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SURVEY ON BOVINE TUBERCULOSIS PREVALENCE AND ASSOCIATED RISK FACTORS IN SELECTED DAIRY FARMs AND ITS ZOONOTIC POTENTIAL ON REGULAR MILK CONSUMERS IN HARAR CITY, EASTERN ETHIOPIA.

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ABSTRACT: A cross-sectional study was carried out from February, 2021 up to December 2021 to estimate the prevalence of Bovine Tuberculosis in 5 dairy farms, identify the associated risk factors and its zoonotic potential. cross-breeds dairy cows were first tested to the Tuberculin skin test, In Ethiopia, where livestock are extremely important for people's livelihood, animal diseases can be a real threat to animal productivity and thus negatively impact on the agricultural sector and economic development. Bovine tuberculosis, caused by M.bovis, is a chronic and contagious disease of cattle and other domestic and wild animals. The isolation of M. bovis in freshly drawn milk from the tuberculin positive reactor cows is being reported for the first time in Harar town. The study emphasizes the risk of transmission of TB to human through consumption of contaminated unpasteurized milk and milk products. There is a need for enhanced public health education to raise awareness on the consequences of consuming potentially contaminated milk. Also, measures need to be adopted to test and eliminate positive reactor cows from the dairy supply chain. Using questionnaire survey, the study considered different aspects of public health risks; mainly husbandry, knowledge and public awareness including the farm hygienic practice level with zoonotic potential of bTB infection were assessed on 100 randomly selected respondents which include dairy farm owners, dairy workers, and customers who regularly purchasing milk and milk products from these selected dairy farms for study. The overall survey finding signifies that people lack that this zoonotic disease related Awareness gap level was very higher which could be described as very lower/below 50%. and the overall Comparative Intra Dermal Tubercline skin test prevalence was about 32(12.21%). Out of the total 5 dairy herds tested (262), 32 of them (12.21%) were found to be positive reactors for bovine tuberculosis. Implementation of control program in such areas could be effective to reduce or possibly stop further bTB transmission between cattle and to reduce the likely zoonotic impact. The detected pathogens revealed that the disease transmission could be cyclical: cow-to-man-to-cow, underlying the existence of higher risk of dissemination of mycobacteria among the cattle and human populations.

Key words: Raw milk, bTB, M.bovis, Zehl Neelson stain, Purified protein derivatives, Culture, Harar, Zonoosis

1. INTRODUCTION

Bovine tuberculosis (bTB) caused by Mycobacterium bovis and it is a zoonotic disease, and remains a cause of concern for livestock, wildlife and human health (Huang et al., 2013). A close interaction between animals and humans primarily contributes to the transmission of infectious Zoonotic diseases between them (Mbugi et al., 2012). The most important causes of bovine TB in cattle are Mycobacterium bovis (M. bovis) and Mycobacterium caprae (M. caprae), both of which cause infectious diseases (Prodinger, et al., 2005; Cvetnic et al., 2007; Javed et al., 2006; Duarte et al., 2008). M. bovis has one of the broadest host ranges of all known pathogens and has been diagnosed worldwide. Cattle are considered to be the true hosts of M. bovis (OIE, 2008).

The pathogen is the cause of bovine tuberculosis (bTB) and in chronically infected cattle can be associated with both poor health and production (Radostits OM,2001 ; OIE,2009). Zoonotic transmission, from cattle to humans, is of great concern with approximately 3% of all human tuberculosis cases being caused by M.bovis (Grange JM,1994; Cosivi1999)

It is generally believed that zoonotic transmission occurs through close contact with infected cattle or through consumption of untreated milk. Hence in many high-income countries the control of bTB in cattle is primarily aimed to protect human health rather than animal health (Marcotty, et al,2009; de la Rua-Domenech ,2006;Palmer,2006). The zoonotic risk of food borne transmission has been mitigated through public health initiatives such as meat inspection and processing milk by heating to a high temperature (Thoen,2006) Increasing awareness of bTB, through education programs, has also been integral to zoonotic tuberculosis (zTB) control(Palmer,2006;Thoen,2006; Collins,2006)

Cattle serve as the main host for M.bovis worldwide (Huang et al., 2010 ; Gumi et al., 2011), while other domestic animals such as pigs, cats, dogs, horses and sheep are considered to be spill-over hosts. It has been recorded as the most recurrent cause of zoonotic tuberculosis in humans (Tengunia et al., 2011).

In Ethiopia, the presence of bTB in cattle (Gumi et al (2012; Nuru (2015) and human due to M. bovis (Gumi et al (2012; Firdessa et al (2013) ; Nuru (2015) were reported previously. After isolating M. tuberculosis from animals it was also suggested the presence of human to animal transmission in Ethiopia (Berg (2009); Ameni (2013). In developed countries, the occurrence of human Tuberculosis Due to M.bovis has Meaningfully declined because of mandatory pasteurization of milk together with tuberculin skin test of cattle followed by culling/slaughtering the infected cattle (Palmer et al., 2013) In such countries, animal TB control and elimination programs, together with milk pasteurization, have drastically reduced the incidence of disease caused by M. bovis in both cattle and humans. The direct correlation between M.bovis infection in cattle and disease in the human population has been well documented in industrialized countries.

In France, the bovine tuberculosis eradication efforts commenced in 1950 when 25% of cattle herds were infected. In 2000, the operation of the program was granted the Officially TB-Free (OTF) status, a highly favorable situation to ensure trading. In recent years, France has become the most important live animal exporting country in the European Union and one of the most important in the world (Boschiroli, 2014). In developing countries, however, animal (tuberculosis) TB is widely distributed, control measures are not applied or are applied sporadically, and pasteurization is rarely practiced. The direct correlation between M. bovis infection in cattle and disease in the human population has not been well documented and little information is available from such countries (Collins, 1983; Cosivi, 1995), risk factors for M. bovis in both animals and humans are present in the tropics.

Many factors account for the failure of developing countries to control and eradicate bTB, many of them political and economic. The most important ones especially in African countries include problem of social unrest due to political instability and ethnic wars, lack of veterinary expertise and communication networks insufficient collaboration with border in countries and hence lack of quarantine and smuggling of live animals across state boundaries (Ayele et al., 2004).

In Ethiopia, exotic breeds were found to be more susceptible than cross and local breeds to M. bovis with manifestation of high incidence and prevalence rates (Regassa 2008; Ameni et al., 2008). In addition, a herd prevalence rate of 42.6% to 48.6% was found to be higher than the prevalence rate of individual animals (7.9% to 18.7%), that may indicate that the herd size can favour the transmission of BTB in intensive dairy farms in particular (Ameni et al., 2007; Shitaye et al., 2007).

The zoonotic risk of BTB is often associated with consumption (ingestion) of dairy products based on unpasteurized milk infected with M. bovis. However, aerosol transmission (inhalation) from cattle-to-human should also be considered as a potential risk factor. Mainly through consumption of un pasteurized milk products - and its prevalence in Ethiopian cattle can therefore be a contributing factor to the human burden of TB in Ethiopia that currently is ranked as the 7th highest in the world (Gumi, et al., 2012). Most meat and milk consumers generally prefer raw milk & meat (as compared to treated milk and cooked meat) because of its taste, availability and lower price. The zoonotic risk of BTB is often associated with consumption (ingestion) of dairy products based on unpasteurized milk infected with M. bovis. Also, aerosol transmission from cattle-to-human should also be considered as a potential risk factor (Zeru et al., 2014).

Bovine tuberculosis causes production efficiency losses ranging from 10 to 25% in infected animals, leading to severe losses related to reduced milk and meat production, condemnation of carcasses in slaughterhouses, sanitary slaughter of tuberculin-positive animals, and restrictions on the export of animals and meat products of bovine origin (Brasil , 2006 ; Paes , 2010). It also carries an indirect threat since they account for the majority of the 20% losses that affect the livestock sector at the production level and since they could severely hamper the animal traction resources that are needed to produce or transport goods. These losses raise an issue of food security to sustain an increasing demand for proteins of animal origin (Lefrançois and Pineau, 2004).

In Ethiopia, where livestock are extremely important for people's livelihood, animal diseases can be a real threat to animal productivity and thus negatively impact on the agricultural sector and economic development. Bovine tuberculosis, caused by M.bovis, is a chronic and contagious disease of cattle and other domestic and wild animals (Ayele, 2004).

Groups at elevated risk of infection with M. bovis include farmers, abattoir & dairy workers, veterinary personnel, zoo staff and others who may handle infected animals or tissues, and those who drink unpasteurized milk. The incidence of tuberculosis due to this organism is poorly understood, as the causative organism is not always identified. Approximately 25- 30% of all tuberculosis cases were thought to be caused by M. bovis before the advent of milk pasteurization and bovine tuberculosis control programs, at a time when close contact with livestock was also more common. Newer studies estimate that this organism is responsible for < 1% to 5-10 % loss (Thierry Lefrançois and Pineau 2004). Most cases in developed countries now occur in elderly or immunosuppressed individuals, as the result of reactivated infections acquired in childhood, and in immigrants from endemic regions. Small clusters of M. bovis infections seem to be caused by person-to person transmission, especially among people in close contact (e.g., families). Healthy people, as well as those who are immunocompromised, have been affected in these incidents. (OIE, 2019).

