both district dairy farm owners, Gondar zuria district dairy farm owners has low awareness as well as they consume raw milk higher than Gondar town dairy farmers.

Based on the above conclusion the following recommendations are indicated:

- Crossbreds should have better management since they are more exposed to BTB infection.
- In medium scale dairy farms appropriate stocking of herd size and hygiene should be followed.
- In dairy farms male animals should have better management
- Dairy cattle feed is better to be stored appropriately to avoid contamination
- It is better to cull BTB infected animal with organized compensation plan as it has economical as well as epidemiological impact on the dairy production.
- Introduction of new animal should be tested before mixing to the herd.

- Hygiene and avoidance of contamination should have due emphasis to prevent between herd infection.
- Although the LAM Ag test has less agreement with standard PPD test, it has promising indications to diagnose BTB easily, so further study needed here.
- Awareness creation on zoonotic importance of BTB should be done especially at Gondar zuria district.
- Further study on isolation and spatial distribution of causative agent should be done to see and control the transmission dynamics.

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8. APPENDIX

Questionnaire Survey

1. Owner name --- woreda ---------------------------
   PA -- Village ----------------------------
2. Herd size ----------------------
   A. Bull ------- B. Heifer ---------- C. Cow------------ D. Calf ----------------
3. Breed type?
   A. Local ---- B. Cross ---- C. Exotic------ D. Others (specify) -------
4. Sex
   A. Male --------- B. Female ----------------
5. Age
   A. ≤ 6 months ---------- C. > 1 years ≤ 2 years -------------- E.> 3 years
   B. > 6months ≤ 1 year --------- D. >2 years ≤ 3 years --------------
6. What type of production do you have for the dairy herds?
   A. Intensive C. Open grazing system
   B. Semi intensive D. Other Specify ---------------------------------------
7. Have you construct a house for the dairy herd?
   A. Yes B. No
8. If the answer for Q. no. 7 is yes, what are the materials used?
   A. Wood C. Metal E. Others (Specify) ------------------------------
   B. Grass D. Metal sheet
9. Did the house have good ventilation, i.e window or mesh structure on the bottom of the wall?
   A. Yes B. No
10. What is the material used for the house floor?
    A. Concrete C. Sand E. Others (Specify) -----------------------------
    B. Soil D. stone
11. What material do you used for mulching the floor?

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12. If the answer for Q. No. 7 is “No”, where will be the shelter of the dairy herd during the night?
    A. In the main house   C. In the kitchen   E. Others (Specify) ---------------------------
    B. At the backyard   D. outside the house
13. Per day feeding frequency of the herd?
    A. Once   B. twice   C. Three times   D. Four times E. Others (Specify) --------------
14. Per day watering frequency of the herd?
    B. Once   B. twice   C. Three times   D. Four times E. Others (Specify) --------------

15. Does the herd have a feeding trough?
    A. Yes   B. No
16. If the answer for Q. no. 15 is “yes”, how much frequent to clean the trough?
    A. Once in a day   C. once in a month   E. Others (Specify) ---------------------------
    B. Once in a week   D. once in a year
17. Does the herd have watering trough?
    B. Yes   B. No
18. If the answer for Q. no. 17 is “yes”, how much frequent to clean the trough?
    C. Once in a day   C. once in a month   E. Others (Specify) ---------------------------
    D. Once in a week   D. once in a year
19. Have you heard about Bovine tuberculosis?
    A. Yes   B. No
20. Do you know that Bovine TB is zoonotic?
    A. Yes   B. No

21. If the answer is “yes” for Q. no 20, what do you do as a precaution?

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22. Have you experienced tuberculosis infection in the dairy herd?
    A. Yes   B. No
23. Do you ever have bovine tuberculosis screening test for your dairy herd?
    A. Yes   B. No
24. If the answer for Q. no. 22 is “yes”, do you have noticed any effect on the dairy production?
    A. Yes   B. No
25. If you said “yes” for Q. No. 23, which production loss you noticed?
    A. Milk yield reduction   C. Long calving interval
    B. Infertility   D. Abortion   E. Others specify ---------------------
26. If your answer is “yes” for Q. No. 24, which symptoms are expressed?

A. Coughing C. Localized lesion E. others specify----------------------------
B. Emaciation D. milk yield reduction F. All symptoms were expressed

27. Which age group of the dairy herd is more affected with BTB than others?
    A. $\leq$ 6 months ---------- C. > 1 years $\leq$ 2 years --------------- E.> 3 years

    B. > 6months $\leq$ 1 year --------- D. >2 years $\leq$ 3 years ---------------

28. Which sex type is more affected with BTB?
    A. Male C. Equally affected
    B. Female D. All Other (specify) ---------------------

29. In the dairy herd which breed is more infected with BTB?
    A. Local C. Exotic E. Others specify -------------
    B. Cross D. All breeds are equally infected
30. Once after acquiring BTB disease which breeds is more resistant?
    A. Local C. Exotic E. Others specify -------------
    B. Cross D. All breeds are equally infected
31. Do you have any experience of BTB vaccination?
    A. Yes B. No
32. Is there any family member who was infected with human TB?
    A. Yes B. No
33. If the answer is "yes" for Q. No. 31, is there any animal who was coughing after some time?
    A. Yes B. No
34. Do you have a trend isolation of sick animals?
    A. Yes B. No
35. Do you have a trend taking sick animals to clinic?
    A. Yes B. No
36. What was the response for the treatment?
    A. Good /there was response B. Poor/no response at all
37. If the answer is "No" for Q. no 34, why?
    A. Lack of awareness C. The clinic is far from here
    B. Lack of vet service D. Others Specify -----------------------------
38. What will do if you have purchased new dairy animal?
    A. Isolation from herd for few days C. checking health status by veterinarian
    B. Introducing to the herd immediately D. Other specify --
39. Do the dairy animals have contact with other domestic animals?

A. Yes B. No

40. Do you have dairy farmers association?
    A. Yes B. No
41. Do you sell milk and milk products?
    A. Yes B. No
42. If you say “yes” for Q. No 39, who is your customer?
    A. Local consumers In the village C. others specify -----------------------------------
    B. Café and restaurants in the woreda
43. If the answer is “No” for Q. no. 40, what will you do with milk?
    A. For home consumption C. It will be discarded
    B. For calves D. Others specify -----------------------------------
44. Do you have a habit consumption of raw milk?
    A. Yes B. No
45. Do you have a habit of consumption of yoghurt?
    A. Yes B. No
46. Is there any wildlife contact with the herd?
    A. yes B. No
47. In this kebele, do you have rule that restricts animal’s movement from another Kebele?
    A. Yes B. No