www.ijmedicines.com Volume-1 | Issue-6 | November - 2017

IJAM

RESEARCH PAPER

PREVALENCE AND ASSOCIATED RISK FACTORS OF BOVINE TUBERCULOSIS IN MECHA DISTRICT, NORTHWESTERN AMHARA, ETHIOPIA.

Belachew Workneh	Collage of Veterinary Medicine and Animal Sciences, Department of Veterinary Epidemiology and Public Health. University of Gondar, Ethiopia
Araya Mengistu	Collage of Veterinary Medicine and Animal Sciences, Department of Veterinary Epidemiology and Public Health. University of Gondar, Ethiopia
Seleshe Nigatu	Collage of Veterinary Medicine and Animal Sciences, Department of Veterinary Epidemiology and Public Health. University of Gondar, Ethiopia
Sarah Waibel	Faculty of Veterinary Medicine, The Ohio State University, USA
Wang Shu	COPH -Division of Epidemiology, The Ohio State University, USA
Wondwossen A. Gebreyes	Global Health Programs, College of Veterinary Medicine, The Ohio State University and VPH-Biotec Global Consortium, Columbus, Ohio, USA.

ABSTRACT

Background: Bovine tuberculosis, which is caused by Mycobacterium bovis, is a chronic debilitating disease of animals characterized by the formation of granulomas (tubercles) in tissues and organs.

Aim: This study aimed to estimate the prevalence of Bovine tuberculosis and associated risk factors. Methods: The study used a cross sectional study conducted from November 2016 to June 2017 on dairy farms that are found in Mecha district, northwestern Ethiopia. The study site was selected purposefully and tuberculosis (TB) caudal fold tuberculin test (TCFTT) and TB Lipoarabinomannan (LAM) antigen test (TLAT) were used to investigate the disease at 95% confidence interval. Information from owners was collected using questionnaire, to evaluate the possible potential risk factors. Sample size was determined using single population proportion sample size determination techniques and a total of 385 cattle were tested using TCFTT and 220 using TLAT.

Results: In this study, the animal level prevalence of BTB was 1.6% (6/385) by TCFTT, while it was 5.9% (13/220) by TLAT. One point four percent (3/220) animals were positive for both tests. Similarly, herd level prevalence of 3.75% (6/160), 10% (12/120) and 2.5% (3/120) were recorded by using TCFTT, TLAT and both tests, respectively. Based on the current finding, the Kappa test indicated that the two tests agreement was poor (Kappa < 0.4). Herd size and management of the farm showed a significant association for the occurrence of bovine tuberculosis ($p < 0.05$); whereas, age, body condition, and breed of animals ($P < 0.05$) were significant contributing factors for bovine TB occurrence at the cow level while using TCFTT. Of the total 71 respondents 15(21.1%) knew about bovine tuberculosis (BTB) and 10(14.1%) knew BTB being a zoonotic disease with a history of at least one human TB patient.

Conclusion: In conclusion, the present study revealed an overall low level prevalence of BTB in the dairy cattle and farms (herds) as well as low knowledge's regarding the disease. Although the test indicated a low level of the problem, this should be taken as warranty and requires designing of an acceptable control strategy of disease before reaching its climax and poses great socioeconomic impacts as well as public health concern. Besides, raising community awareness regarding the disease is suggested.

KEY WORDS : Mecha, Bovine tuberculosis, TB caudal fold tuberculin test, TB LAM AG test, prevalence, Risk factor

1. INTRODUCTION

Ethiopia has the largest livestock population being the first in African countries.(1) Livestock and its products play an important role in Ethiopia's economy and social development both at household and national level. Despite the huge number of cattle and their economic importance, the productivity is low due to different constraints among which diseases are the one that can hamper it. Bovine tuberculosis (BTB) is among the major important diseases that causes devastating economic loss through occur in cattle and buffalo from deaths, chronic disease, and trade restrictions as well as pose great public health impacts.(2)

Tuberculosis is a well-known wide spread infectious disease in human beings and animals which is the leading cause of death due to a single infectious agent among adults in the world. *Mycobacterium tuberculosis* is the most common cause of human TB. The vast majority of people carrying dual (HIV and *Mycobacterium bovis)* infections live in developing countries; however, dual HIV and *M. bovis* infection has been reported in industrialized countries too. (3) Bovine tuberculosis (BTB), which is caused by bacilli members of the *Mycobacterium tuberculosis* complex: *M. bovis,* is a debilitating and a chronic bacterial disease of cattle that occasionally affects other species of mammals including humans (zoonosis).(4,5)

The infection to bovine can occur through the colostrums or milk to calves, ingestion of feed contaminated with feces of infected animals, aerosol, contact with each other and wildlife. In calves, lesions involve the mesenteric lymph nodes with possible spread to other organs. In older cattle, infection usually spreads in the respiratory tract. *Mycobacterium* remains viable in the environment or soil for about two years and cause occurrences of disease associated with various responsible risk factors).(6,7) The disease can be diagnosed by using various microbiological tests. Among that tuberculin test has been frequently used for the screening purpose. In this test the purified protein derivative (PPD) of *Mycobacterium* is used for single intradermal (SID) injection and the reaction within 72 hours after injection and a positive reaction constitutes a diffuse swelling or discolorations at the injection site.(4)

Zoonotic tuberculosis is prevalent in animals of many developing countries where surveillance and control activities are often

Article History	Received	Accepted	Published
	03/09/2017	19/10/2017	20/11/2017

***Corresponding author** Araya Mengistu

Collage of Veterinary Medicine and Animal Sciences, Department of Veterinary Epidemiology and Public Health. University of Gondar, Ethiopia araya@uog.edu.et

inadequate or unavailable; Ethiopia is one among these countries where many epidemiologic and public health aspects of the infection remain largely unknown. But, nowadays several studies conducted at different parts of the country and showed that BTB is endemic in Ethiopia.(8,9,10) The disease status in the selected dairy farms of Mecha district was not known and this drives conducting the test in the area with the aim of estimating the prevalence and associated risk factors of bovine tuberculosis situation on smallholder dairy farms in Mecha district.