In the study area, Due to the different livestock diseases that made a significant decline of production and productivity of milk very far below the standard WHO recommendations, the per capita consumption of milk should be 90liters per year. Due to all these in harar town there is a higher disparity between the milk demand and supply around this time, as the needs of population increase with the demographic pressure in one way also aggravate the problem that made people in secured of the milk protein b/s of the defiecit production faced along with un hygienic practices that predisposes to milk borne diseases such as mycobacterial mastitis and many others, too.(HARDO,2018) To the best of our knowledge information on BTB among dairy herds in Eastern Ethiopia is limited. The current study was designed to investigate/and estimate tubercline based prevalence of BTB also isolate through culturing milk sample to assess isolates drug susceptibility profile of the pathogen.

This study area topic lacks appropriate attention and is currently an understudied area of research and this enables to answer the important reasons why and how to avoid bovine tuberculosis from the urban Cross Holstein Friesian cattle dairies that are the potential source of raw milk to harar town population along with understanding the extent of the problem through recording the prevalence of M. bovis in milk samples collected from those tuberculin reactors for detection of the agent , and with due emphasis, to the higher the risk of infection to the human population (Food safety, milk borne zoonotic diseases such as Mycobacterial Mastitis and others), the higher losses in productivity of infected animals (food security, along with demographic pressure) , the risk that trade restrictions might be imposed by countries which are well-advanced in the eradication progress and the chances of transmission of M. bovis will increase with the use of unpasteurized milk and the transfer of resistant gene through consumption of food animals that might affect the standard treatment regimen. (Adams, (2001).

Therefore, this study aims to determine the prevalence of Bovine Tuberculosis in selected dairy farms based on tubercline (ppd) and Questionnaire Survey on farm owners, farm workers, and other regular milk

consumers/customers with identifying potential associated risk factors and determine the milk contamination level.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

Harari Region is located 515 km East of the capital city Addis Ababa. According to the information from Harari Agricultural and Rural Development Office, (HRADO**. (2004**) Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia **(CSA,(2007**), Harari has a total population of 183,415, of which 92,316 were men and 91,099 women.. Of these total population 46% are lives in rural population while the rest 54% are lives in the urban population. It has three rural administrative districts and three urban administrative districts. These three urban districts have a total of seventeen /17/ urban kebeles. The other three rural districts also have a total of seventeen /17/ rural kebeles. The total area of this region is 311.25 sq. km, of which 53% of the land is used for agricultural, 21% is covered with shrubs and 26% is covered with the constructed residence and others infrastructures.. The mean annual rain fall range is estimated about 650 mm –850 mm in bimodal pattern. The long rainy season extends from June to August followed by a dry season ranging from December to February. The short rainy season lasts from March to May. The eastern, northern, Southern and Western boundaries are adjacent to the four Districts of the Oromiya region, Namely, Babile, Jarso, and Fadis and Haramaya respectively.

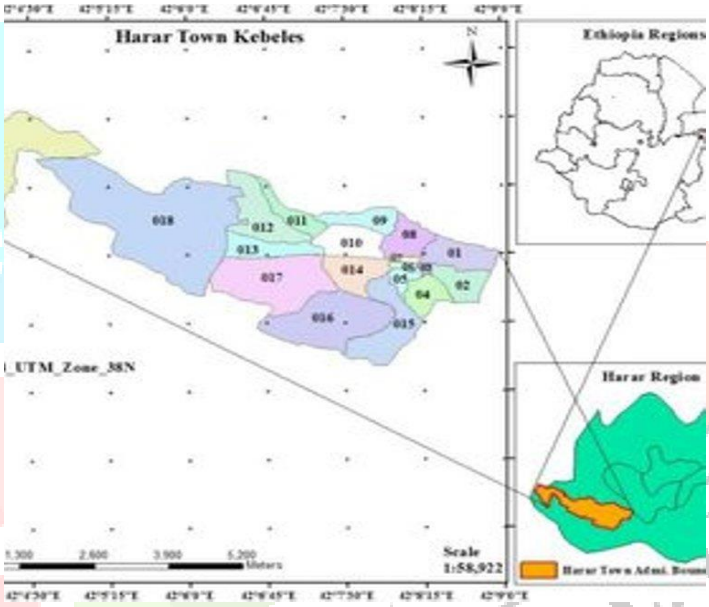

Figure 1: Kebeles of the Harar city of the Harari regional state

The average minimum and maximum temperature range is about 21^0C to 27^0C . During the month of December, the minimum temperature goes 23^0C to 25^0C and in the month June the maximum temperature goes up to 27^0 C. Harar town is densely populated having accommodating 54% of the total population than the rural part where the milk scarcity was hit the town where in the past years milk has been surplus even in the market place. But now a days with a significant demographic pressure the town faced because of different reasons the milk demand and supply is highly mismatched, there is a high deficit in milk demand, due to this gap much more additional retailers not less than 86 who are working in milk trade supply sourced from babile district. Further to this trend even milk supply from babile goes to meet the demands of towns such as Aweday. So the protein demand is unsecured especially milk.

Currently, Due to this all problems peoples are obliged to relay on the commercial synthetic packed milk smuggled in the eastern part of the country which are the products of yemen and Arabian emrates which has two forms of product, the powered and fluid form (Powder form Nido, Ancor,zemzem,Yemeni which is a liquid form). The higher demand of milk is substituted by this product which is available in most roadside shops. This milk production decline gap is due to different diseases where TB is the major share of this with higher decline rate and put potential consumers lacks supply with risk predisposing to milk borne diseases including mastitis disease impairing production. The region has different livestock that comprise the following Cattle 52000 Donkeys and Horses 11250 Goats 57000 Sheep 63000 Bee hive 1290 and A poultry population of 72,000.

2.2. Study population

The study animals are lactating dairy cattle managed under intensive dairy farms in the study area. All selected dairy farms were owned by private investors. Dairy cattle in the selected farm were the study units. And their breeds composition were crosses of Holstein Friesian and zebu herds. The husbandry and farm setting differed somewhat from one study site to the other depending on the level of Awareness, educational status of farm owners and access of extension services. The sampling technique was purposive having the following inclusion and exclusion criteria were used for farms and individual animals (lactating) Farms were included if they have been established for over ten years and having the herd size of greater than > 40 and >60 cattle, and who gave a written informed consent for cattle to have SICCT test, agreed to conduct all technical duty until finalizing it. Both the Cross breed Dairy cattle found in dairy farms of Harar Town and customers who regularly purchasing milk and milk products from these dairy farms are the study population

The intensive dairy farms in the harar town comprised small, medium and large size dairy herds that range between 7-145 head of cattle. Surprisingly, Most of the intensive dairy farms were concentrated in and around the Harar town and rearing cross-breed dairy cattle. But most of the small sized farm holders' dairy herds were located in the rural areas of the region. Where all the majority of milking cows are indigenous/local cattle but the very small numbers of cross-breeds are also found around rural areas. For this study purpose all the selected five dairy farm herds' size ranged between 44-65 cattle and All were managed under intensive system.

2.3. Study Design and Study Period

This study designed as a cross sectional study intended to do with a defined resource & skills personnel support within a time frame of February 30/ 2021__June 30 2021. to determine the BT prevalence in Harar town. It has also a questionnaire based data collected from targeted respondents and analyzed methods considering the major associated risk factors contributing to zoonotic effect of the disease. and any lab detected finding results

2.4. Sample Size Determination and Sampling Procedure

The sample size required for cattle of this study will be determined on the expected prevalence of Mycobacterium bovis and the desired absolute precision according to Thrusfield (2005) as follows:

$$n = \frac{1.962 \text{ x } Pexp \text{ x } (1 - Pexp)\ 44}{d^2}$$

Where n = the required sample size,
Pexp = expected prevalence
d = desired absolute precision
Where n is the required sample size,
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Since there was no any btb related study conducted so far. And By taking the upper limit of prevalence range explored by different representative studies that have been done indifferent parts of the country considered as about 50% prevalence rate of Mycobacterium bovis to spring as a base line for cattle at dairy farms in harar so that the assumed expected prevalence is as 50%. Therefore, to calculate the total sample size, the following parameters will be used: 95% level of confidence (CL), 5% desired level of precision and with the assumption of 50 % expected prevalence level of Mycobacterium bovis in Cattle by using the above formula the required sample size becomes 384 numbers of cattle, which will be taken and accomplished to all study animals for intradermal skin testing procedures accordingly following the milk sample collection from only those reactors of the test.

2.5. Operational definitions

Body condition score defined as Body condition score: Body condition scoring was categorized into three, viz. poor, medium and good BCS. A poor condition (BCS: 0-3) animal an angular and bony shape with no or negligible amount of fat over the backbone, ribs, pins and hooks. Moreover, there is no noticeable fat around the tail, head or even in the brisket. However, medium condition (BCS: 4-6) cattle have moderately good appearance, evident hips, though there is a small part of fat over the hooks, pins and the backbone is no

longer detectable. Good condition cattle (BCS > 6) are fleshy and the ribs are no longer observable. There is also fat everywhere even in tail head and in the brisket.

Herd size was defined as the number of cattle being kept in a farm. Considering the farming practices of these districts farm size was classified into two (2) levels. Small <40, medium when the number of cattle between 40-50; large: when >50 cattle. New animal inclusion was defined as whether animals are bought from where the farm history is known as safe history farm or simply from livestock market place or the unknown place. Interpretations of animals to account as a reactor animals at a cut of point be >2 be considered positive & < 2 be considered as negative according to critics/ amendment made on the prior- O.I.E- standard set. By different African studies. All other operational definitions, recording format including the written consent will be attached on the annex section.(the O.I.E standard interpretation that have three categories- > 4mm as positive, ≥ 2mm and < 4mm , as doubtful & < 2mm , as Negative) for this study the OIE standard was not used, rather this study used the amended one having only two categories (negative & positive),where the one is positive at > 2mm cut off point & the other was the negative with sufficient justification by African authors/researchers. (Which considered the African context which the OIE also accepts the critics and even allows to proceed owns country's level context).

