Epidemiology Of Bovine Tuberculosis And Its Public Health Significance In Debre- Zeit Intensive Dairy Farms, Ethiopia

Meseret Bekele[(1)], Gezahegn Mamo[(2)], Samueal Mulat[(3)], Gobena Ameni[(4)], Gashaw Beyene[(5)], Etsegenet Tekeba[(5)]

[1]Department of Veterinary Public Health, Ministry of livestock and Fishery, P.O. Box. 1084. Addis Ababa Ethiopia,
[2]Department of Veterinary Public Health, Addis Ababa University, Debre Ziet
[3]USAID AGP-LMD (CNFA) Regional Program Officer (Oromia), P.O. Box 81155 E- Addis Ababa, Ethiopia
[4]Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia
[5]Department of Veterinary Epidemiology, Ministry of livestock and Fishery, P.O.B. 1084. Addis Ababa, Ethiopia,
Yohanan14sm@gmail.com

Abstract: A cross-sectional study was conducted to assess the epidemiology of bovine tuberculosis (BTB) and its public health significance in 10 intensive dairy farms, owning 558 head of cattle in Debre Zeit. On the basis of the CIDT test, the overall animal level prevalence was 17.02% (95% CI: 16.99%- 17.05%). A statistically significant variation in prevalence was observed across age group (χ^2 =19.9; P= 0.001); lactation (χ^2 =13.42; P= 0.001) and parity class (χ^2 =12.80; P= 0.012). The herd prevalence was significantly associated with herd management (χ^2 = 32.19; P = 0.000) and air circulation (χ^2 = 31.94; P = 0.000). Out of the 25 tuberculin skin tested dairy farmer workers 16 were skin reactors (>10mm induartion) with a prevalence of latent tuberculosis of 64% (16/25) was recorded. The prevalence was significantly different across their habit of raw animal product consumption (χ^2 = 10.43; P = 0.001) and their knowledge of tuberculosis transmission (χ^2 = 5.74; P = 0.017). With regard to their knowledge of bovine and its public health risk of transmission from cattle to human 64% (16/25) of the respondents do not know about bovine TB and its risk of transmission, while only 36% (9/25) were aware of the disease and its zoonotic transmission. Mycobacteriological culture of milk and nasal swab from strong tuberculin reactor animals showed 4% (1/22) growth in the culture and direct m-PCR result showed signal positive for 7 samples (3milk and 4nasal swab) out of 20 samples have signal for genus Mycobacterium. In conclusion, this study had demonstrated the high prevalence of BTB in the expanding dairy farms of Deber Zeit and hence it warrants a need for action towards the control of bovine tuberculosis in the study area.
[Meseret Bekele, Gezahegn Mamo, Samueal Mulat, Gobena Ameni, Gashaw Beyene, Etsegenet Tekeba. **Epidemiology Of Bovine Tuberculosis And Its Public Health Significance In Debre- Zeit Intensive Dairy Farms, Ethiopia.** *Biomedicine and Nursing* 2016;2(2): 8-18]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 3. doi:10.7537/marsbnj02021603

Keywords: *Bovine tuberculosis, CIDT, Debre Zeit, Epidemiology, Mycobacterium bovis, Public health.*

1. Introduction

Tuberculosis has been a major health risk to human and animals for more than a century. It is widely distributed throughout the world affecting many vertebrate animals of all age groups of humans and animals. (Thoen *et al*., 2006).

Bovine tuberculosis (BTB) is a chronic infectious and contagious zoonotic disease of cattle, other domestic animals, certain wild life populations, and humans (OIE, 2009). The disease represents a major threat to wildlife because it spreads rapidly, affecting a wide variety of animal species and likely to create a permanent reservoir of infection (Michel *et al*., 2008). Bovine tuberculosis is caused by *Mycobacterium bovis*, a slowly growing nonphotochromogenic Gram-positive acid-fast bacilli and a closely related group of *Mycobacteria* members of the *Mycobacterium tuberculosis* complex (MTBC). (Mostowy *et al*., 2005).

Mycobacterium bovis, the most universal pathogen among *Mycobacteria*, is a chronic contagious respiratory disease of cattle spreading within and between animal species mainly by aerosol and ingestion. Even though, eradication programs in developed countries have reduced or eliminated TB in cattle and human disease and results rare occurrence, the disease still prevails worldwide, particularly in Africa (Ayele *et al*., 2004; Zinsstag *et al*., 2006a).

Human tuberculosis (HTB) of animal origin, particularly *M. bovis* is becoming increasingly important in developing countries, as humans and animals are sharing the same micro-environment, dwelling premises and waterholes (mostly during draught and dry season) especially in rural areas . Humans primarily acquire BTB through consumption of raw or undercooked milk and other products obtained from infected cattle and/or occasionally by respiratory route. In a country like Ethiopia, due to the aggravating factors of cultural habit in raw meat and

milk consumption, hardly practice of milk pasteurization, high BTB prevalence of the cattle population and absence of effective control measures makes BTB the most important zoonotic disease (WHO, 1993; Etter *et al.*, 2006).