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in Mecha district, Western Gojjam zone of Amhara National Regional State (ANRS), Northwestern Ethiopia, which is found 525 Km away from North West of Addis Ababa, and 34 km south east of Bahir Dar the capital city of Amhara region. Mecha is bordered by Sekela on the South, by the Agew Awi Zone on the Southwest, by Abay River (it separates Debub Achefer from Semien Achefer) on the west, by Bahir Dar Zuria on the Northeast, and by Yilmana Densa on the East. Merawi is the capital town of Mecha district. Mecha district covers an area of 1,481.64 km^2 with a population density of 197.13 persons per square kilometer and organized in to 40 rural and 4 urban kebeles. It has a total population of 292,080 (147,611 are men and 144,469 women), of whom, 22,677 (7.76%) are urban inhabitants and there were a total of 66,107 households since 2011. The local community's livelihood is largely depended on agriculture employing nearly 100% of labor force. The climatic condition of the district alternate between along summer rain fall (June- September) and winter dry season (December-March) with annual rainfall ranges from 1200-2000 mm. The study area was located at latitude 10° 30°N and longitude 37°29° E with altitude of 1800 to 2500 meter above sea level. Annual mean temperature of the district is between 24-27^{0}C. The land is covered by different vegetation types such as Savanna grassland, forest reverine and bush lands, with major agricultural products seasonally harvested like sorghum, maize, Teff, Wheat, linseed and other legume groups. The total livestock population comprises of 568814 including 192, 556 cattle, 148 971 ovine, 23, 106 equine and 204, 181 poultry.(11) In this district, five specific sites namely Kudmi, Bachema, Enamert, Enashenfalen and Merawi were purposively selected based on accessibility and the presence of sufficient number of cattle population mostly crossbred with a history of frequent supplementation of milk and milk products to the consumer and willingness of the farmers and owners to cooperate.

3.2. Study design and period

The study was conducted using a cross sectional study design from November, 2016 to June, 2017.

3.3. Study population and sampling method

The study populations were cattle originated from Mecha district and its surroundings. The study animals was local and cross breeds kept under zero grazing (intensive) management system that were maintained on stall feeding of roughage and concentrate feed of balanced ration with close supervision and mixed(semi-intensive) management system that were maintained on both stall (cut and carry) feeding and free grazing on permanent communal pastures and grazed with other livestock. Purposive sampling was used to identify those dairy owners who were willing to participate (cooperate) in the study, questionnaire survey and that was willing to keep their cattle for the study period. Thus female dairy cattle's above six month except, late pregnant cows above 8 months gestation period, clinically sick animals with any disease problem and recently calving cows were sampled. The individual sampled animal was recorded with its breed, age group (young: (6 months-3years), adult: (3-7 years) and old: above 7 years), herd size was grouped (small (2-5 animals), medium (6-10 animals) and large (above 10 animals) Grange (2001), management system (intensive and, semi-intensive) and body condition group (poor (score 1 and 2), medium (score 3 and 4) and good (score 5)) based on Nicholson and Butterworth (1986).(12,13)

3.4. Sample size determination

The sample size calculation formula described by Thrusfield, (2007) was used to determine the sample size.(14)

$$N = \frac{Z^2 Pex\,(1-Pex)}{d^2}$$

Where:

N= required sample size, Z= statistic at level of confidence for 95% (Z = 1.96), Pex = expected prevalence and d^2 the desired precision.

For this study 8.9 % (0.089) (Fetene *and* Kebede, 2008) was taken as an expected prevalence and 3% of desired absolute precision were used. Based on this the total sample size was 344 animals. However, 41 additional animals were tested.(15)

3.5. Test methodologies

TB-Caudal Fold Tuberculin Test (TCFTT) and TB LAM AG test (TLAT) were performed to estimate the prevalence of bovine tuberculosis for cattle's and questionnaire survey to the small-holder farmers or Diary farm owners and or their family members were used for the study.

3.5.1. Tuberculosis Caudal Fold Tuberculin Test (TCFTT)

Tuberculosis Caudal Fold Tuberculin Test (TCFTT) was performed to estimate the prevalence of bovine tuberculosis in the study area. The caudal-fold test measures the immune response to the bacteria. For TCFTT 0.1ml of Purified Protein Derivative tuberculin (PPD) was inject at one of the caudal folds of hairless skin about 7 centimeters distal to the base of the tail away from the hairline in the center of the fold intradermal using 1ml TB syringes and the injection area was read 72±6 hours later. The injection site that shows swelling or discoloration had been recorded as a positive reaction. A positive reaction indicates that animal's immune system had recognized the tuberculin protein and had mounted an immune response. Those no reactions were seen recorded as a negative reaction.

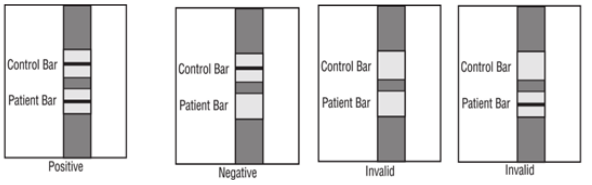
3.5.2. Tuberculosis lipoarabinomannan (LAM) Antigen (AG)-Test Protocol

This protocol was used to determine the sensitivity of the LAM-Test on cattle urine and milk using both, the simple non-modified LAM-Test (total time to diagnosis 25 min) and the 'quick and easy' modified LAM-Test (total time to diagnosis 60 min by adding mannosidase and removing lipids from urine/milk and by adding caseinase/lactase in milk). The detection of lipoarabinomannan (LAM), a major glycolipid component of the cell wall of *M. tuberculosis* and other *mycobacteria*, is a potential approach to diagnosing active TB. Generally Alere determine ™ TB LAM Ag is an invitro, visual read, immunoassay for the detection of lipoarabinomannan (LAM) antigen of *Mycobacteria* in human urine.(16,17)

A test in this study was performed on urine of 220 cattle's which able to voided urine parallel to TB-CFTT with a special attention to positive animals and herds of TB-CFTT following the procedures. The result of the test was read within 25-35 minutes after pouring the urine on the card. Visualization of strip on both patient and control Bar indicates positive, on control Bar indicates negative. But visualization of strip on patent Bar, and absence of strip on both Bars was indicating invalid TB LAM Ag test result (Figure-1). Invalid results were obtained during the study from the test had been corrected by retesting to became either positive or negative.