2.6. Research methodology

2.6.1. Comparative intradermal tuberculin skin test, CIDT

These materials were provided and borrowed from the nearby regional human and veterinary laboratory. But both the reagent (avian tuberculine and Bovine purified protein derivative) have been gotten from NAHDIC. As a regional animal disease investigation and surveillance laboratory, its staffs have supported & cooperated in this study. CIDT was used to test animals for BTB. The test was performed by the intradermal injection of bovine purified protein derivative (B-PPD) and avian PPD (A-PPD) (supplied by Prionics Lelystad B. V., The Netherlands) after the skin was shaved and the thickness measured with a caliper. The PPDs were injected into two sites in middle third of the neck (about 12cm apart)

Both injective sites should be located in the border of the anterior and middle third of one side of the neck. For Inconclusive Reactor (IR) retests, the injections should be made (if possible) on the opposite side of the neck to that used at the previous test. The upper site (avian tuberculin) should be, in the adult bovine, at least 10cm below the crest, and the lower site (bovine tuberculin) should be approximately 12.5cm from the other, on a line roughly parallel with the line of the shoulder, as in the picture below. In order to maximize test sensitivity, injection sites should be as close as possible to those indicated. (Generally , injection site demarcation be used based on the achievement of the following objectives such as facilitate measurement of the skin fold thickness at the correct site, minimize any risk of contamination during injection, be clearly and easily identifiable on the second day of the test for visual inspection, palpation and re-measurement and avoid trauma to the skin surface).

Interpretation: In this study Interpretation of the test result is made at two different cut-off points i.e. the animal is considered positive if the skin reaction at the PPD-B site minus the skin reaction at the PPD-A is > 4 mm as the O.I.E standard set was Amended by (Gobena Ameni et al., 2008) as the O.I.E standard put below shows the reading that was Amended by the Ethiopian researchers studied based on the local context (OIE, 2008). However, if a reaction to B-PPD tuberculin is less than the reaction to A-PPD the result is interpreted as negative reaction. A dairy herd is considered positive if at least one tuberculin reactor animal was present in that herd. All skin measurements (before and after) and test readings were carried out by the researcher himself.

2.6.2. Questionnaire survey

A structured questionnaire was administered by interview in the respondents preferred language. The questionnaire was developed through discussions with Dairy workers, veterinary professionals and researchers. The questionnaire was pretested and modified prior to final use. The questionnaire took 15–20 minutes to administer. Questions asked focused on Dairy workers, owners background, dairy practices routine herd practices, herd reproduction, feeding/ housing, cattle , and other risky behaviors predisposing to infectious diseases.

Based on the formula of (Arsham 2002), A total of 100 people which includes five farm owners HH / dairy workers / milk consuming customers / were also interviewed to assess their awareness on bTB and its transmission to humans. The mean age of the respondents is 81 (40-49) and the proportions of male to female were (63% and 37%)

A five page Questionnaires which each of these pages assess five different aspects of the risk to both peoples and cattle that were described in order as follows the overall Demographic and Socioeconomic Characteristics of Respondents, General management related risk factors assessment out put on frequency table (herd structure, access to vet. Service), Risk factors related to Public Health Risk Exposures to Zoonotic TB Prevention, Knowledge of bovine tuberculosis symptoms, prevention and its spread, and the Hygienic Milking Conditions with how kept Cleanliness of Housing Environment.

5 dairy cattle herds from A total of 69 dairy herds in Harar City and its surrounding Areas were examined for bTB with CIDT test. Mean age of the study animals was 5.1 years (95%CI: 4.85-5.34). The sex of all of the study animals was female (100%). The breed composition was cross-breed (50%) and zebu (50%). The questionnaire has got five parts. These are personal information of the farm owner, general information about the farm, questions related to risk factors to bovine tuberculosis, management and husbandry practices of the dairy farms, questions related to public health risk and awareness and knowledge of public health aspects as well as individual animal data recording. An enquiry was administered to those willing participants.

Information on of farm structure and management was collected from randomly selected large and medium sized herds using a standardized questionnaire with a closed type of questions. According to Msangi (1997) body condition score (BCS) of the dairy cattle was recorded generally as thin and medium (BCS = 1.0-2.0 and 2.0- 3.5, respectively).

2.7 Data collection

All the required data were collected based on the five pages Questionnaires and other farm related Animal related potential risk factors (i.e. sex, age, breed, and body condition score) were recorded when the CIDT test was performed.. Dairy herd owners were also asked for TB related personal information (such as educational level, knowledge on bTB and its zoonotic implication, and family TB history) and herd characteristics (such as dairy herd management system, herd size, keeping of different animals together, introduction of new cattle in to the herd) using a structured questionnaire after they were well informed and oral consent was obtained from each owner/ respondents..

2.8 Data analysis

Data collection on the prepared record sheet both the Skin measurements before and after was recorded along with animals ID No then Data were classified, filtered, coded using Microsoft Excel sheet and was transferred and analyzed using STATA version 14.2 regression analysis (Stata Corp., Collage station, TX). and for the questionnaire data used SPSS 20.and Pearson chi- square was used to evaluate the statistical significance of the associations of different categorical varia4bles with tuberculin skin test results.

Logistic regression interpreting odd ratio including the Multivariable logistic regression analyses were performed to quantify effects of pre-specified risk factors on tuberculin reactivity. P-value less than 5% was considered statistically significant. In cases of estimating the effect of different risk factors in terms of odds ratio (OR) with corresponding 95% confidence interval, statistical significance was assumed if the confidence interval did not include one among its values.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Prevalence Based on the SCITDT Tubercline Skin Test

Since the sampling technique was purposive having the different inclusion and exclusion criteria were set for farms and individual animals (lactating) such as farms were included if they have been established for over ten years farm, having the herd size of at least greater than (> 45) cattle , those who have willingness to have written informed consent for cattle to have SICCT test, agreed to conduct all technical duty until finalizing it. Even though the intended sample size was 384 due to different reasons such as the husbandry and farm setting differed somewhat from one study site to the other depending on the level of Awareness, educational status of farm owners and access of extension services. almost more number of farm owners, refused to agree to the informed consent for the study engagement/participation and affects the study in not to fulfill the required sample size, lack of Awareness' make them hesitant., and the study was proceed with the avail sample size,262.

In a total of 262 cattle from 5 herds were tested by using the SICCT test. In the Overall skin test performed there were 32 reactors (12.21. % total animal prevalence- not adjusted for herd size in 5 herds (100% herd prevalence; 95%), with each positive herd having at least one reactor (Table 1). Farm E had the highest prevalence (21.5% at animal level with 95% CI; 5.90 (12.7%- 27.42%) and 100%at herd level with 95% CI: 55–100%) among all the five dairy farms of the region's town where as farm A had the lowest prevalence (4.4% at animal level with 95% CI: 1.00* % and 100% at herd level with 95% CI: 0-100%). There was significant variation between study areas (among the study dairy farms) in prevalence of tuberculin reactors ($\chi 2 = 11.34.$, df = 4, p-value= 0.024). In this case one can see the larger number within inconclusive (8.748%) and the smaller number in positive at cut offs values of >4mm (decreased sensitivity). So from this one can understand and accepts /take the local cut offs as comprehensive without repeating the additional test to refine the larger inconclusive into +ve and -ve, by taking additional time &cost, too. Based on this understanding we also prefer the local cut offs than the OIE interpretation standard set. Because the standard has basic defects and so not being representative to all environments and breeds.

Table 1: Result of Tuberculin (SCITT) test prevalence based on the Amendment to African context (Ameni G, 2008)

Tested Farms	Total (%)	Positive (%)	Negative (%)	Prevalence based on the OIE standard		Amendment made to the African context (Ameni G, 2008)
				at > 4 mm cut off value	At > 2mm and < 4mm , as doubtful	
Farm A	45	2(4.44)	41	2.2	2.2	4.4
Farm B	45	4(8.89)	43	4.4	4.4	8.9
Farm C	63	6(9.52)	57	9.5	6.3	9.5
Farm D	44	6(13.64)	38	13.6	6.6	13.64
Farm E	65	14(21.54)	51	21.5	21.5	21.5
Total	262	32(12.21)	230	3.4	8.78	12.21

In this case one can see the larger number within inconclusive (8.748%) and the smaller number in positive at cut offs values of >4mm (decreased sensitivity). So from this one can understand and accepts /take the local cut offs as comprehensive without repeating the additional test to refine the larger inconclusive into +ve and -ve, by taking additional time and cost, too. Based on this understanding we also prefer the local cut offs than the OIE interpretation standard set. Because the standard has basic defects and so not being representative to all environments and breeds. All herds were positive for btB, since each herd were having 2- 14 range of skin test reactant against the test.

Table 2: Culture-based prevalence of Mycobacterium bovis among raw cow milk samples collected from reactors of tubercline skin test, post culturally examined microscopically by Ziehl-Neelsen staining method

milk samples from private dairy farm	No. of examined samples	Pre culture P/smear AFB	Post culture Positive smear for AFB (LJ –P)		Negative samples		Possible Identity
		No	No	%	No	%	
Dairy farm C	6	-	2	33.3	4	66.7	M. bovis
Dairy farm E	6	-	2	33.3	4	66.7	M. bovis
Dairy farm A	2	-	1	50	1	50	M. bovis
Dairy farm B	4	-	1	25	3	75	M. bovis
Dairy farm D	6	-	1	16.6	5	83.4	M. bovis
	23		7	30.4	16	69.6.	