Currently, in Debre-Zeit town, due to its proximity to the capital city Addis Ababa, accompanying the expansion of the town with increasing population and its opportunity of being destination areas for national and international tourists the demand for dairy products is increasing. Following the demand, intensive dairy farming using Holstein cattle, or Holsteins cross-bred with zebu, is becoming a common practice. As a result, understanding the epidemiology of diseases of intensification with public health significance like BTB in the intensive dairy farms contributes a lot in reducing the economic impact of the diseases on dairy farms and most importantly in safeguarding the public from risk of acquiring zoonotic diseases that arise from consumption of raw animal product and close physical contact. the present work was undertaken with the following objectives to determine the prevalence of BTB in large scale intensive dairy farms in the Debre Zeit., to identify the causative agent and the risk factors associated to bovine tuberculosis, to assess the extent of human latent tuberculosis on selected dairy farm worker using human tuberculin skin test and to investigate the potential risk factors for transmission of zoonotic tuberculosis in dairy farms

2. Materials And Methods

2.1. Study area

The study was done in Debre Zeit town which is located 47 Km East of Addis Ababa. Debre Zeit is found in the East Shewa Zone of the Oromia Regional State, in central Ethiopia at latitude and longitude of 8°45′N 38°59′E with an elevation of 1,920 meters (6,300ft) above sea level (Figure 4.). The total area of Debre Zeit is about 14,000 hectare of land (140 km^2). The average annual temperature range is 14 ^{0}C to 26 ^{0}C with relative humidity of 61.3%. There were 146,312 cattle, 23,885 sheep, 54,994 goats, 2,008 horses, 2561 mules, 25,410 donkeys and 24,045 poultry in Ada'a Liben. Based on figures from the Central Statistical Agency (CSA, 2007), the national census reported that a total human population of Debre Zeit is estimated to be 73,372 of whom 35,058 were men and 38,314 were women (CSA, 2007).

The dairy farms were selected based on random sampling and the dairy workers are selected based on their willingness. All laboratory work was done at the Aklilu Lemma Institute of Pathobiology-Addis Ababa University.

2.2. The study design

The study design used was cross-sectional study design. This study was conducted from November 2012 to May 2013 using comparative intradermal tuberculin test on 558 cattle, and human intradermal tuberculin test (PPD test) on 31 volunteer. Collection of milk and nasal swab samples for mycobacteriology culture were taken from strong tuberculin test reactor animals. A questionnaire survey was carried out on dairy farm owners and workers to assess the associated risk factors on epidemiology of BTB and its zoonotic significance in the study farm.

2.3. The study subject and sample size determination

For comparative intradermal tuberculin test in animal one stage clustered sampling method was used to sample the study herds and in selected farms all female animals above 6 months of age with exclusion of pregnant animals above 8 months of gestation period were sampled. In this study, out of the total 19 total large scale intensive dairy farms with a herd size greater than 20 dairy cows, above 50% of the farms were included. Accordingly, 10 large scale dairy farms were selected randomly for the study.

The number of large scale intensive dairy farm are selected according to 5% absolute precision at 95% confidence interval (CI) is going to be used during determination of the sample size . The between cluster variance to be 0.01 (i.e. since between cluster variance is not available, the variance of different studies conducted in Ethiopia was taken). The previous work in the study area was 65.8 % by Ameni *et al.* (2003a). Thus, giving 10 clusters were sampled randomly and all animals in each selected farms which fulfill the inclusion criteria were tested (n=558).

$$g = \frac{1.96^2\{nVc + P\,ex\,p(1 - Pexp)\}}{n(d^2)}$$

Sample size was determined using one stage cluster sampling formula (Thrusfield, 2005)
g= 21.49
Where, g = number of clusters to be sampled;
n = predicted average number of animals per cluster (56);
P exp = expected prevalence (65.8%);
d = desired absolute precision (5%);

Vc = between-cluster variance (0.01)

Since the population of clusters from which the sample to be selected was small, the clusters were adjusted based on the following formula (Thrusfield, 2005).

$$gadj = \frac{Gx\ g}{G+\ g} = 10.08=10$$

Where, G is the total number of clusters in the population (19clusters).
Hence 10 farms were selected based on the result of the calculation.

2.4. Study Methodology

2.4.1. Comparative intradermal tuberculin test in animal

The comparative intradermal tuberculin test (CIDT) was applied in all cattle using both avian and bovine purified protein derivates (PPD) supplied by Aklilu Lemma Institute of Pathobiology-Addis Ababa University. Intradermal injections of 0.1 ml (2,500 IU/ml) bovine PPD and 0.1 ml (2,500 IU/ml) avian PPD were made in two shaved sites, 8 to 12 cm apart from each other in the middle neck region, after having recorded skin thickness with a caliper. Skin thickness was measured again at both injection sites after 72 hours. The reaction result at each site was obtained as the difference of the skin thickness after 72 hours minus before injection. The data was interpreted using two different diagnostic cut-off points to define positive test results: 1) a reaction is usually considered to be positive if the increase in skin thickness at the bovine site of injection is more than 4 mm greater than the reaction shown at the site of the avian injection. The reaction is considered to be inconclusive if the increase in skin thickness at the bovine site of injection is from 1 to 4 mm greater than the avian reaction. The reaction is considered to be negative if the increase in skin thickness at the bovine site of injection is less than or equal to the increase in the skin reaction at the avian site of injection (OIE, 2009) and 2) an animal was considered positive if the bovine minus the avian tuberculin reaction was greater than 2 mm (Ameni *et al.,* 2008,) while if the difference is less than 2mm, the animal was considered as negative. A herd (farm) was considered as positive if it had at least one bovine tuberculin reactor animal.

2.4.2. Delayed Hypersensitivity Skin Test in human

After disinfecting the area Tuberculin skin test 0.1 ml (2TU/0.1 ml) tuberculin PPD RT23 (Statens Serum Institute, Copenhagen, Denmark) was administrated intradermally in the middle of the left forearm by an experienced nurse. The diameter of the indurations was measured transversely after 72 h using a ball-point pen and flexible plastic ruler. Diameters of skin indurations ≥10 mm were considered as positive, while diameters of indurations less than 10 mm were considered as negative (CDC, 2000).

Ethical considerations

The study protocol was approved by the Ethical Clearance Committee of the Aklilu Lemma Institute of Pathobiology, Addis Ababa University (Ref. No. ALIPB/728/2005/12). The aim of the study was explained to each of the study participants and written consent was obtained. Tuberculin PPD injections were carried out under aseptic conditions by well experienced nurse. Individuals whose TST indurations were >10 mm were advised to consult the nearest health facilities for further checkup.