LAM test Procedure:

1. Remove the protective foil cover from each test.
2. Apply 60 μL of urine sample (precision pipette) to the sample pad (White pad marked by the arrow symbol). A 60 μl MICRO SAFE CAPILLARY TUBE was used.
3. Wait a minimum of 25 minutes and read result. Visualize the strip under standard indoor lighting conditions or in the shade. Do not visualize the strip under direct sun light. Results are stable for up to 35 minutes after sample application. Do not read beyond 35 minutes.[18]



Fg-1 Results interpretation of TB LAM Ag Test

3.5.3. Questionnaire survey

The semi-structured questionnaire was prepared and administered to household's members in Mecha district that was engaged directly or indirectly with dairy cattle's. The owners of dairy cattle were interviewed about their habit of raw milk consumption, management system of their cattle, knowledge of bovine tuberculosis and its zoonosisness and recent history of bovine tuberculosis upon their cattle's or their family members.

3.6. Data collection, management and analysis

TB caudal fold tuberculin test result (after 72±6 hours of injection) and LAM-Test result (within 25-35 minutes) were interpreted and recorded from each individual of dairy cattle during the study. The development of swelling and discoloration or no reactions at injection site was used to determine the Caudal Fold Tuberculin test result as well as Visualization of strip on both patient and control Bar, on patent Bar, and on control Bar or absence of strip on both Bars was also used to determine TB LAM Ag test result. The tests were interpreted with care and the associated risk factors of the individual animal such as body condition, age, breed, were also recorded and documented gently. During the study period, good data management practices (including survey monitoring as well as on site supervision, timely data archiving and transfer, sorting and filing) was implemented.

The prevalence was calculated by dividing the proportion of positive reactors by the total number of tested multiplied by 100% for each tests. That means Individual animal level prevalence was computed as the number of positive reactors out the tested animals per 100 animals tested and the herd level prevalence was calculated as the number of herds with at least one positive reactor animals out the tested herds per 100 herds.

The collected data from the questionnaire survey, caudal fold tuberculin and LAM tests were entered to Microsoft Excel Spreadsheet computer program and Then, coded and were analyzed using Statistical Package for Social Sciences (SPSS) version 20 statistical software. Pearson Chi- square (X^2) test was conducted to assess and determine the significance of the variations of risk factors associated with prevalence of Bovine tuberculosis in the cattle and herds. Kappa test also used to see the agreement between TB caudal fold tuberculin test and TB LAM AG test. During the analysis the household or the herd was considered as positive if one head of cattle among others was give a positive result for the CFT test and/or TB LAM Ag test. In all analysis the degree of risk factor association with the disease occurrence signified by 95% confidence level and p-value < 0.05 was considered statistically significant at 5% significant level.

4. RESULTS

4.1. Herd Level Prevalence of bovine tuberculosis

Out of 160 herds tested a herd level prevalence of bovine tuberculosis was 3.75% (6/160) revealed by TB caudal fold tuberculin test and 120 herds had also tested by TB LAM AG test a herd level prevalence of 10%(12/120) was found in the study area. As a result 2.5% (3/120) prevalence of bovine tuberculosis was obtained from the herds that give positive reactors of both TB caudal fold tuberculin and LAM AG tests with a Kappa test result of 29%.

According to TB caudal fold tuberculin test analysis the differences in prevalence among the different herd size and general management of the herds were statistically significant (P= 0.000) as presented below (Table-1) and TB LAM AG test analysis was also show a significance difference in prevalence of bovine tuberculosis with herd size (P= 0.005) and general management (P= 0.016) of herds (Table-2). Herds size also statistically significant (P= 0.005) for both tests prevalence. However, there was insignificant difference in prevalence with the general management system of dairy farm (P= 0.064) as presented below (Table-3).

Table 1: The association of herd and management factors to the prevalence of BTB using TB caudal folds tuberculin test in Mecah district, Ethiopia, 2017

Variables	Category	No of herds tested	No of Positives	Prevalence (%)	X2	p-value
Herd size	<10 10-20 >20	143 13 4	2 3 1	1.4 23.1 25	20.648	0.0001
management	intensive semi-intensive	28 132	5 1	17.9 0.8	8.713	0.0001

Table 2 : The association of herd and management factors to the prevalence of BTB using TB LAM AG test in Mecha district, Ethiopia, 2017

Variables	Category	No of herds tested	No of positives	Prevalence (%)	X2	p-value
Herd size	<10 10-20 >20	103 13 4	7 3 2	6.8 23.1 50	10.756	0.005
management	intensive semi-intensive	27 93	6 6	22.2 6.5	5.783	0.016

Table 3: The association of herd and management factors to the prevalence of BTB by both tests in Mecha district, Ethiopia, 2017

Variables	Category	No of herds tested	No. of positives	Prevalence (%)	X2	p-value
Herd size	<10 10-20 >20	103 13 4	1 1 1	0.97 7.69 2.5	10.734	0.005
Management	Intensive Semi-intensive	27 93	2 1	7.41 1.08	3.442	0.064

4.2. Individual Animal Level Prevalence of bovine tuberculosis

A total of 385dairy cattle were tested for the presence of bovine tuberculosis by using TB caudal fold tuberculin test. Among those 220 dairy cattle which able to voided urine were also tested by TB LAM AG test. The tests result revealed a bovine tuberculosis prevalence of 1.6% (6/385), 5.9% (13/220) and1.4 %(3/220)for TB caudal fold tuberculin test, TB LAM AG test and both tests, respectively at 95% confidence interval of each prevalence (Table 4-6).