The prevalence in the culture result of this finding on result part table growth on selective media important for Mycobacterium growth indicated that out of the 23 Milk samples subjected to culture on L-J media containing pyruvate only 7 (30.4%) showed growth positive, where as in L-J with glycerol there were no growth at all. This results indicated that L-J medium could be used for primary isolation, sensitivity testing, identification and sub-culturing of the majority of Mycobacteria as reported by Maureen (1981).This result has similarity with that of reported by Ameni et al. (2003) who finds (13.3%) from milk samples collected from Canadian cattle, this similarity might be due to sampling of milk from dairy reactors to CIDT in both studies , but (Saad El-din et al.,2013) reported low growth of 3(6%) and 1 (2%) milk samples from tuberculin positive and negative reactors. But the present study is higher compared to the study conducted by (Akililu et al., 2014 and Gad et al., 2000) who reported milk culture positivity of 9% and 9.3% respectively. At the same time, the present milk sample culture positivity indicated that it is slightly higher as put in the table 2. Above (30.4%) compared to the report of (Hamid et al., 2003) who recorded 28.07% and 25% culture positivity from milk of tuberculin positive buffaloes and cows respectively. Furthermore, the disease affects the livelihood of the dairy herders, and could have a negative impact on the herd replacement because of its effect on the production of calves Animal and herd Level btb Prevalence

A total of 262 lactating crossbred cows from 5 purposively selected dairy herds were tested using CIDT test to assess the prevalence of bovine tuberculosis (bTB). The number of tested dairy animals in the study area was 134 animals from medium sized herds and 128 were from large sized dairy herds. Out of 32 dairy animals 20 were found positive reactors for bTB. The overall apparent individual animal prevalence was observed to be 15.6 % (95% CI = 1.88(0.58-4.03)) when using the >2 mm. standard cut-off value. Out of the total 262 `dairy cows tested, 32 were positive reactors for tuberculin skin test with an overall prevalence of 12.21 %, while of the total tested, 20 of them were found positive reactors for bovine tuberculosis with an overall prevalence of 15.6 % (Table 4).

Table 3: Animal and herd level bTB prevalence for five dairy herds in the study areas.

Level	Farm C.	Farm E	Farm A.	. Farm B	. Farm D	Overall study prevalence %
Animal level: % Prev. (95%CI)	9.5	21.5	4.4	8.9	13.6	12..21
Positives	6	14	2	4	6	32
Total number tested	63	65	45	45	44	262
Herd level: % Prev.(95%CI)						
Positives	6	14	2	4	6	32

Table 4.: Relative differences in individual animal prevalence rate for cows between large sized and medium sized herds of dairy animals in 5 selected dairy farms in Harar City, Eastern Ethiopia.

Farm size(herd size)	Total No tested	Number positive	Prevalence (%)	Chi-square	P-value
Large Sized Dairy farm	128	20	15.6	11.34	<0.05
Medium Sized Dairy farm	134	12	8.9		0.103
Total	262	32	12.21		<005

Herd prevalence was 100 % (at >2 mm cut-off and 4.24 % (95%CI: 2.04-8.38) at >4 mm cut-off value. After adjusting for herd size, practice of proper drainage across the study dairy farms and introduction of new animal, the odds of herd positivity in a confined management system was 10.8 times higher than that of the smallholder local management. All the required secondary data related to the private dairy farms Data was collected from the Harari Regional Agricultural Bureau, based on it, in the total 69 dairy farms in the Harar town. All dairy farms hold about 1111 individual number of dairy cattle. Out of these total herds avail, for this study selected only 5 dairy farms selected purposefully by using certain inclusion and exclusion criteria such as considering the greater range (44-65) of herd size body condition, status hygienic conditions, proper drainage and others. as serious risk factor for the disease we were going to study. From these five private dairy farms only these 262 number of cattle we succeed only 262 number of cattle fulfilled inclusion criterion within five dairy herds but some more private farm owners are refused for the legal consent, and only those volunteer hence examined for bTB by the use of CIDT. All skin thickness measurement (before and after) was recorded along doing the tuberculin inoculation duty.

Individual Animal related potential risk factors (i.e. sex, age, number of calvings , body condition score ,lactating stage , water source new animal inclusion, biosecurity status farm establishment time, age, calving stage, number of calving, housing condition, No of calving and Proper drainage, Vet service delivery and etc.) are recorded when the CIDT test was performed.(Table 4)

3.1.2. Questionnaire survey

A structured questionnaire was administered by interview in the respondents preferred language. The questionnaire was developed through discussions with Dairy workers, veterinary professionals and researchers. The questionnaire was pretested and modified prior to final use. The questionnaire took 15–20 minutes to administer. Questions asked focused on Dairy workers, owners background, dairy practices routine herd practices, herd reproduction, feeding/ housing, cattle , and other risky behaviors predisposing to infectious diseases.

Results of the descriptive statistics analysis indicated that among those interviewed, 32% (32/100) of the respondents do not have knowledge on BTB and awareness of its zoonotic implication. A total of 100 people was interviewed and all responded to the questionnaires. Of these, 36 % of the respondents had consumed raw milk and categorized as having high-risk behavior whereas the remaining who did not consume raw milk (31%) were referred as “the low-risk group.” Including those mixed interest proportions (33%) also can be seen as slightly risky behaviour. Sixty-three percent (63/100) of the respondents were male, while 37% respondents were females and more than half of them (60/100) were in b/n the age range of 40–49 years. The univariate regression analysis also showed that among the socio-demographic variables, only the age variable was shown to have a significant difference across proportions of categories of variable as compared between low and high-risk groups ($P < 0.01$). 63% of respondents were male while 37% of respondents were females and the majority age category of respondents’ were b/n the age range of 40-49 which was 60.60% of respondents age was between 40-49 (the majority) while the least age category were b/n (18-29),which was (17%), following (30-39) which was about (12%) and the higher age range,(50-59) is about (11%)proportion. Regarding their education level Non-formal education (2%) Primary (35%) Secondary (16%) and the tertiary level (46%) including the un specified 1%.

Table 5: Demographic and Socioeconomic Characteristics OF Respondent.

Education level	Male		Female		Total	%
	Total No of cases	%	Total No of cases	%		
Non-formal edu.	0	0.00	2	5.4	2	2
Primary	24	38	11	249.7	35	35
Secondary	10	15.9	6	16.2	16	16
Tertiary	29	46	17	45.9	46	46
Others (specify)	0	0.00	1	2.7	1	1
Total	63	100	58	100	100	100

As described on the table 5 above the overall respondents' sex and education level distribution tells us respondents' higher education level (at Tertiary level education) for male 15/42(35.7%) while for females was 20/58 (34.5%) (At Tertiary level education). A questionnaire survey study that preceded the SICTT reported here showed that awareness was very limited. The hygienic conditions and sanitary services available to dispose waste water and organic material in the dairy farms was seen in some places within the study sites 30% Of the total respondents responded that they do have the feeding trough while the rest 70% responded that don't have the feeding trough (On table 6 below) 0nly 12% of the total respondents responded that they do have the watering trough while the rest 88% responded that don't have the watering trough.

3.1.3 Household Characteristics of the dairy farm owners

Out of the total 38 dairy herd owners assessed, 21 (55.3%) of them were females and female-headed dairy farms. Furthermore, 24 (63.3%) of the total dairy farm owners assessed were young people involved in the dairy business. 27 dairy herd owners had total household members less than five whereas 11 of the dairy farm owners had a family member ranging from 6-10. Educational status of the dairy owners was assessed and 19 of them were grade 9-12 whereas those with diplomas and above accounted 6. Six of the dairy owners were with basic writing and reading skills. None of the dairy farm owners were illiterate. As to the job status of the dairy farm owners, 17 of them were self-employees, none of them are retired ones and 13 of them were in other different job categorical positions (Table 5)

Table 6: General management related risk factors assessment out put on frequency table (herd structure, access to vet. Service) N=100

Statement	+Ve Frequency 'Yes'	-Ve Frequency, 'No'	%
Good ventilation Good/bad	26	66	26
Feeding frequency per day twice/.triple	49	51	49
Watering frequency per day once/twice	60	16	60
Have feeding trough Absent/present	30	70	30
Cleaning feeding trough once/twice	74	26	74
Have watering trough yes /No	12	88	12
Does you heard of bTB yes/No	26	17	26
Do have bTB screening test yes/No	60	40	60
New animal purchased Quarantined/isolation	68	32	68
Taking animals to veterinary clinic. yes/No	16	35	16

3.1.4 Husbandry and Management Practices of the Dairy Farms

This part encompassed personal information of the dairy farm owners. It has included information related to the dairy farms and associated risk factors to the occurrence of the disease problem. Furthermore, information regarding to the dairy farm management, husbandry practice and public health hazards of the dairy farms was included in the questionnaire survey. Regarding to awareness when respondents were asked whether they have heard of the btB disease only 26% of respondents responded as they have already heard of it, but 17 % of them responded as they have not heard of it.

Similarly to ventilation only 26% of respondents responded that the farm has sufficient ventilation; while 66% of them responded that their farm has no sufficient ventilation level. From the total respondents, Only 49% of respondents responded that the feeding frequency per day was about twice while 51% of them responded that they feed triple times per day of the total respondents, Only 60% respondents were respondents as their farm watering frequency of about twice to their cattle while 16% of them response about thrice/triple times per day

Similarly, with regards to the practice exercised, at times of new animal purchase, 68% of them responded as they let them join the herd immediately, while 32% of them responded to have a quarantine like practice for short time and to join the herd later.