2.4.3. Isolation and identification of Mycobacterium

I. Milk specimen and nasal swab collection and transportation

For laboratory investigation, milk and nasal swab samples were collected from CIDT positive animal. About 30 ml of milk samples were collected from all four quarters the tuberculin reactor cow at the end of milking from each quarter of tuberculin positive dairy cows with sterile universal bottle. The nasal swab was taken from the anterior nasal cavity by sterile swab and put into sterile test tube which contains 5-10ml normal saline solution. And the samples were transported in cold-chain to ALIPB laboratory and kept at 2-8^{0}C (Ameni *et al*., 2003a) until they were cultured.

II. Sample Culturing

Milk culturing for isolation of *Mycobacterium bovis* was carried out according to procedures described (Kazwala *et al*, 1998) with minor modification. The milk samples were centrifuged at 3000rpm for 10 minutes at room temperature. The supernatant with the exception of the cream was decanted and then the cream was mixed with sediment. The mixture was then decontaminated with 2% NaOH, centrifuged again. One or two drops of 0.05% phenol red indicator were added and then neutralized using concentrated HCl. After neutralization, each sediment was inoculated onto 2 Löwenstein-Jensen media (one with pyruvate and the other with glycerol). The culture was incubated at

37˚C and 5% CO_2. Observation for bacterial growth was made for 8-12 weeks checking every week for *Mycobacteria*l growth.

The nasal swab sample were collected from tuberculin positive cattle and processed for culture similar to sputum culture according to WHO guideline (WHO, 1998), by mixing with 2%NaOH (1:3 ratio), agitated in a vortex mixer and decontaminated by thorough shaking for 15 minutes at room temperature. They were centrifuged at 3,500rpm for 15minutes at 4^0C. The supernatant was taken off and the sediment neutralized with 1N HCl using drops of 0.05% phenol red as pH indicator. It was then centrifuged again at the same speed and time; the supernatant decanted and the sediment was inoculated into two slants of Löwenstein-Jensen media, one with pyruvate and the other with glycerol.

Finally, isolates both from milk and nasal swab culture were harvested for molecular typing analysis by scrapping the growth from a slope into 200µl of sterile distilled water and heating at 80°C for 45 min (Cadmus *et al*., 2006). Then, the harvest was kept at -20°C until molecular analysis is performed.

III. Multiplex Polymerase chain reaction (m-PCR)

The m-PCR procedure which is described by Wilton and Cousins (1992) was used. *Mycobacterial* genus typing by multiplex polymerase chain reaction differentiated *M. tuberculosis* complex from *M. avium* complex, *M. intracellularae* and other *Mycobacterial* species. Heat killed directly from milk and nasal swab samples with little modification (Rosa et al., 1999) and AFB positive culture sample were used as source of DNA template. DNA amplification was done in a thermo cycler with 20µl reaction volumes consisting of: 5µl of genomic DNA as a template, 8µl HotstartTaqMaster ($MgCl_2$, dNTP, Taq polymerase), for each sample 0.3 µl of forward and reverse primers for each sample. The primers used were MYCGEN-F, 5' AGA GTT TGA TCC TGG CTG AG 3' (35 µg/µl); MYCGEN-R, 5' TGC ACA CAG GCC ACA AGG GA 3', (35µg/µl); MYCAV-R, 5' ACC AGA CAT GCG TCT TG 3', (35 µg/µl); MYCINT-F, 5'-CCT TTA GGC GCA TGT CTT TA 3', (75 µg/µl); TB1-F, 5'-GAA CAA TCC GGA GTT GAC AA 3', (20 µg/µl); TB1-R, 5'-AGC ACG CTG TCA ATC ATG TA 3', (20 µg/µl) and 5.2 µl per sample of Qiagen water. The PCR reaction was carried out using Thermal Cycler (VMR Thermcycler 732-1200 Leicestershire, UK) based on the following amplification programme: 95^0C for 10 minutes for enzyme activation; 95^0C for 1minute for denaturation; 61^0C for 0.5 minute for annealing; 72^0C for 2 minutes for extension, involving 35 cycles all in all; and final extension at 72^0C for 10 minutes.

M. avium, M. tuberculosis and *M. bovis* strain NCTC 8559, H37 Rv and 2122/97 were used respectively as positive controls while H_2O (Qiagen, USA) was used as a negative control. The PCR product was then electrophoresed in 1.5% agarose gel in TAE running buffer 10X containing Ethidium Bromide at concentration of 0.5µg/ml in 1.5% agarose gel, 100bp DNA ladder (USA), and orange 6x loading dye (USA) were used in gel electrophoresis. All members of the genus *Mycobacterium* produce a band of 1030bp. On the other hand, *M. Avium* or subspecies such as *M. avium sbsp. paratuberculosis, M. intracellularae* and members of *M. tuberculosis* complex produce a band, 180bp, 850bp and 372bp, respectively.

2.4.4. Questionnaire survey

Farm owners and workers were interviewed according to their willingness to participate and given verbal consent, on the same day their cattle were tested for BTB. Interviews were made in all farms in Amharic. Questionnaires include on livestock husbandry/management and household characteristics, such as structure, presence of other livestock, mixing of cattle and other livestock, watering and feeding system, reproduction, cattle contact with other cattle herds, purchasing of animals. Furthermore, questions were asked related to human consumption habit, contact between humans and cattle, TB knowledge and known TB status in their home.