Analysis of host factors showed that the individual level prevalence of BTB using TB caudal fold tuberculin test was varied significantly by age (P=0.008), body condition (P=0.039) and breed (P=0.018). The difference in reactivity to TB LAM AG test to the body condition (P= 0.006) of animal was statically significant. Whereas, age and breed of animals was statically insignificant (P=0.528 and P=0.579), respectively. Breed (P= 0.660) and body condition (P= 0.066) of the animal also has statically insignificant association, but age showed statically significant association (P= 0.012) with positive reactors of both tests.

Table 4 : Association of host factors to positive TB caudal fold tuberculin test reactivity of cattle in Mecha district, Ethiopia, 2017

Variables	Category	No of animals tested	No of positives	Prevalence (%)	X2	p-value
Age (years)	Young adult old	68 211 106	0 1 5	0 0.47 4.71	9.587	0.008

Body condition	Poor Medium Good	132 249 4	5 1 0	3.79 0.40 0	6.512	0.039
Breed	Cross Local	143 242	5 1	3.5 0.41	5.570	0.018

Table 5: Association of host factors to positive TB LAM AG test reactivity of cattle in Mecha district, Ethiopia, 2017

Variables	Category	No of animals tested	No of positives	Prevalence (%)	X2	p-value
Age (years)	Young adult old	47 117 56	2 6 5	4.26 5.13 8.93	1.278	0.528
Body condition	Poor Medium Good	79 137 4	10 3 0	12.66 2.19 0	10.132	0.006
Breed	Cross Local	119 101	8 5	6.72 4.95	0.309	0.579

Table 6: Association of host factors to positive test reaction for both tests in Mecha district, Ethiopia, 2017

Variables	Category	No of animals tested	No of positives	Prevalence (%)	X2	p-value
Age (years)	Young adult old	47 117 56	0 0 3	0 0 5.4	8.907	0.012
Body condition	Poor Medium Good	79 137 4	3 0 0	3.8 0 0	5.428	0.066
Breed	Cross Local	119 101	2 1	1.7 1	0.194	0.660

4.3. Assessment of respondent's knowledge and awareness about bovine tuberculosis and its risk factors through questionnaire survey

A total of 71 households that kept dairy cattle that produce milk and milk products for home consumption or to sell milk and milk products for local market or consumer were interviewed. Among these, 21.1% (15/71) knew BTB can infect cattle and 14.1% (10/71) of the respondent's recognized the zoonotic importance of BTB among these 90 %(9/10) knew consumption of raw milk and respiration as means of transmission from cattle to human beings. From the total interviewed households, about 94.4% (67/71) did not practice house sharing with their cattle for the seek of dignity instead of fear of diseases and 80.3% (57/71) used boiled milk and of which (89.5% (51/57)) boiled for fear of diseases and (10.5 %(6/57)) boiled and consume without justification. Generally the questionnaire survey revealed that owner's awareness and knowledge about bovine tuberculosis, its zoonotic importance and mode of transmission in the study area still not that much appreciable (Table-7).

Table 7: Assessment of owner's knowledge and awareness about BTB and its zoonotic importance as well as Transmissions

Respondent's knowledge	No of interviewed	No (%) of respondents
Having House sharing	71	4(5.6%)
Know BTB in cattle		15(21.1%)
Know BTB is zoonotic		10(14.1%)
Use boiled milk		57(80.3%)
Eat raw meat		53(74.6%)
History of TB patient in their family		12(16.9%)
Use unboiled (raw) milk		9(12.7%)
Know milk and air droplet transmission of BTB	10	9(90%)
Fear of diseases as a Reason to boil milk	57	51(89.5%)
Know type of TB	12	11(91.7%)
Use modern treatments	12	11(91.7%)

5. DISCUSSION

In the present study an attempt was made to estimate the prevalence of bovine tuberculosis, assess risk factors associated with prevalence of bovine tuberculosis and to create awareness about bovine tuberculosis as well as the potential risk factor for the transmission of infections from animal to human and vice versa in Mecha district.

The herd level prevalence of 3.75% and 2.5% recorded using TB caudal fold tuberculin tests and 2.5% recorded using both tests of positive reactor herds (TB caudal fold tuberculin and LAM AG tests) in the present study were nearly comparable with a recent report of 5.21 %.(18) But lower than herd prevalence of 10% and 11.4% recorded respectively by Marshet, (2014) and Akililu *et al.*, (2014) and also much lower than previous studies done (Gumi *et al.*, 2011; Tigre *et al.*, 2011; Firdessa *et al.*, 2012; Mamo *et al.*, 2013; Romha *et al.*, 2014; Zeru *et al.*, 2014; Sintayehu *et al.*, 2016) were they reported 41.9%, 51.4%, 50%, 44%, 15.3%, 20% and 46%, respectively.(19,20,21,22,23,24,25,26,27) The variations of herd prevalence reported among the different studies including this study might be attributed due to the differences in the available different epidemiological conditions that could play a role in the transmission of BTB, such as herd size, the level of intensive management system practiced, the amount of susceptible breeds available in the herd, mobility and close contact between different susceptible animal species in the study area.