From the total respondents Only 16% of respondents responded having a trend to take animals to vet clinic for access service delivery while 35 % of them are against this experience, not to take animals to veterinary clinic.

Table 7: Educational Status of respondents with age

Education level	18-29	No of anima	30-39	No. (%)	40-49	No. (%)	50-59	No. (%)	Sum	No. (%)
Non-formal	1		0		1		0		2	
Primary ed	11		12		1		11		35	
Sec	3		0		2		0		5	
Tertiary	2	2	0	4	56	26	0		58	32
Total	17	2	12	4	60	26	11		100	32

As the educational status of respondents shown on table .7. among total respondents the higher education level category was the top that engaged about 56 respondents while the least level of education category engagement data shows only 2 in number (Non-formal). The max number of male participants engaged in the study were 63/ while females were 37 both gender categories falls within the same category (40-49)-the same age category for both male and female. The least number of respondents engaged for the study that has also the least age level categories 18-29 (both genders share the same least age category for least participation in both gender). Based on education the higher (tertiary) level category the major respondents' engagement for the study are about (58) which were the higher level of education.

Regarding the risk related to Public Health Risk Exposures of Zoonotic Tb whether respondents have a raw milk consumption habit, and beliefs of consumers of raw milk, How do you prevent being infected With TB from cattle? Concepts of Disease prevention including awareness of disease transmission concepts and row milk drinking preference. the proportion of the respondents who had knowledge on bTB and its zoonotic implication were below 50% (48/100). This result is in line with the reports of (Gebremedhin Romha et al., 2014; Fikre Zeru et al., 2014), who indicated 35%, , 29.7% and 30.8% level of awareness in their study subjects, respectively.

Table 8: Risk factors related to Public Health Risk Exposures to Zoonotic TB Prevention n =100

Statement	Agreed 'yes' Frequency	Disagree 'No' Frequency	% %
How do you prevent tb infection from cattle a skin testing/vaccination	85	15	85
Accustomed to drink un pasteurized milk	36	64	36
What do you do when a dairy cattle has bTB culling / selling	39	61	39
Notifying veterinarian in case of infection	34	66	34
Would owners allows inspection of their animals	29	71	29
Do owners/farm workers use glove to pick up animal dung?	45	55	45
What do you do when you are infected with bTB? go hospital/not go hospital	67	33	67
you protect your wounds while handling animals.	65	35	65
do not eat while handling animals.	20	80	20
House have good ventilation Good/not Good	66	66	66

85% of respondents agreed on performing a skin testing as an option of prevention of bTB while 15 % of them responded the option for treatment of infected animal 36^% of respondents responded as they are accustomed to drink only raw milk while 64 % of them responded that they were using the practice of boiling before drinking. From the total only 39% of them responded that when their cattle has a bTB taking culling, as an option while 61% of them responded as preferring selling cattle to cattle market.

From the total respondents 34% of them when asked whether they notify veterinarian in case of infection responded positively as yes response, while 66% of respondents responded as not notifying to vets, which is against.

Body condition score (BCS) of the dairy cattle was recorded generally as thin and medium (BCS = 1.0- 2.0 and 2.0-3.5, respectively). Of the tested 51 thin body conditioned dairy cattle, all of them were found to be positive for bovine tuberculosis, while of the total 287 medium body conditioned dairy cattle tested, 45 of them were positive reactors. The prevalence of bovine tuberculosis in thin and medium body conditioned dairy animals was observed to be 100.00% and 15.6%, respectively (Table 4). A strong significant difference was observed for the prevalence of bovine tuberculosis between thin and medium body conditioned cows and heifers ($P < 0.05$) when using the 4 mm. arbitrary cut-off value test result. The significant difference due to body condition score of the dairy animals in this study was found to be one of the intrinsic host risk factors for the occurrence of bovine tuberculosis in the study area. As an important host risk factor, the differences in body condition score of the dairy animals in the study area have made the occurrence of the disease problem to be more prevalent in thin body conditioned cows and heifers than that of medium ones. This indicates that thin cows and heifers were more affected by the disease problem than medium body conditioned cows and heifer. This is in line with the fact that animals affected by the disease problem lose their body condition gradually and become emaciated due to the chronic nature of the disease.

3.1.5 Public Health Significance of BTB

All the respondents of the dairy farmers in this study answered that they and the member of their family used to consume unpasteurized or non-boiled milk as well as uncooked meat, and their contact with the dairy animals was closer than expected. 14 of the dairy owners (36.8**%)** responded they knew that bovine tuberculosis was a cattle disease and can be transmitted to humans through consumption of unpasteurized or non-boiled milk as well as via uncooked meat (Table 8).

In this finding, 5 (13.2%) of the respondents working in the dairy farms have noticed clearly having some of the clinical sign of the disease in the last two years. Besides, out of the 22 CIDT test positive herds, 3 (13.6%) of them had respondents with obvious clinical signs of tuberculosis. Thus, to reduce the public health risks arising from the disease, it is imperative to strengthen dairy farmers' awareness through extension services,

training and education program. Of the total respondents, 29% of them responded that they do allow inspection of their animals by professionals positively, as yes, while 71% of them responded negatively as No. Response

From the total respondents only 45% of respondents were using a hand glove positively when asked whether farm workers were used a hand glove or not to pick up animal dung, while 55%of them were responded as they were not using this protective hand glove responding No, as negatively. Regarding to public health related risks about 67% of respondents preferred and responded that if they were infected with bTB just as to go to the hospital while 33% of them responded not to go to hospital.

From the total respondents only 65% of respondents responded that they were protecting their wounds while handling Animals, but 35% of them were not used wound protective while handling animals At the same time with this risky behavior from the total respondents only 20% of them were not eat while handling animals without using wound protective while 80% of them were responded as they ate food and weared wound protectives while handling animals.

Regarding to farm ventilation, 66% of them responded that their house have a good ventilation while 26 % of them responded against (No). Similar study questionnaire Survey findings have shown that regular milk consuming owners, dairy workers; regular milk consuming communities are at the greatest risk from bTB. Furthermore, people with poor levels of knowledge living in marginalized areas are particularly at high bTB risk (WHO, 2010). Public awareness of hygienic measures, that can substantially reduce the risk of diseases in these settings, is often very limited (World Bank, 2010a). A questionnaire study elsewhere that preceded the SICTT reported here showed that awareness was very limited. The hygienic conditions and sanitary services available to dispose waste water and organic material in the dairy farm wanted emphasis in some places within the study sites. For success, sensitization meetings /awareness campaign should be held to increase/improve the current awareness status. In this study the different points asked in the above table there are a significant number of respondents with this risky behavior. On such cases, Success in reducing the public health significance of zoonotic diseases, greatly depends on the level of cooperation between medical and veterinary sectors, in the diagnosis of zoonoses, exchange of information, organization of shared surveillance systems, common training of staff and creation of community awareness (WHO, 2003).

Table 9 : Knowledge of bovine tuberculosis symptoms, prevention and its spread N-100

Statement	Frequency (agreed) (Yes)	Frequency Disagree (No)	%
7.Knowledge of clinical signs of TB in animal,	45	51	45
2.Does bovine TB affect humans?	64	36	64
3.Modes of transmission	72	28	72
4. bTB infection in man is preventable	61	34	61
5..knowledge of symptoms in humans	63	37	63
6. Boiling milk before drinking kills the bacteria	19	78	19
7 .Separating animal pens from humans reduce zoonotic TB	3	94	3
8. BTB in man is curable yes/No	71	27	71

Regarding the knowledge, of the total respondents only 45% of respondents have the relevant knowledge to the disease while 51% of do have the knowledge gap. At the same time 36% of respondents were did not have know how of the disease that it affects humans, while. 64 % of them were awared of it. similarly from the total respondents about 37% of them lacks Knowledge of symptoms, while 28% of them lacks the disease mode of transmission, regarding to the awareness, from the total respondents 34 % of them lacks the know-how related to that bTB infection in man was preventable, again of the total respondents 27% of respondents have a gap in relation to knowing the bTB curability in man as a gap. Of the total respondents 78% of them did not know that boiling milk before drinking kills the bacteria organism, while 19% of them were awared of it. Of the total respondents 94% of them did not know about the practice of Separating animal pens from that of humans reduce infection with zoonotic TB, while only 3% of respondents know the value of this practice, positively. Of the total respondents 58% of them responded that they know as positively responding as yes. While the rest 42% respond as did not know that it affect humans.(did not know the zoonotic nature of the disease). This means that a significant percent of the population were an aware of the value of raw milk boiling before drinking, again also similarly did not know the zoonotic nature of the disease, did not know

about the practice of Separating animal pens from that of humans reduce infection with zoonotic TB,also significant number of respondents lack knowledge of symptoms and mode of disease transmission others lack/knowledge /don't know as bTB infection in man was preventable.