2.5. Data analysis

The data were entered into Microsoft Excel data sheets and analyzed using STATA 11 statistical software (STATA Corporation, Collage Station, Texas, USA). The prevalence was calculated by dividing the proportion of cattle found infected (positive reactors) by the total number of cattle tested multiplied by 100. Likewise, awareness of cattle owners was determined by dividing the proportion of people who knew BTB by the total number of respondents. Logistic regression analysis was used to assess the association between positivity and animal risk factors. The difference between the effects of different risk factors on prevalence was analyzed using the Pearson chi-square ($\chi 2$) test. The odds ratio (OR) was calculated to assess the strength of association of different factors with the positivity of bovine TB. A statistically significant association between variables was said to exist if the calculated $P<0.05$ and the confidence interval (CI) for OR doesn't include 1.

3. Results

3.1 Animal Prevalence

The overall individual animal prevalence using a cut-off ≥ 4mm (OIE, 2009) was 17.02% (95% CI, 16.99%- 17.05%) and using a cut-off > 2mm (Ameni *et al*., 2008) (Appendix IV), the prevalence was 21.15

% (95% CI, 20.81%- 21.49%). The herd prevalence was 100% (Table 1).

Table 1: Herd and individual animal prevalence of BTB by >4mm and >2mm cut-off value.

Prevalence	Total	By > 4mm off			By > 2mm off
	Tested	Positive	Prevalence (95 CI %)	positive	Prevalence (95CI %)
Animal level	558	95	17.02 %(16.99- 17.05)	118	21.15 %(20.81- 21.49)
Herd level	10	10	100 % -	10	100% -

The association of risk factors with bovine tuberculin reactivity with respect to individual animal prevalence indicated that there were a statistical significant variation across farms (χ^2=182.53; P= 0.000), age group (χ^2=19.9; P= 0.001), lactation status (χ^2=13.42; P= 0.001), parity class (χ^2=12.80; P= 0.012), management status (χ^2=30.96; P= 0.000), ventilation type (χ^2=76.12; P= 0.000), and previous test and culling practice in each farm (χ^2=57.60; P= 0.000), while body condition score and pregnancy status of the animal were not significantly associated with bovine tuberculin reactivity (P>0.05) (Table 2).

Table 2: Association of potential risk factors affecting reactivity of cattle to bovine tuberculin reactivity.

Variables	Number of animals			
	Examined	Positive (%)	χ^2 -Value	P- Value
Farms (Code 0-9)	558	95 (17.03%)[§]	182.53	0.000**
Age (Year)				
0.6 ≤ 2	179	23(12.85%)	19.9	0.001*
2 ≤ 5	246	50(20.33%)		
> 5	133	22(16.54%)		
Body condition				
Poor	134	22(16.42%)	5.68	0.225
Medium	356	55(15.4%)		
Good	68	18(26.47%)		
Pregnancy				
Pregnant	111	25(22.52%)	5.95	0.203
Non Pregnant	439	70(15.95%)		
Lactation				
Lactating	278	48(17.25%)	13.42	0.001*
Non Lactating	272	47(17.28%)		
Parity class				
Heifer	238	38(15.97%)	12.80	0.012*
Parity 1 - 3	263	49(18.63%)		
Parity ≥ 4	57	8(14.04%)		
Management				
Good Medium	334 118	42(12.57%) 17(14.41%)	30.96	0.000*
Poor Ventilation	106	36 (33.96%)		
Good	35	23 (65.71%)	76.12	0.000*
Medium Poor	217 306	19 (8.76%) 53 (17.32%)		
Test-culling practice				
Test-culled	201	16 (7.96%)	57.6038	0.000*
Test-not culled	221	31(14.03%)		
Status unknown	136	18(35.39%)		

*statistically significant, **highly significant

As shown in Table 5, the univariate and multivariable logistic regression analysis of the risk factors showed statistically significant differences on tuberculin reactivity among animals within age groups and with respect to previous test and culling practice of each farm. Multivariable logistic regression analysis

showed that cattle in age group (2 ≤ 5years) had 2.83 times the odds of being bovine tuberculin reactor compared to age group (0.6 ≤ 2years) (adjusted OR = 2.83; 95% CI: 1.47-5.44). Cattle in the farm which has no previous practice of testing and culling procedures had 6.29 times the odds of being bovine tuberculin reactors compared with those cattle found in farm which carry out previous testing and culling procedures (adjusted OR=6.29; CI=1.59-24.9) (Table 3). The culling is mainly carried out by slaughtering the tuberculin reactor animals.

Table 3: Univariate and multivariate logistic regression analyses of risk factors for CIDT test result in individual animal level.

Variables	Number of animals		Crude OR	Adjusted OR
	Examined	Positive (%)	(95% CI)	(95% CI)
Age (Year)				
0.6 ≤ 2	179	23(12.85%)	1	1
2 ≤ 5	246	50(20.33%)	2.53(1.66-3.85)**	2.83(1.47 - 5.44)*
> 5	133	22(16.54%)	1.91(1.17-3.10)*	2.22(0.96 – 5.15)
Body condition				
Poor	134	22(16.42%)	1	1
Medium	356	55(15.4%)	0.97(0.64-1.47)	1.22(0.70 – 1.79)
Good	68	18(26.47%)	1.63(0.90-2.95)	1.35(0.65 – 2.78)
Pregnancy				
Non Pregnant	439	70(15.95%)	1	1
Pregnant	111	22.52(%)	1.52(1.00-2.32)	1.32(0.77 – 2.27)
Lactation				
Non Lactating	272	47(17.28%)	1	1
Lactating	278	48(17.25%)	1.68(1.19-2.39)	1.59(0.72-3.49)
Parity class				
Heifer	238	38(15.97%)	1	1
Parity 1 - 3	263	49(18.63%)	1.78(1.23-2.58)*	0.65(0.27 – 1.60)
Parity ≥ 4	57	8(14.04)	1.00(0.54-1.88)	0.51(0.16 – 1.67)
Management				
Good	334	42(12.57%)	1	1
Medium	118	17(14.41%)	1.53 (0.99-2.35)	0.71(0.32-1.55)
Poor	106	36(33.96%)	2.32(1.49-3.62)**	2.09(1.01-4.30)*
History of test-culling				
Test-culled	201	16 (7.96%)	1	1
Test-not culled	221	31 (14.03%)	1.84(1.21-2.82)	2.01(0.55-7.28)
Status unknown	136	48 (35.39%)	4.69(2.93-7.52)**	6.29(1.59-24.9)**

3.2. Mycobacteriological examination results

3.2.1. Milk and nasal swab culture results

Out of 22 milk and nasal swab samples collected and cultured, only 4.5% (1/22) showed growth on LJ media from nasal swab sample. Culture positive colony was further confirmed by ZN staining and showed typical acid-fast bacilli.