There was statistically significant association of dairy cattle management system and herd size with herd positivity in the present study meant that dairy cattle kept intensively showed higher prevalence of bovine TB than kept under semi-intensive and as herd size increased, there was a corresponding increase in the prevalence of bovine TB. This is in line with the findings of recent studies (Romha *et al.*, 2014; Zeru *et al.*, 2014, Ataklti, 2015) who reports significant association between both herd size and management system with herd positivity.(25,26,28) Intensive farming system supported by increased herd size promotes close contact between animals, intensification, stress and overcrowding of animals, which favors spread of infection from infected animal to other healthy animals within a herd, making the prevalence of infection greater in intensive farming and larger herds. This is because; bovine TB is a disease of overcrowding. Thus, when the number of animals in a herd increases, aerosol transmission of the disease is increased. (29) But diverting from the above reality the association of management for common positive reactor herds of both tests was statistically insignificant (Table-3).

In the present study, the prevalence of bovine tuberculosis at animal levels was 1.6% and 1.4% by TB caudal fold tuberculin test and both tests of positive reactor animals (TB caudal fold tuberculin and LAM AG tests), respectively. This finding was in agreement with the pervious findings of Tschopp *et al.*, 2010 (0.9%), Gumi *et al.*, 2012 (2.0%), Tamiru *et al.*, 2013 (1.0%), Admasu *et al.*, 2014 (1.56%) Marshet, 2014 (3%) and Anwar *et al.*, 2015 (1.27%) Whereas, Contrary to the findings of Ameni and Erkihun, 2007, Elias *et al.*, 2008, Firdessa *et al.*, 2012,Mamo *et al.*, 2013,Romha *et al.*, 2014, Zeru *et al.*, 2014 and Ataklti, 2015 who recorded animal level prevalence of 11.0%, 23.7%, , 30.0%, 11.0%, 4.3% 11.3% and 11.72%, respectively in Ethiopia and 14.3% (Thakur *et al.*, 2010) and 5.9% (Mondal *et al.*, 2014) India, and Bangladesh, respectively; the current finding was lower. Even it is lower from findings made by Gumi *et al.*, 2011, Romha *et al.*,2013, Tschopp *et al.*, 2011, Mohammed *et al.*,2012 and Sintayehu *et al.*, 2016 who reported lower animal level prevalence of 5.5%, 6.6%, 6.8%,7.1% and 5.5%, respectively.(18, 19, 20, 23, 24,25,26, 27,28,30,31,32,33,34,35,36,37,38,39,40) The variation observed among the studies might be attributed to differences in management system, feeding habit, numbers of cattle (sample size), types of cattle breeds largely involved in the study, or differences in ecological zones. Moreover, the management system in the majority of our study animals was semi-intensive composed of largely local breed and relatively smaller herd sizes where no positive cases was recorded in most of them. Some previous studies were conducted on larger herds were composed of largely pure Holstein Friesian and cross breed dairy cows only kept under intensive management system.[23,35] This might also indicate the low prevalence recorded in the present study compared to studies, which reported high BTB prevalence.

Similar to the studies of Admasu *et al.,* (2014), Romha *et al.,* (2014), Zeru *et al.,* (2014) and Anwar *et al*., (2015) there was statistically significant association between breed type with BTB prevalence as showed by TB caudal fold tuberculin test result in the present study and this is strengthened by the fact that the prevalence of BTB is influenced by the breed of cattle (Cosivi *et al.,* 1998) and may be associated to the relative resistance of the zebu cattle to BTB as compared to Holstein Friesian and other cross breeds.(18,25,26,33,41,42)

Body condition analyses in this study have indicated the presence of significant association between body condition and positivity using TB caudal fold tuberculin test. Most of the Cattle's were positive for this study had poor body condition, this indicates poor body condition was associated with the increased risk of bovine tuberculosis positivity as compared to medium and good body conditioned cattle. This result is in consistent with the previous studies.(18,19,26,27, 33) This may be due to the reality animals with good body condition score have relatively good immunological response to the infectious agent than animals with medium body condition score and Poor body conditioned animals have relatively weak immunological responses to BTB and subsequently susceptible to the infection. So, bovine tuberculosis is more common and serious in poor body conditioned animals compared to their counter parts. Poor nutrition predisposes to tubercular infection and higher TB prevalence expectation in animals with poor body conditions; since tuberculosis positive animals develop a poor body condition score as a result of being infected, with *M. bovis*.(43,44)

Analysis of age in relation to bovine TB prevalence of cattle using TB caudal fold tuberculin test in the present study was found statistically significant. This is in agreement with previous studies like Ameni and Erkihun, (2007); Elias, *et al., (*2008); Thakur *et al., (*2010); Gumi *et al., (*2011); Mamo *et al., (*2013); Mareshet *et al*., (2014) and Sintayehu *et al*., (2016); reported age was significantly associated with positivity.(19,21,24,27,34,35,36) This is because; a young animal might be exposed to the pathogen, but express the disease in adult age in addition to longer duration of exposure time than an inherent age predisposition.(45,46)

The prevalence of bovine tuberculosis 10% and 5.9% of the current study at herd and animal level respectively were recorded using TB LAM AG test was impossible to compare with other pervious findings done on bovine TB. The reason behind is the other pervious findings were done using tuberculin skin tests of Purified protein Derivative (PPD) rather than TB LAM AG test of urine. But in order to compute the prevalence of the disease with other pervious findings and the above reported herd level prevalence of this study had equal herd prevalence with Marshet, (2014) and in consistent to the findings of Akililu *et al*., (2014) who reported herd level prevalence of 11.4% using comparative intradermal tuberculin test and higher than TB caudal fold tuberculin test and common positive herds of both tests in this study.(19,20) The disparity of prevalence between the two test of the current study might be due to poor agreement (Kappa= 0.29) between TB caudal fold tuberculin test and TB LAM AG test.