Table 10: Hygienic Milking Conditions with how kept Cleanliness of Housing Environment N=24

Statement (Risk Factors)	Total	+ve Frequency	-ve Frequency	%
Hygienic milking conditions?		7	17	29.2
have good drainage ?		6	18	25
remove manure regularly (kept clean and dry)		23	1	95.8
access of people to the farm		15	9	62.5
how frequent checking animals health		16	8	66.6

Regarding the overall hygienic milking conditions (7/24) 29.2% proper and while the rest 17/24) 70.8% have still lacks proper procedural performance. Of the studied private dairy farms a Proper drainage was also assessed and this practiced with in the level of (25%) while the rest 75% were against to this practice. Of the total respondents .95.8*% of respondents response reveals that they remove manure regularly (kept clean and dry) regarding access of people to the farm,62.5% (15/24),allows access to the farm while 62.5 respondents were not allow to enter into the farm. Whatever reason was there, this might be attributed to minimize the risk of pathogen entrance by different fomites. 66.6% (16/24) of respondents response tells that two times per day (at morning and in the evening), while 33.3% (6/24) responded at the rate of twice per week.

The hygienic and sanitary condition of the dairy farms was assessed in this study. This was done based on the ventilation, odor, and animal stoking density, and waste disposal, floor and animals as well as general cleanness of the dairy farms. Most of the time the Hygienic measures are recommended for the control of TB among cattle. The infected animal, which is a potential source of infection, should be identified through periodic application of the intradermal tuberculin test and removed from the herd by destruction or by in either segregation.

In subsequent comparison of dairy farms with regard to hygienic and sanitary standards as poor versus medium, occurrence of higher bTB exposure was detected in hygienically poor herds than medium dairy herds. Accordingly, of the total 5 dairy herds tested in sanitarily larger Dairy farms, 2 of them (38.5%) were found relatively to have more positive reactors for bTB, while of the total 5 dairy herds tested in sanitarily medium dairy herds tested, all of them (100.0%) were found to have a positive reactors for bovine tuberculosis which was found to be statistically and significantly different ($p < 0.05$).

Considering important aspects to evaluate and Ensure housing environment was clean at all times, Ensuring Whether milking area was kept clean , Ensuring Whether the milkers follow hygienic rules , Ensuring Whether milk storage area is clean and neat , Ensure Whether milk storage equipment is adequately cleaned to hold milk , How frequent /Regularly check animals for signs of disease? Ensure whether the farm have a good drainage or not? and ensuring whether remove manure regularly and and beds be kept clean and dry or not.

Therefore, there is a need for increased public health education to raise awareness on the consequences of consuming potentially TB bacteria contaminated, unboiled or un pasteurized milk, especially for those who are immune-compromised. Regular annual TB testing of all cattle and reactor's elimination from milk supply chain should be supported by government organizing these private farm owners them up to the level of strengthened and legally registered cooperative through provision of access to credit to fulfill different materials they are in need such as milk pasteurizing plant or machine to safeguarding the health of milk consumers and control the spread of the Agent/ pathogen.

Results from different Hygienic measures taken by dairy workers and owners on their dairy farm signifies that there was a significant difference observed for the prevalence of bovine tuberculosis between hygienically and sanitarily medium and poor standard of dairy farms and dairy cattle ($P < 0.05$) when using the 4 mm. arbitrary cutoff value test result. Hence, the significant difference due to farm hygiene condition of the dairy animals in this study was found to be one of the decisive risk factors for the occurrence of bovine tuberculosis in the study area. As a decisive environmental risk factor, the differences in farm hygiene and sanitation

condition of the dairy animals in the study area have made the occurrence of the disease problem to be more prevalent in hygienically poor farms than that of medium ones. This indicates that cows and heifers in hygienically poor farms were more affected by the disease problem than those in hygienically medium farms.

In this study, the significant difference in milk production between the test positive and the test negative dairy cattle could mainly be attributed to the negative impact of bTB on milk production. The animals affected could have a capricious appetite and lack the usual consumption of feed. Thus, progressive loss of body weight results in low body condition score of the animals. Subsequently, the animals affected by the disease gradually lose the normal physiological milk production capability. Hence, the whole dairy business as well as its value chain players will be affected greatly. In this study, the frequency and occurrence of CIDT test positive reactor dairy cattle was significantly increased in animals which were recorded as poor body conditioned cattle. This explains body condition of dairy animals to be one of the possible potential risk factors for the occurrence of bovine tuberculosis. (Balako **et al**(2013) Pertinent to this risk factor, reports are available that disclosed the influence of this intrinsic risk factor which influences the infection dynamics of the disease in dairy cattle population (Ameni ,Mammo et al (2009)

In fact, there are evidences of high prevalence rate of bovine tuberculosis in poor body conditioned dairy cattle Ameni et al (2009); Regassa et al (2008;, Ameni et al (2003a). However, this does not under rate the effect of several intrinsic and extrinsic factors such as level of nutrition and availability of feed, the animals' age, breed and some chronic diseases which affect the body condition score of dairy cattle other than BTB. Another risk factor that has shown to contribute for increased frequency of positive reactors for CIDT test among the study herds and individual animals was the hygienic and sanitary standards. Herds and individual animals in poor hygienic and sanitary standards of the farms were more affected than those in medium farm hygiene and sanitation conditions. Since the dairy farms in hygienically poor farms were less ventilated and the circulation of clean and fresh air was not enough, the occurrence of the disease was observed to be more prevalent.

This can be attributed to the fact that the poor hygienic and sanitary standards of the dairy farms could have enhanced the multiplication of the causative agent of BTB. As the dairy herds get sanitarily poor, the floor of the house becomes moist and shady which favors the multiplication of the causative agent (M. bovis) of the disease problem. This, in turn, facilitates further contamination of apparently healthy dairy animals in the herd. Hence, further transmission of the disease occurs and animals in the dairy farm get infected and diseased. This finding is in line with the finding of Ameni et al. (Ameni et al (2009**)**] who has reported cattle husbandry and management in Ethiopia was a predominant factor for affecting the pathology of bovine tuberculosis. In fact, this study could have produced a stronger evidence for the association of this extrinsic risk factor to the occurrence of the disease problem.

The prevalence and associated potential risk factor findings of bovine tuberculosis in this study among the dairy herds and individual animals is a strong evidence that is needed for prevention and control intervention for dairy cattle and for their surroundings. However, it should be noted that the effects of the aforesaid potential risk factors which are associated with the occurrence of the disease are varied in different dairy cattle production systems, and other possible risk factors associated which have to be presumed.

Associated risk factors

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

Thirteen pre-assumed potential risk factors, based on knowledge and understanding of the husbandry system and biological relevance were considered and screened by both univariate and multi variable analysis. For this investigation Associated risk factors// independent variables/ considered to collect at both the animal , herd levels, and management level were recorded those risk factors at the animal levels such as the following age Number of calving, Body condition score (BCS) Lactation stage (months),,, the Second category risk factors were at the herd levels were listed as follows water source ,proper drainage , new animal inclusion, biosecurity status, New animal inclusion , housing conditions , Time Establishment , Herd size, Biosecurity Status, and proper drainage.. and the third management related risk factors category were the farm site, time

of establishment, herd size, housing condition, vet. Service delivery including the dependent outcome variable /bTB status, the Body condition of the animals was determined according to (Nicholson, M. J. and Butterworth, M. H., (1986.) as poor, medium or good. Poor body condition score (p=0.001) were considered for extremely lean cattle with projecting dorsal spines pointed to the touch and individual noticeable transverse processes. Cattle with usually visible ribs having little fat cover and barely visible dorsal spines was expressed as a medium body condition score. A good body condition score was articulated for the animals when fat cover is easily observed in critical areas and the transverse processes were not visible or felt.

The management condition (sanitation status) of the studied farms was categorized as poor, medium (satisfactory), or good as described by Ameni et al. based on A biosecurity status such as odor, neatness, waste drainage, nature and cleanness of the floor and animals, light source, ventilation, presence of confinement), feeding practice (concentrate and hay), possession of an exercise yard, and contact with other nearby herds and provision with clean water. The herd size of farms under the study is categorized into two groups.(40–50 and > 50) was statistically significant (p=0..0042) , number of calving, lactation stages (1st stage , 2nd Calving , 3rd calving stages) , Age of the farm establishments is defined as number of years the farm owners started rearing cattle. It was classified in three categories, 7-10 years, 10-15 years, and >15 years of work at dairy cattle farming.

3.1.7 Univariable regression analysis for risk factors

Thirteen potential risk factors, based on knowledge and understanding of the husbandry /management system and biological relevance were considered and screened by univariable regression analysis (Table, 11 below). four risk factors with p-value of < 0.30 and with OR > 1 were selected for multivariable analysis. Proper drainage, farm and time of farm establishment did not fulfill the stated criteria and were excluded from analysis. A full description of the measured risk factors is provided in Table below. Total number of examined animals (3rd column in Table 4) used for analysis of respective risk factor may differ from the overall number of animals tested (N = 262) to check the presence of missing values. Multivariable analysis of potential risk factors for positive cattle reactors using GLMM and Based on their high OR, absence of multi collinearity and statistical significance (p-value).

Additionally In cases of estimating the effect of different risk factors in terms of odds ratio (OR) with corresponding 95% confidence interval, statistical significance was assumed if the confidence interval did not include one among its values. When poor Biosecurity status has was1.15 times more at risk than the medium in status and at the same time when there is a practice of inappropriate drainage happen there is a 3.16 times increase to the more risk of bTB positivity. than the other category. So as one number of calving increase there is a 1.86 times increased the risk of bTB positivity (comparing the different calving number cows the factor to decrease or increase be 1.36) though not statistically significant, as water is a vehicle to microorganisms water source has 1.37 times increase of the risk to btB positivity.; as the herd size increases the risk concurrently by 1.88 times increase to the risk of bTB positively as a predisposing factor.; as the number of calving increases by one there is 1.36 times increase of risk to bTB.