3.2.2. Multiplex PCR typing

Multiplex PCR (Genus typing) was done on one culture positive isolate from nasal swab and the electrophoresis separation of the PCR product showed positive for genus *Mycobacterium* and was negative for *Mycobacterium tuberculosis* complex (MTBC) or *Mycobacterium avium* complex (MAC) group. Hence, it was non-tuberculosis *Mycobacteria* (NTM) species. In addition, m-PCR procedure was also tried directly on milk (n=10) and nasal swab (n=9) samples. The gel electrophoresis result of the PCR product from 7 samples (3 milk and 4 nasal swabs) samples showed non-specific signals, hence the result was inconclusive result and needs repeating the procedure (Appendix I).

3.3. Human tuberculin skin test result of dairy farm workers

From all the 10 dairy farms 31 volunteers dairy farm workers (milkers and animal attendants) were tested by human tuberculin skin test using tuberculin PPD RT23 (Statens Serum Institute, Copenhagen, Denmark) and consecutively questionnaire was administered to assess the zoonotic importance and associated risk factors for transmission of BTB.

Out of the 31 who take the tuberculin PPD only 25 were came on after 72hr for reading the skin test result. Based on the result, out of the 25 tested dairy farm worker 16 were positive with a prevalence of

latent tuberculosis at a cut-off point ≥ 10 mm was 64% (95% CI, 43%-84%). As indicated on the Table 4 the prevalence of latent tuberculosis was significantly different across their habit of consumption of raw milk (χ^2 = 10.43; P = 0.001), their knowledge of BTB transmission (χ^2 = 5.74; P = 0.017). But the prevalence was not significantly different between age groups (χ^2 = 2.7; P = 0.10), educational status (χ^2 = 3.34; P = 0.188) and their service year in the current farm (χ^2 = 0.15; P = 0.702).

Table 4: Association of human tuberculin skin test result with risk factor for zoonotic transmission of BTB.

Variables	Number of Attendants			
	Tested	Positive (%)	χ^2-Value	P- Value
Consumption of raw milk and meat				
no	7	1(14.3%)	10.43	0.001*
yes	18	15(83.3%)		
Age				
< 30	11	9(81.8%)	2.70	0.100
>30	14	7(50%)		
Knowledge of TB transmission				
yes	9	3(33.3%)	5.74	0.017*
no	16	13(81.2%)		
Education status				
illiterate	5	4(80%)	3.34	0.188
Primary	14	10(71.4%)		
Secondary and above	6	2(33.3%)		
Service year				
<3	6	4(66.7%)	0.15	0.702
>3	19	11(57.9%)		

*statistically significant

4. Discussion

With ever increasing demand for milk and other dairy products in the expanding population of urban dwellers, intensification of dairy cattle consisting of high milk producing dairy cow is expected to increase in urban dairy farms of Ethiopia. Hence, unless proper control strategies are designed, livestock disease such as bovine tuberculosis which has direct association with the intensification of dairy farms are increasing in prevalence and causing severe economic impact on the dairy industry and has significant public health hazard for the consumer of the dairy products (Tschopp *et al*., 2009).

In the present study, the overall animal and herd prevalence recorded in Debere Zeit were 17.025% and 100%, respectively using CIDT test. The individual animal prevalence finding was relatively in agreement with previous reports by Ameni *et al*. (2007), who reported 22.2% in the exotics Holstein breeds of dairy cattle in Sellale and Holeta area, 23.08% (Ameni *et al*., 2003a) in Deber Zeit Research Center and prevalence of 23.7% (Elias *et al*., 2008) in Addis Ababa. However, the individual prevalence of this study was lower than the previous studies carried out in different urban dairy farms of Debre Ziet area ranging from 29.7%-65.8% (Kiros, 1998; Ameni *et al*., 2003a).This difference in which relatively low prevalence reported as compared to previous studies in Debre Zeit area could be due to the variation in recent improvement in management system of the dairy farms and 59.8% (334/558) of the dairy cows included in this study were under good management system in which they had better housing, ventilation and hygienic conditions that might reduce the predisposing factors for BTB infection (Barewinek and Taylor,1996; Humblet *et al*., 2009). However, the result of the present study's prevalence was much higher than those of other CIDT-based BTB prevalence studies carried out in different parts of Ethiopia on cattle managed under traditional extensive and pastoralists production system in which prevalence ranging from 0.8%-11% were reported (Ameni *et al*., 2003b, Gumi *et al*., 2011, Mamo *et al*., 2013). The difference may be mainly associated to the variation in production system and breed of the animal in which most of the animals in present study were exotic/ cross breed managed under intensive system while the in other studies majority of the animals tested were the indigenous zebu breed managed under extensive production system. Hence, the intensification and exotic breed have been known as

risk factors for infection with *Mycobacterium bovis* (Ameni *et al*., 2006; Ameni *et al.,* 2007).