Animal level prevalence of TB LAM AG test was also in agreement with the findings of Gumi *et al.,* 2011, Tschopp *et al.,* 2011, Mondal *et al.,* 2014 and Sintayehu *et al*., 2016 who reported animal level prevalence of 5.5%, 6.8%, 5.9% and 5.5% respectively. (21,27, 37, 39) But lower than the previously reported prevalence based on tuberculin skin tests Zeru *et al*., (2014) and Atakltì, (2015) and higher than the prevalence reported by this study based on TB caudal fold tuberculin test as well as common positives of both tests.(26,28)

Analysis of breed showed that there were insignificantly associated with positive reactors of TB LAM AG test and both tests of the present study inconsistent to Marshet, (2014) who Report insignificant of breed with the positivity of tuberculin skin test and positivity LAM test in cross breed cattle is higher than that of local breed cattle as mentioned above(Table-5) and this may be due to the involvement of low blood level maximum up to 75% cross breeds with poor nutrition as a predisposing factor in this study supported by Marshet, (2014) his study insignificant association of breed due to absence of pure Holstein Friesian in his study.(19)

Age analysis also showed statistically insignificant association with positivity of cattle by TB LAM AG test, which is In agreement with some previous studies of Admasu *et al., (*2014); Romha *et al.,* 2014 and Anwar *et al*., (2015) who reports insignificant association of age.(18,25,33) The agreement of the finding of Anwar *et al* (2015) and the current finding might be due close proximity of the study area agro-ecological zone, feeding and production system. Body condition of the animal had significant association to positivity of TB LAM AG test, But there was insignificant association between body condition and common positivity of both TB caudal fold tuberculin and LAM AG tests in this study which agrees with the findings of Asseged *et al*. (2001); Firdessa*et al.,* (2012) and Romha *et al.,* (2014) although had higher prevalence at poor body conditioned animals compared to medium and good body condition.(18,23,25,47)

The current finding was also tried to see the agreement of TB caudal fold tuberculin test and TB LAM AG test using kappa test and it was found with poor agreement (Kappa < 0.4) both at animal and herd level.

In the present study, the result of the questionnaire survey analysis showed that 21.1% of the respondents' were aware of BTB with 14.1% knowledge about zoonosis of the disease. This indicates the respondent's knowledge about BTB and its zoonotic importance were low. Mode of transmissions was recognized by below 50%. This result agrees with the previous report (Romha *et al.,* 2014; Zeru *et al.,* 2014; Anwar *et al*.,2015; Jemberu **,** 2015), who indicated 29.7% , 30.8%, 25% and 22% level of awareness of BTB with low level knowledge about zoonosis of the disease in their study site, respectively.(18,25,26,48) The questionnaire Survey result also indicated that 16.9% (12/71) of the respondents had a history of at least one TB patient in their family members and most of them also treated by modern drugs 91.7% (11/12). But none of the respondents in this study were found to be aware about the reverse transmission of the disease from human to cattle. Regarding meat consumption habit 74.6% respondents have a habit of consumption of raw meat and unaware about transmission of the disease through untreated meat which is strengthened by marshet (2014) and Jemberu **, (**2015) reported with little knowledge (1.7%) and (17%) about M. *bovis* transmission through meat.[19,48] This is inline The zoonotic risk of BTB is often associated with consumption of untreated meat in addition to consumption of untreated milk and via aerosol in the proximity to livestock based on Cosivi *et al.,* 1998 and Wilkins *et al.,* 2008.(40,49) Lack of understanding regarding the zoonotic of BTB, raw animal product consumption habit and poor sanitary measures are the potential risk of BTB to public health. According to Ashford *et al., (*2001) and Tigre *et al., (*2011) explanation 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.(22,50)

6. CONCLUSION AND RECOMMENDATIONS

This study using TB caudal fold tuberculin and TB LAM AG tests has showed low level prevalence of bovine tuberculosis both at herd and individual animal level in smallholder dairy cattle in Mecha district. This study also showed that BTB prevalence increased with increasing age of cattle and with decreasing body condition at individual animal level. Similarly, being cross breed was identified as animal level risk factors for being positive. Herd size and management systems are also an important risk factor contributing to the prevalence of BTB in cattle. The results of present study revealed that the respondents had low knowledge and awareness about BTB and its zoonotic importance as well as mode of transmissions with consumption of raw milk and meat habit, which highlights the high risks of the public. Generally, the present study signifies the economic and the possibility of public health importance of the disease and urges launching of some control strategies of disease before reaches its climax and pose great socioeconomic impacts. Therefore, in view of socioeconomic aspects the disease in this country, use of cattle, milk and meat, consumption habit of the community and concerning dairy and its products the following recommendations have been forwarded:

- Further study on bovine TB both in animals and humans should be conducted with priorities to the epidemiological patterns and zoonotic importance to elucidate the role of *M. bovis* in human and animal populations
- Further studies on bovine TB of cattle should be conducted to investigate the sensitivity and specificity of TB LAM AG test.
- Awareness of the community should be created through Public education about the potential risk of consumption of raw animal products by public health office and the veterinarian of the district jointly.
- Milk pasteurization plant should be established and Boiling of milk and cooking of meat before consumption should be practiced by the society to secure milk origin bovine tuberculosis.
- As justified by many studies due to financial constraints test and slaughter policy is impractical in developing countries like Ethiopia therefore, test-and-segregation and isolation of calves immediately after birth should be practiced as a control option.
- Better management practices such as avoiding overcrowding, stress and suffocation should be implemented
- Strong inter-sectoral collaboration between public health and veterinary professions should be established to assess and evaluate the socioeconomic impacts of the disease at national level and to put appropriate decisions.