In this study, Larger herd size is 1.9 times exposed to bTB positivity than the medium herd size having <50 herd size was observed (OR 1.88(0.58-4.03)). Regarding body condition score category the poor body condition s category were 1.24 times exposed for btB positivity than the medium body conditions core animals (OR 1.46 (0.58-3.65)) Medium to good medium (1.18) to poor (1.46times at risk than the good) those herds that exercise the new animal inclusion practice were 1.15 times more exposed to the btB positivity than those herd didn't practice. The new animal inclusion in to their herd. (OR 1.15 (0.52-2.55). Dairy herds that access vet. Service deliveries by using only on call basis are 1.19 times more exposed to the disease in question than the herds that access the vet service regularly on weekly basis. (OR 1.19 (0.56-2.56)).

Table 11: Univariable regression analysis for the Evaluation of the different risk factors with the prevalence of bovine tuberculin positivity in / 5 / selected Harar town dairy farms

Variable/	Category	Examined	Positive	Prevalence/ Percentage (%)	OR (95% CI)	P-value
Farm	A	45	2	4.44	1.00*	
	B	45	4	8.89	2.10 (0.36-12.08)	0.407
	C	63	6	9.52	2.26 (0.44-11.77)	0.332
	D	44	6	13.64	3.39 (0.65-17.83)	0.149
	E	65	14	21.54	5.90 (1.27-27.42)	0.024*
Age	<6yrs	169	13	7.69		
	>6yrs.	93	19	20.43	3.08(1.44-6.57)	0.004*
No of calving	<3	173	17	9.83		
	>3	15	15	16.85	1.86 (0.88-3.93)	0.104
Body condition	Good	104	11	10.58		
	medium	90	11	12.22	1.18(0.48-2.86)	0.719
	poor	68	10	14.71	1.46 (0.58-3.65)	0.421
Lactation stage	early	63	9	14.29		
	mid	83	11	13.25	0.92(0.35-2.37)	0.857
	Late	116	12	10.34	0.69 (0.27-1.75)	0.436
Vet.service	weekly	108	12	11.11		
delivery	On call	154	20	12.99	1.19 (0.56-2.56)	0.648
Herd size	<50	134	12	8.96		
	>50	128	20	15.63	1.88(0.58-4.03)	0.103
Biosecurity	medium	89	10	11.24		
	poor	173	22	12.72	1.15 0.52-2.55)	0.729
Proper	Yes	89	8	8.99		
drainage	No	173	24	13.87	1.63 (0.70-3.80)	0.256
Water source	Pipe	107	11	10.28		
	stored	155	21	13.55	1.37(0.63-2.97)	0.428
New.animal	No	89	10	11.24		
inclusion	Yes	173	22	12.72	1.15 (0.52-2.55)	0.729
Housing	Good	62	6	9.68		
condition	medium	156	20	12.82	1.37 (0.52-3.60)	0.520
	Poor	44	6	13.64	1.47(0.44-4.91)	0.528
Time of	7-10	45	6	13.33		
establishment	10-15	65	14	21.54	1.78(0.63-5.06	0.277
	>15	152	12	7.89	0.56(0.20-1.58)	0.271

Results from multivariable logistic regression analysis are summarized on (Table: 12) below. The body condition, animal with poor body condition as compared to animal with medium or good body condition was identified as a significant risk for being positive reactor although the precision of the estimate was low as indicated by wide confidence interval (0.48-2.86). By using Multicolliniarity- test revealed that they are collinear with herd size for these reason omitted the following three variables time of farm establishment---proper drainage, and farm site.

Table 12. Multivariable logistic regression analysis of risk factors for bovine tuberculin reactors in selected dairy farms of harar town.

Risk factor	Odds Ratio (OR).	Std. Err	Z-values	p-values	95% CI for OR
Age	2.232709	.8916459	2.01	0.044*	1.020703 - 4.883876
No. of calving	5888072	.267939	-1.16	0.244	0.2413407 - 1.436533
body condition	.390217	.114877	-3.20	0.001*	0.2191384- . 6948546
herdsize2	.390217	.9585256	2.03	0.042*	1.031169 - 5.213366
_cons	1166067	.1266747	-1.98	0.048	0.0138684 -- .9804359

The present animal and herd-level prevalence was considerably larger similar range of estimates (12.21 - 100%) respectively. This happens may be due to the lower sample size, design & the probable herd size favors transmission of the disease. The herd range variation in the prevalence of tuberculous reactors was thought to be attributed to differences in the herd size of the study animals. As the majority of the herds in the present study were ranging between 44- 65 holding two categories (i.e. medium and large comprising greater than 50 and less than 50 animals, and management conditions favoring the spread of bTB, such as overcrowding and poor ventilation, were less likely to have influenced the prevalence of infection. In the present study, age of the animals was found to be significantly associated with positive tuberculin reaction. In the multivariable analysis of the risk factors considered, lactation status of the animals was another factor found to be significantly associated with tuberculin reactivity although this was not evident in the univariable analysis'.

3.2 DISCUSSION

In the present study the highest prevalence was recorded in farm E, which is 21.5 %. As one can easily compared to the following Descending ordered listed from 'the smallest prevalence to the highest' be ordered was put as Farm C,, Farm B, Farm A, Farm D and,, Farm E. with their respective Disease prevalence rate of 4.4 % ,8.9% 9.5% , 13.6% and 21.5% , respectively. and where the highest prevalence of tuberculin positive reactivity was 21.5 % in the age group between 5-6 years as compared to the younger 2-3 y and older > 6 age group. The prevalence of the medium & large herd size has a great difference, which is lower than that of the large herd size, which is 21.5 %. The animals with medium body condition score have the lowest prevalence 43.8% than those of the poor body condition score (56.3%) of tuberculin positive reactivity (Table 11).

The present study has identified herd size as one of the herd level risk factors for bTB spread which concurs with previous studies carried out in several parts of the world. It has been shown that bTB positivity is higher in larger herds than smaller ones (Cleaveland et al (2007); (Cleaveland et al (2007;Firdessa et al., 2012; Inangolet et al ., 2008; Kaneene et al ., 2002; Munyeme et al ., 2008; Olea-popelka et al .,2004; Porphyre et al .,2008). This may be related to the increased chance of bTB transmission in larger herds, possibly due to high stocking density in combination with poor ventilation Ameni et al.,2006; Dejene et al., (2016) ; Huang et al .,(2013). However, other confounding factors in the management, trading and grazing practices between large and small herds may also contribute to this so-called density dependence in transmission Begon et al ., (2002; Skuce et al., (2012).

The present study has identified herd size as one of the herd level risk factors for bTB spread which concurs with previous studies carried out in several parts of the world. It has been shown that bTB positivity is higher in larger herds than smaller ones.The herd and individual animal prevalence rate (100 % and 12.21 %, respectively) of bovine tuberculosis observed in the current study substantiates the fact that bovine tuberculosis is one of the infectious diseases affecting the dairy cattle population in the study area. This study showed that large sized dairy herds with Holstein-Friesian breeds of cattle were more affected than small sized ones by the bovine tuberculosis. Thus, the prevalence of the disease was higher in large sized dairy herds than the small sized counterparts.

The significance difference observed for the occurrence of bTB between large sized dairy herds and small sized ones could be attributed to the fact that as the dairy herds get a greater number of dairy animals, it made them more prone to the disease. This result is in line with the study findings reported by Firdessa et al, **(2013).**. Besides, as the large sized dairy herds were relatively more intensified and with more input supply, it might have made them more prone to the disease problem. Since the large sized dairy herds were crowded and overstocked by more number of dairy animals, the probable occurrence of the disease in them was relatively higher and the animals in them were being more infected.

Indeed, among the different studies undertaken, the herd level prevalence rate of bovine tuberculosis ranged from 43.4% to 50% by Firdessa et al Firdesa et al. (2013) , while the individual animal prevalence rate of bTB ranged from 3.4% in a small holder to 50% in intensive dairy production systems which have been reported in various places of the country (Ameni G. and Wudie A. 2003.;. , Ameni G., Bonet P. and Tibbo M. 2003a, Asseged B.,et al (2000), Firdessa R.,et al (2012) and Regassa, A., (2005). Hence, the large sized dairy herds necessitate more attention with regard to management and husbandry practices. Suitable housing system has to be facilitated in order to keep the appropriate dairy farm standards. Thorough follow up and Animal health care should be taken for the large sized dairy farms to protect them from further infection by the disease.

3.2.1 Animal prevalence and associated host risk factors

The current animal level BTB prevalence of 3.55% at ?_2 mm cut-off is comparable to the previously reported prevalence of 3.0% and 4.02% by other studies in different sites of Ethiopia (Tschopp et al., 2009; Firaol Tamiru et al., 2013) but significantly lower than the previously reported prevalence of 7.0% in southern Ethiopia (Balako Gumi et al., 2011), 18% in Afar (Gezahegne Mamo et al., 2013) and 6.8% Meskan, Gurage region, central Ethiopia (Tschopp et al., 2011). Animal prevalence of 1.27% recorded at >_4mm cut-off value was similar to the prevalence of 2.0%, 1.0% and 1.56% in Ethiopia (Balako Gumi et al., 2012; Firaol Tamiru et al., 2013; Petros Admasu et al., 2014) and 2.6% in Zambia (Pandey et al., 2013) while it was lower than 11.0%, 23.7%, 5.5%, 30.0%, 11.0%, 4.3% and 11.3% in Ethiopia (Gobena Ameni and Erkihun Aklilu, 2007; Elias Kebede et al., 2008; Gumi et al., 2011; Rebuma Firdessa et al., 2012; Gezahegne Mamo et al., 2013; Gebremedhin Romha et al., 2014; Fikre Zeru et al., 2014), 7.13% in Ecuador (Proano-Perez et al., 2009), 14.3% in India (Thakur et al., 2010) and 5.9% in Bangladesh (Mondal et al., 2014). The variation observed among the studies might be attributed to differences in management practices, production system, types of cattle breeds largely involved in the study, or differences in ecological zones. Moreover, the herd size in the majority of our study herds was small and maximum two tuberculin positive cases was recorded in few herds. This might also explain the low prevalence recorded in the present study compared to other studies, which reported high BTB prevalence.