On the other hand the herd prevalence recorded by this study, 100% by considering a herd as positive even with a single reactor animal, is high as compared with previous results 43.4% reported by Elias *et al*. (2008). This is because of the absence of strict, regular and sustainable BTB control measures within the farms. Hence, even if one animal is infected in such a farm with a large herd size, there is a high chance of circulating the disease within the herd. Additionally, given the scarce resource on dairy breeds in Ethiopia, there is a tendency to keep animals with a long production life without culling, reinforcing the chance that they participate in BTB spread. The high prevalence recorded in some farms has serious implications and thus calls for attention of the concerned sectors.

In this study, the animal prevalence of BTB varied among the 10 farms. The BTB prevalence among the animals increased and showed statistically significant associations with herd management (χ^2 = 30.96; P = 0.000) which goes to the worst due to confinement, inconvenient and communal watering and feeding troughs, optimal stocking rate and absence of animals movement regularly. This result was in agreement with previous studies (Kiros, 1998; O'Reilly and Daborn, 1995; Ayele *et al*., 2004) who reported higher prevalence of BTB in animals kept under poor management than animals in good management. A poor management system promotes the chance of transmission of infection and development of the disease.

Similarly, the prevalence of the animals which stay in barn having poor air circulation increase than those stay in barns with good air circulation (χ^2 = 76.12; P = 0.000), this was in agreement with previous studies (Ayele *et al*., 2004). Radiostits *et al*. (2007) indicated that inhalation is almost the most invariable portal route of bovine infection in housed cattle which have prolonged close contact between infected and healthy animals, and even in those of pasture aerosol route is considered to be the principal mode of transmission.

In this study the prevalence of BTB was significantly high in middle age groups of (2 ≤ 5 year) (OR=2.53) and followed by the older group (> 5) (OR=1.91) as compared to the young age group (0.6 ≤ 2years), which was in line with previous findings by different researchers (Ameni *et al*., 2007; Regassa *et al*., 2010, Mamo *et al*., 2013). The possible reasons could be the fact that adult animals had longer and repeated chance of exposure to *Mycobacteria*l infection during their life time. Moreover, most of the farms have the middle age group due to their high production and kept longer in the farm and the dairy farm owners are not willing to cull the middle age even if they are reactive.

In this study, there was a significant difference between lactating and non lactating cows. This finding was in line with result reported by Tweddle and Livingstone, (1994) and Kiros, (1998), who explained that factors that may suppress the immune responsiveness of cattle herds in various production systems due to environmental and management factors (malnutrition, lactation, pregnancy and concurrent infection) might cause difference in prevalence of BTB.

Interestingly, cattle in the farm which has no previous practice of testing and culling procedures had 6.29 times the odds of being bovine tuberculin reactors compared with those cattle found in farm which carry out testing and culling procedures. This result clearly showed that intervention in the control of BTB through testing and culling can result a significant impact on reducing the prevalence of the disease at individual farm level (Barwinke and Taylor, 1996). The possible reasons might be associated to the reduction in the number of bovine tuberculin reactor animals from the farm which will consequently reduces the source of infection for the remaining animals in the herd. In addition, in this study it has been observed that a reduction of bovine tuberculin reactors in farms which practice testing without culling as compared to those with no such practice. This difference might be related to the fact that by carrying out tuberculin skin testing the owner might develop awareness on the disease transmission and practice a segregation of the reactor animal from the rest of the herd, and this will reduce the rate of close contact with healthy animals and hence decreases the transmission rate of BTB.

In the present study the prevalence of latent tuberculosis in dairy farm workers using TST showed that dairy workers has higher prevalence (64%) which in agreement with report by Tores-Gomzalez *et al*. (2013) in Mexico who reported 74 % prevalence using TST and in association of TST result in dairy worker with high consumption habits of raw milk and other animal products showed a statistical significance difference in prevalence as compared to those who do not have habit of consumption raw animal product. This finding is also in agreement with previous findings in Ethiopia by Regassa *et al*., 2007; Kazwala *et al*., 1998 and Ameni *et al*., 2003b. This might be because of the risk of infection with zoonotic TB through consumption of raw animal product and close contact with infected animals.

5. Conclusion And Recommendations

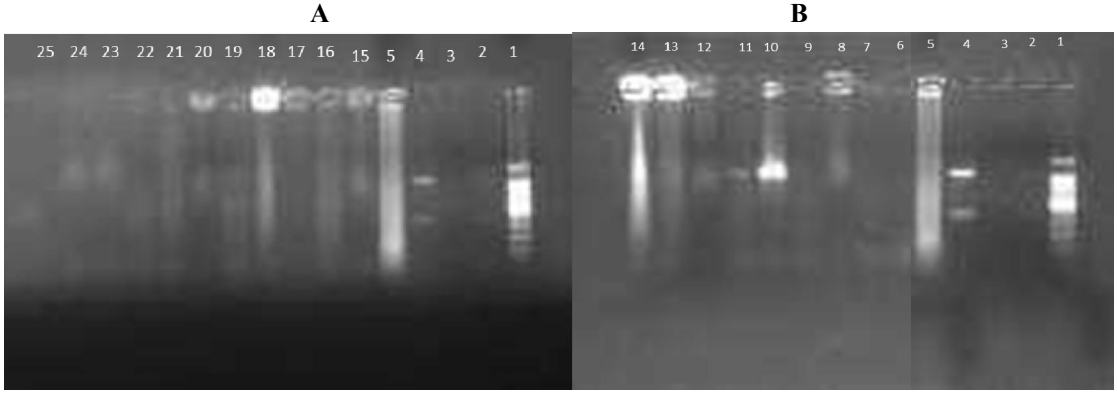
This study demonstrates a widespread occurrence of BTB infection in large scale dairy farms of Debre

Zeit town with high prevalence at individual farm level. In present study also demonstrated that all the farms studied in the town were infected with BTB, which result a herd prevalence of 100%. A higher number of bovine tuberculin reactor animals were found in dairy animals managed under poor management system and in those farms which did not cull the test positive animals. Also the prevalence was high in adult cows age ranging 2-5 years than younger cows. Prevalence of human TST was high in dairy farm workers and they were not aware of the existence and route of transmission of BTB and its public health significance. Moreover, a large proportion of the public had habit of consuming raw animal product predisposing them for potential exposure to infection by zoonotic tuberculosis. The culturing and m-PCR result revealed poor growth only one nasal swab sample were grew on LJ medium and only one sample were under genus *Mycobacterium* which need further investigation. In conclusion, this study had demonstrated the prevalence of BTB in the expanding dairy farms of Deber Zeit and hence it warrants the need for paying attention towards the control of bovine tuberculosis in the study area.