7. REFERENCES

1. Central Statistics Agency (CSA), (2013): Report on livestock and livestock characteristics (private peasant holdings) CSA Agricultural Sample Survey of 2012/13 Addis Ababa, Ethiopia.
2. Ayele, W., Neill, S., Zinsstag, J., Weiss, M. and Pavlik, I., (2004): Bovine tuberculosis, an old disease but new threat to Africa. Int. J. Tuberc. Lung Dis.; 8(8): 924-937.
3. Grange, J.M., Daborn, C. and Cosivi, O., (1994): HIV-related tuberculosis due to Mycobacterium bovis. EurRespir J; 7: 1564-1566.
4. Radostits, O.M., Gay C.C., Blood D.C., and Hinchelift K.W., (2000): Disease caused by bacteria – Mycobacterium. In: Veterinary Medicine: A Text Book of Disease of Cattle, Sheep, Pig, Goat and Horses. 9thed. Harcourt Publisher Ltd., London; Pp. 909–918.
5. Thoen C.O., Steele J.H., Gilsdorf M.J. (2006): Mycobacterium bovisInfection in Animals and Humans. 2nded. Blackwell Publishing Professional, Ames, Iowa,USA.; Pp. 317.
6. Elizabeth, L., Marston, G., Churchyard and Cock, D., (2006): Tuberculosis in sub-Saharan Africa: opportunities, challenges and change in the era of antiretroviral treatment. Lancet, 367: 926–37.
7. Goldman, E. and L.H., dan Green, (2009):Practical Handbook of Microbiology, 2th Ed., Taylor and Francis Group, Boca Raton, London, New York, Pp: 245.
8. Ethiopian Journal of Health Development (EJHD), (2008): Special Issue on Bovine Tuberculosis in Ethiopia Volume 22: 97-145 ISSN 1021-6790
9. Yousefi, F., Sankian, Z., Moghadam, A., Farshadzadeh, R., Gholoubi, M., Zarif, K., Naderinasab and Varasteh, G., (2009): Cloning, expression and purification of 10 KD culture filtrated protein (CFP-10) of Mycobacterium. Afr. J. Bio. technol.; 8: 3916-3920.
10. Armauer Hansen Research Institute (AHRI), (2014/15): annual report; Pp 3-7.
11. Central Statistics Agency (CSA), (2007): Population and Housing Census: Population Size by Age and Sex: CSA Summary and Statistical Report of 2007 Addis Ababa, Ethiopia.
12. Grange J.M., (2001): Mycobacterium bovis infection in human beings. Tuberculosis, 81(1-2): 71-7.
13. Nicholson, M.J. and Butterworth, M.A. (1986): A guide to condition scoring zebu cattle, International livestock center for Africa (ILCA). Addis Ababa, Ethiopia, Pp. 72-74.
14. Thrusfield M. (2007): Veterinary Epidemiology. 2nd ed. London: Blackwell Science Ltd. Pp. 178-198.
15. Fetene, T. and Kebede, N., (2008): Bovine tuberculosis of cattle in three districtsOf northwestern Ethiopia, Trop Anim Health Prod, (2009) 41: 273–277
16. Peter, J., Green, C., Hoelscher, M., et al., (2010): Urine for the diagnosis of tuberculosis: current approaches, clinical applicability, and new developments. CurrOpinPulm Med. May; 16 (3):262-70.
17. Alere, (2013): Determine TM TB LAM Ag Product Information Sheet.
18. Anwar, N., Mamo, G., Teshome, L., Zewdie, A., Medhin, G., Piepe, R. and Ameni, G., (2015): Bovine tuberculosis and its risk factors among dairy cattle herds in and around Bahir Dar City, Northwest Ethiopia. Ethiopian Veterinary Journal; 19 (2): 27-40.
19. Marshet, B., (2014): prevalence of bovine tuberculosis in and around ambagiorgies town smallholder dairy farms and assessment of farm owner's awareness about bovine tuberculosis, north Gondar; Ethiopia, University of Gondar faculty of Veterinary Medicine, (DVM Thesis); Pp. 20-23.
20. Akililu, B., Gobena, A., Teshale, S., Faanta, D., Akafate T. and Ketema, T., (2014): Epidemiology and public health significance of Bovine tuberculosis in and around Sululta District central Ethiopia. African J. of Micro. Res. Vol.; 8(24): 2352-2358.
21. Gumi, B., Schelling, E., Firdessa, R., Aseffa. A., Tschopp, R., et al., (2011): Prevalence of bovine tuberculosis in pastoral cattle herds in the Oromia region, southern Ethiopia. Tropical Animal Health and Production;43: 1081–1087.
22. Tigre, W., Alemayehu, G., Abatu, T. and Deressa, B., (2011): Preliminary study on publichealth implication of bovine tuberculosis in Jimma town, South West Ethiopia. Global Vet, 6 (4): 369-373.
23. Firdessa, R., Tschopp, R., Wubete, A., Sombo, M., Elena, H., Girume, E., Teklu, K., Lawrence, Y., Martin, V., R. Glyn, H., Douglas Y., Stephen, V. G., Mesfin, S., Abraham, A. and Stefan, B., (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.
24. Mamo, G., Abebe, F., Worku, Y., Hussein, N., Legesse, M., et al., (2013): Bovine tuberculosis and its associated risk factors in pastoral and agro-pastoralcattle herds of Afar Region, Northeast Ethiopia. J. Vet. Med. Anim. Health, 5(6):171-179.
25. Romha, G., Gebreegziabher, G. and Ameni, G., (2014): Assessment of bovine tuberculosis and its risk factors in cattle and humans, at and around Dilla town, southern Ethiopia. Anim. Vet. Sci., 2(4):94-100.
26. Zeru, F., Romha, G., Berhe, G., Mamo, G., Sisay, T. and Ameni, G., (2014): Prevalence of bovine tuberculosis and assessment of Cattle owners' awareness onits public health implication in and around Mekelle, Northern Ethiopia. J. Vet.Med. Anim. Health, 6 (6):159-167.
27. Sintayehu, W. Dejene, Ignas M. A. Heitkonig, Herbert H. T. Prins, Fitsum A. Lemma, Daniel A. Mekonnen, Zelalem E. Alemu, Tessema Z. Kelkay and Willem F. de Boer, (2016): Risk Factors for Bovine Tuberculosis (bTB) in Cattle in Ethiopia. Eco-Epidemiology of Bovine Tuberculosis, Pp. 8-10.
28. Ataklti, H., (2015): Assessment of bovine tuberculosis in dairy farms and its public health importance in and around adigrat district; Mekelle, Ethiopia. (DVM Thesis); Pp 21-25.

29. Radostits, D.M., Blood, D.C. and Gay, C.C., (2006): Veterinary Medicine. A text book of Disease of Cattle, sheep, Pigs, goats and horses, 8th ed. Bailliere Tindal, London; Pp. 748-785.
30. Tschopp, R., Schelling, E., Hattendorf, J., Aseffa, A. and Zinsstag, J., (2010): Risk factors of bovine tuberculosis in cattle in rural livestock production systems of Ethiopia. Prev. Vet Med, 89(3-4):205-211.
31. Gumi, B., Schelling, E., Firdessa, R., Erenso, G., Biffa, D., et al., (2012): Low prevalence of bovine tuberculosis in Somali pastoral livestock, southeast Ethiopia.Trap Anim Health Prod, 44(7): 1445-1450.
32. Tamiru, F., Hailemariam, M. and Terfa, W., (2013): Preliminary study on prevalenceof bovine tuberculosis in cattle owned by tuberculosis positive and negativefarmers and assessment of zoonotic awareness in Ambo and Toke Kutaye districts,Ethiopia. J. Vet. Med. Anim. Health, 5(10):288-295.
33. Admasu, P., Berihun, W. and Niguse, A., (2014): Prevalence of bovine tuberculosis in dairy cattle of Yeki District, Southern Ethiopia. African J. Basic and Appl. Sci; 6 (5): 135-140.
34. Ameni, G. and Erkihun, A., (2007): Bovine tuberculosis on small-scale dairy farms inAdama Town, central Ethiopia, and farmer awareness of the disease. Rev. sci. tech.Off. int. Epiz.; 26(3): 711-719.
35. 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(3): 915-923.
36. Thakur, A., Sharma, M., Katoch, V., Dhar, P. and Katoch, R., (2010): A study on the prevalence of Bovine Tuberculosis in farmed dairy cattle in Himachal Pradesh. Vet World, 3(9): 409-414.
37. Mondal, M. A. H., Parvin, M. S., Sarker, S. C., Rahman, A. K. M. A. and Islam, M. T., (2014): Prevalence and risk factors of bovine tuberculosis in cattle in Mymensingh sadar. Bangl. J. Vet. Med, 12(2): 179-183.
38. Romha, G., G. Ameni, G. Berhe and G. Mamo, (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.
39. Tschopp, R., Bobosha, K., Aseffa, A., Schelling, E., Habtamu, M., et al., (2011): Bovine tuberculosis at a cattle-small ruminant-human interface in Meskan, Gurage region, Central Ethiopia. BMC Infect Dis, 11: 318.
40. Mohammed, N., Hailu, M. and Gebreyesus, M., (2012): Prevalence and zoonotic implications of bovine tuberculosis in Northwest Ethiopia. College of Agriculture and Environmental Science, Bahir Dar University, Bahirdar, Ethiopia, International Journal of Medicine and Medical Sciences; 2:188-192.
41. Cosivi, o., Grange, J., Daborn, C., Raviglione, M., Fujikura, T., Cousins, D., Robinson, R., Huchzermeyer, H., de Kantor, I. and Meslin, F., (1998): Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. Emerg. Infect. Dis, 4(1): 59-70.
42. Ameni, G. and Erkihun, A., (2007): Bovine tuberculosis on small-scale dairy farms in Adama Town, central Ethiopia, and farmer awareness of the disease. Rev. sci. tech. Off. int. Epiz. 26(3): 711-719.
43. Cadmus, S., Onoja, I. and Salisu, I., (2010): Risk factors associatedwith bovine tuberculosis in some selected herds in Nigeria. Trap AnimHlth Prod; 42: 547-549.
44. Radostits, O.M., Gay, C.C., Blood, D.C., Hinchelift, K.W., (2007): Veterinary Medicine: A Text Book of Disease of Cattle, Sheep, Pig, Goat and Horses. 10th ed. London, UK: Saunders ELSEVIER; Pp. 909-918.
45. Radostits S.M., Blood D.C. and Gray C.C., (1994): Veterinary Medicine: A Text book of Disease of Cattle, Sheep, Pigs, Goats and Horse.8thed. Bailliere Tindal. London; Pp. 748-785.
46. Griffin, J., Martin, S., Thorburn, M., Evens, J. and Hammond, R., (1996): A case-control study on the association of selected risk factors with the occurrence of bovine tuberculosis in the republic of Ireland. Preventive veterinary medicine; 27: 80-90.
47. Asseged, B., Lubke-Beker, A., Lemma, E., Kiros, T. and Britton, S., (2001): Bovine tuberculosis: A cross sectional and epidemiological study in and around Addis Ababa. Bulletin of Animal Health and Production in Africa; 48: 71–80.
48. Jemberu, A., (2015): molecular epidemiology of bovine tuberculosis in cattle and its public health implications in gambella town and its surroundings, gambella regional state, Ethiopia. AAU College of Veterinary Medicine and Agriculture, Bishoftu (MSc thesis); Pp. 34
49. Wilkins M., Meyerson J., Bartlett P., Spieldenner S., Berry D. et al., (2008): Human Mycobacterium bovis Infection and Bovine Tuberculosis Outbreak, Michigan, 1994–2007. Emerging Infectious Diseases 14 (4): 657-660.
50. Ashford D., Whitney E., Raghunathan P. and Cosivi O., (2001): Epidemiology of selected mycobacteria that infect humans and other animals. Review of Science and Technology, Office International des Epizooties, 20, 325–337.