In light with the above mentioned findings, the following recommendations are forwarded:

- Integrated control strategies such as test and segregation combined with culling by slaughter method at individual farm level, improved farm management by limiting the stocking densities and rearing BTB-free animals for replacement need to be applied.
- Public education and creation of the awareness on BTB and its zoonotic importance with due consideration on the danger of consuming unpasteurized dairy products and other animal product should be initiated.
- Pasteurization milk and milk product should be initiated and adapted at a large scale dairy farm level and policy needs to be designed with this respect.
- Furthermore, more detailed studies to assess and evaluate the scale of the problem, to identify and characterize the causative agent of BTB and its role in human tuberculosis are needed.

Genus typing m-PCR agarose gel electrophoresis result.



Gel electrophoresis separation of PCR products of multiplex PCR genus typing of *Mycobacteria* from cattle. Lane 1=100bp DNA ladder; Lane 2= *M. tuberculosis* (positive control), Lane 3= Qiagen H_2O (Negative control), Lane 4= *M. bovis* (positive control), Lane 5= *M. avium* (positive control), Lane 6-25 were isolates from samples such as, Lane 6=H18L-N, lane 7= 64GG-M, Lane 8= A28-687-N, Lane 9= 38GG-N, Lane10= A4-39 culture, Lane 11= A4-39-N, Lane 12= A4-39-M, Lane13= A2-633-M, Lane14= A31-87-M, Lane 15= A28-687-M, Lane 16= 38GG-M, Lane 17=081-N, Lane 18=A1-77-M, Lane 19=A1-77-N, Lane 20=019-M, Lane20= A20-30-M, Lane21= 0959-N, Lane22 =A21-28-M, Lane 23= A21-28-N, Lane24=019-N, Lane25=64GG-N. (Where N is nasal swab, M is milk)

Acknowledgements:

The authors acknowledge Ministry of Agriculture and Rural Development for its financial support to this research. The material support given by Akililu Lema Institute of Pathobiology for this work is also acknowledged. We are grateful to farm owners, who were willing to involve in this study.

Corresponding Author:
Dr. Meseret Bekele
Ministry of livestock and fishery, Addis Ababa, Ethiopia
Telephone: +2519081676
E-mail: yohanan14sm@gmail.com

References

1. Ameni, G., Aseffa, A., Engers, H., Young, D., Gordon, S., Hewinson, G. and Vordermeier, M. (2007): High prevalence and increased severity of pathology of bovine tuberculosis in Holsteins compared to Zebu breeds under field cattle husbandry in Central Ethiopia. *Clinical Vaccine Immunology*, 14: 1356–1361.
2. Ameni, G., Aseffa, A., Engers, H., Young, D., Hewinson, G. and Vordermeier, M. (2006): Cattle husbandry in Ethiopia is a predominant factor for affecting the pathology of bovine tuberculosis and Gamma Interferon responses to *Mycobacteria*l antigens. *Clinical and Vaccine Immunology*, 13: 1030-1036.
3. Ameni, G., Bonner, P. and Tibbo, M. (2003a): Across-sectional study of bovine tuberculosis in selected dairy farms in Ethiopia. *International Journal of Applied Research in Veterinary Medicine*, 1: 85–97.
4. Ayele, W., Neill, S., Zinsstag, J., Weiss, M. and Pavlik, I. (2004): Bovine tuberculosis, an old disease but new threat to Africa. *International Journal of tuberculosis Lung Disease*, 8: 924-937.
5. Barwinek, M. and Taylor, N. (1996): Assessment of Socio-Economic Importance of Bovine Tuberculosis in Turkey and Possible Strategies for Control or Eradication. Turkish-Germany Animal Health Information Project. General Directorate of Protection and Control. Ankara Eschborn: Deutsche Society for Technical Cooperation. Pp. 3-45.
6. Cadamus, S., Palmer, S., Okker, M., Dale, J., Gover, K., Smith, N., Jahans, K., Hewinson, R. and Gordon, S. (2006): Molecular Analysis of Human and Bovine Tubercle Bacilli from a Local Setting in Nigeria. *Journal of Clinical Microbiology*, 44: 29–34.
7. CDC, (2000): Targeted tuberculin testing and treatment of latent tuberculosis infection. *MMWR*; 49 (No. RR-6). www.cdc.gov/MMWR/PDF/rr/rr4906.pdfR.
8. Cousins, D. (1992): *Mycobacterium* infection and control in diagnostic livestock. *Review of Science and Technology, Office International des Epizooties,* 20:71-85.
9. CSA, Central Statistical Agency of Ethiopia, (2007): *Population and Housing Census of Ethiopia*. Retrieved 2011-12-02, P. 22.
10. Elias, K., Hussein, D., Asseged, B., Wondwossen, T. and Gebeyehu, M. (2008): Status of bovine tuberculosis in Addis Ababa Dairy farms. *Review of science and technology*, *Office International des Epizooties*, 27: 915-923.
11. Etter, E., Donado, P., Jori, F., Caron, A., Goutard, F. and Roger, F., (2006): Risk analysis and bovine tuberculosis: a re-emerging zoonosis. *Annals of the New York Academic Sciences*, 10: 61-73.
12. Gumi, B., Schelling, E., Firdessa, R., Aseffa, A., Tschopp, R., Yamuah, L., Young, D. and Zinsstag, J. (2011): Prevalence of bovine tuberculosis in pastoral cattle herds in the Oromia region, southern Ethiopia. *Tropical Animal Health and Production*, 43: 1081–1087.
13. Humblet, M. Boschiroli, M. and Segerman, C. (2009): Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. Review article .*Veterinary Research*, 40: 1-24.
14. Kazwala, R., Daborn, C., Kusiluka, L., Jiwa, S., Sharp, J. and Kambarage, D. (1998): Isolation of Mycobacterium species from raw milk of pastoral cattle of the Southern Highlands of Tanzania. Tropical Animal Health Production. 30 (4): 233-242.
15. Kiros, T. (1998): Epidemiology and zoonotic importance of bovine tuberculosis in selected sites of Eastern Shoa, Ethiopia. MSc. Thesis. Free University of Berlin and Addis Ababa University.
16. Mamo, G., Abebe, F., Worku, Y., Hussein, N., Legesse, M., Tilahun, G., Medhin, G., Bjune, G. and Ameni, G. (2013): Bovine tuberculosis and its associated risk factors in pastoral and agro-pastoral cattle herds of Afar Region, Northeast Ethiopia. *Journal of Veterinary Medicine and Animal Health,* 5(6): 171-179.
17. Michel, A., Coetzee, M., Keet, D., Mare, L. and Warren, R. (2008): Molecular epidemiology of *Mycobacterium bovis* isolates from free ranging wildlife in South African game reserves. *Veterinary Microbiology*, 1: 9-14.
18. Mostowy, S., Inwald, J., Gordon, S., Martin, C., Warren, R., Kremer, K., Cousins, D. and Behr, M. (2005): Revisiting the Evolution of *Mycobacterium bovis*. *Journal of Bacteriology*, 187: 6386-6395.
19. O'Reillly, L. and Daborn C. (1995): The epidemiology of *Mycobacterium bovis* infections in animals and man, a review. *Tubercle and Lung Disease,* 76: 1-16.
20. OIE, (2009): Office International des Epizooties Terrestrial manual: World Health Organization for Animal Health, Paris: Office International des Epizooties.
21. Radostits, O., Blood, D. and Gay, C. (2007): Diseases caused by mycobacterium species. In Veterinary medicine: a textbook of the diseases

of cattle, sheep, pigs, goats and horses, 10th ed. Baillière Tindall, London, Pp. 1007-1017.
22. Regassa, A. (2007): Study on *Mycobacterium bovis* in animals and human in and around Fiche, North Shewa zone, Ethiopia. MSc. Thesis. Faculty of Veterinary Medicine. Addis Ababa University, Debre-Zeit, Ethiopia.
23. Regassa, A., Tassew, A., Amenu, K., Megersa, B., Abunna, F., Mekibib, B., Marcotty, T. and Ameni, G. (2010): A cross-sectional study on bovine tuberculosis in Hawassa town and its surrounding, Southern Ethiopia. *Tropical Animal Health and Production,* 42: 915-920.
24. Rosa, E., Daniel, L., Garz6n, G., Mejia, W., Manuel, E. and Luis A. (1999): Identification of *Mycobacterium bovis* in Bovine Clinical Samples by PCR Species-Specific Primers. *Canadian Journal of Veterinary research,* 63:101-106.
25. Teshome, M. (1986): Bovine tuberculosis in state dairy farms in and around Addis Ababa. Study Report, MoA, Ethiopia.
26. Teshome, Y. (1995): Status of bovine tuberculosis in Ethiopia. In: Thoen C.O., Steele J.H. eds. *Mycobacterium bovis* Infection in Animals and Humans. Iowa State University Press, Ames, Iowa, USA. Pp. 273–279.
27. Thoen, C., Lobue, P. and de, K. (2006): The importance of *Mycobacterium bovis* as a zoonosis. *Veterinary Microbiology,* 112: 339-345.
28. Thrusfield, M. (2005): Veterinary Epidemiology. 3rd ed. UK, Blackwell Science. Pp: 228-247.
29. Tores-Gomzaleze, P., Soberanis-Ramos, O., Martinez-Gamboa, A., Chavez-Mazari, B., Barros-Herrera, M. T., Tores-Rajas, M., Cruz-Hervert, L. T., Gracia-Gracia, L., Singh, M., Gomezaleze-Agurirre, A., de Leon-Gardun, A. R., Sisuentes-Osornio, J. and Bobadila-Del-Valle, M. (2013): Prevalence of latent and active tuberculosis among dairy farm workers exposed to cattle infected by *Mycobacterium bovis. PLoS FOUR* .7: e2177.
30. Tschopp, R., Schelling, E., Hattendorf, J., Aseffa, A. and Zinsstag, J. (2009): Risk factors of bovine tuberculosis in cattle in rural livestock production systems of Ethiopia. *Preventive Veterinary Medicine,* 89: 205-211.
31. Tweddle, N. and Livingstone, P. (1994): Bovine tuberculosis control and eradication programs in Australia and New Zealand. *Veterinary Microbiology*, 40: 23–29.
32. WHO, (1993): Report of the WHO meeting on zoonotic tuberculosis (*M. bovis*) with the participation of FAO. Geneva, Switzerland. WHO/CDS/VPH/ 93-130.
33. Zinsstag, J., Kazwala, R., Cadmus, I. and Ayanwale, L. (2006a): *Mycobacterium bovis* in Africa. In: Thoen C.O., Steele J.H. Gilsdorf M.F. (Eds.): *Mycobacterium bovis* Infection in Animals and Humans. 2nd ed. Blackwell Publishing Professional, Ames, Iowa, USA. Pp. 199–210.

4/19/2016