Similar to other recent studies in Ethiopia (Petros Admasu et al., 2014; Gebremedhin Romha et al., 2014; Fikre Zeru et al., 2014) and India (Thakur et al., 2010), there was strong association between breed type and tuberculin skin test positivity in the present study at ?_4 mm cut-off value. The probable. Justification could be associated to the relative resistance of the zebu cattle to TB as compared to Holstein and other cross breeds (O'Reilly and Daborn, 1995; Gobena Ameni et al., 2007; Cadmus et al., 2010). However, our finding differed from the reports of Elias Kebede et al. (2008) and Rebuma Firdessa et al. (2012) in such a way that their study herds were composed of largely Holstein and cross breed dairy cows exclusively kept under intensive management system. Although the estimate is less precise, study body condition score in the current study was significantly associated with positive skin reactivity. Animals with poor body condition status were associated to the increased risk of bovine tuberculin positivity as compared to good body conditioned cattle. This result is in agreement with the previous studies (Elias Kebede et al., 2008; Petros Admasu et al. , 2014; Fikre Zeru et al., 2014) although other findings did not report similar result (Rebuma Firdessa et al. (2012). Poor body conditioned animals have relatively weak immunological responses to TB and subsequently susceptible to the infection (Griffin et al., 1993; O'Reilly and Daborn, 1995).

Analysis of sex and age in relation to bovine tuberculin positivity in the present study were found statistically not significant (p=0.619 and p=0.444, respectively). In agreement with some previous studies in Ethiopia (Elias Kebede et al., 2008; Petros Admasu et al., 2014) there was not significant association of age and sex with reactivity in the current study. Other previous

Ethiopian studies (Gobena Ameni and Erkihun Aklilu, 2007; Elias Kebede et al., 2008; Balako Gumi et al., 2011; Gezahegne Mamo et al., 2013), a study from Ecuador (Proano-Perez et al. (2009) and another study from India (Thakur et al. 2010) reported significant association between age and positive reaction. Likewise, an Ethiopian study (Fikre Zeru et al. 2014) reported significant association between sex and tuberculin skin test positivity. In our study, the majority of cattle were female (87.9%, 6931788) and age group between 5-9 years (33%, 2601788), which may explain the observed variation.

3.2.2 Herd level prevalence and associated risk factors

The herd prevalence of 5.21 % recorded in the present study was comparable with a recent report of 7.02% (Firaol Tamiru et al. 2013) but much lower than 41.9%, 51.4%, 50%, 44%, 15.3% and 20% reported by other recent Ethiopian studies (Balako Gumi et al., 2011; Worku Tigre et al., 2011; Rebuma Firdessa et al., 2012; Gezahegne Mamo et al., 2013; Gebremedhin Romha et al., 2014; Fikre Zeru et al., 2014). The disparity of herd prevalence reported among the different studies might be attributed to the differences in the available different epidemiological conducive conditions favored for the transmission of BTB (such as herd size, the level of intensive husbandry system practiced, the amount of susceptible breeds available in the herd, mobility and close contact between different susceptible animal species in the study area). Significant association of dairy cattle management system and herd positivity observed in the current study is in agreement with the findings of earlier studies (Elias Kebede et al., 2008; Gebremedhin Romha et al., 2014; Fikre Zeru et al., 2014). A higher herd prevalence of BTB in intensive dairy herds as compared to smallholder in this study

might be related to their confinement and breed type. In the current study nearly 100% of the animals were cross breeds.

Previous authors also argued in the same ways (Gobena Ameni et al., 2006) and demonstrated that cattle kept under intensive conditions showed significantly higher prevalence as compared to those managed under extensive system. In contrast to our finding other studies (Elias Kebede et al., 2008; Gebremedhin Romha et al., 2014; Fikre Zeru et al., 2014) reported significant association between herd size and herd positivity. Likewise, significant associations were reported between herd positivity and keeping of other animals with cattle (Tschopp et al., 2009) and herd positivity with introduction of new cattle in to the herd (Tschopp et al., 2009; Proano-Perez et al., 2009).

In the present study the proportion of the respondents who had knowledge on BTB and its zoonotic implication were below 50% (25%, 48/192). This result is in line with the reports of (Gobena Ameni and Erkihun Aklilu, 2007; Worku Tigre et al., 2011; Firaol Tamiru et al., 2013; Gebremedhin Romha et al., 2014; Fikre Zeru et al., 2014), who indicated 35%, 37.1%, 80.7%, 29.7% and 30.8% level of awareness in their study subjects, respectively. The zoonotic risk of BTB is often associated with consumption of untreated milk and meat products as well as via aerosol in the proximity to livestock (Cosivi et al., 1998; Wilkins et al., 2008). This urges the integrated work of medical and veterinary personnel to build awareness with regard to BTB and its public health aspects in the study area.

4. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

The isolation of M. bovis in freshly drawn milk from the tuberculin positive reactor cows is being reported for the first time in Harar town. The study emphasizes the risk of transmission of TB to human through consumption of contaminated unpasteurized milk and milk products. There is a need for enhanced public health education to raise awareness on the consequences of consuming potentially contaminated milk. Also, measures need to be adopted to test and eliminate positive reactor cows from the dairy supply chain. As the proportion of cattle affected with bTB in each herd was small, culling of the positive reactors could be part of a feasible control measures.

This study showed a relatively lowest prevalence in the emerging dairy regions of harar as compared to the prevalence observed in the established dairy belt in the central parts of Ethiopia, The important risk factors identified here are the Herd size, proper drainage, housing, biosecurity, introduction of new cattle from other herd, age and animal origin can be mentioned. Implementation of control program in such cities at this stage could be effective to reduce or possibly stop further bTB transmission between cattle and to reduce the likely zoonotic impact.

A zoonotic risk of BTB is often associated with consumption (ingestion) of dairy products based on unpasteurized milk infected with M. bovis. Also, aerosol transmission from cattle-to-human should also be considered as a potential risk factor. Milk consumers Around harar generally prefer raw milk (as compared to treated milk) because of its taste, availability and lower price. The disease transmission may be cyclical: cow-to-man-to-cow, underlying the existence of higher risk of dissemination of mycobacteria among the cattle and human populations.

The fact that severe tuberculosis is linked to high degree of disease transmission potential warrants implementation of proper disease surveillance programs in large-scale farms. Isolation of M. bovis from mammary gland implies a potential threat of zoonotic transmission, where raw milk constitute a customary dietary regimen in the study area. Prevention of transmission in milk offers the better approach for human risk mitigation in the area but requires strategies that improved risk awareness amongst producers and regular Milk consumers.

It is therefore a need for increased public health education to raise awareness on the consequences of consuming potentially TB bacteria contaminated, unboiled or unpasteurized milk, especially for those who are immune-compromised. Regular annual TB testing of all cattle and reactor's elimination from milk supply chain should be supported by government through provision of farmer's compensation and budget allocation to veterinary department for annual tuberculin testing.

4.2 Recommendations

Based on this conclusion the following recommendations are forwarded.

- Before embarking on any control programme it is essential that all dairy farms (because of high prevalence) should be registered and that all dairy cattle older than six months of age are identified with permanent marks, at least tagged with ear tags. At present, tagging is practiced in intensive dairy farms, but it does not yet cover all dairy farms,.
- There is a need of dairy plants that collects milk and performs standardized pasteurized milk packed in plastic sachets to the market from all private dairy farms in harar town.
- Routine livestock disease surveillance should be established to identify and avoid possible hazards to the public health.at the same time.the current molecular level biological tools need to be part of the routine TB diagnosis program in the regional laboratories. This is accomplished through the systematic collection and evaluation of morbidity and mortality reports and other relevant health information, and the dissemination of these data and their interpretation to those involved in disease control and public health decision making.
- Success in reducing the public health significance of zoonotic diseases, greatly depends on the level of cooperation between medical and veterinary sectors, in the diagnosis of zoonosis, exchange of information, organization of shared surveillance systems, common Education training of staff private dairy farm owners /investors workers and creation of community awareness, about the routes of transmission of TB, importance of timely diagnosis, prevention and control methods is vital.
- To decrease risk of transmission we have to encourage farm owners who perform restocking by purchasing animals to request that the animals should be tested for bTB. A possibility for Ethiopia to in part meet the demand for cattle of upgraded breeds could be to establish farms with dairy cattle free of bTB and from which farmers could restock their farms without the risk of introducing the disease into their herds.
- Government authorities should encourage and support farm owners to regularly test their animals for bTB and create incentives to keep their herds free from bTB. So, support should include budget allocation to veterinary department for annual tuberculin testing.
- Awareness campaign holding the following points for dairy farms around the city Conducting a systematic tuberculin testing, Adoption of quarantine, testing of the newly acquired, apply farm certification by establishing -farm free of bTB, Health education and heat treatment of milk.
- Milk at the Market place should come from registered herds that are subject to have an official tuberculosis control plan; the control plan should include herd inspection and herd testing for tuberculosis every year to minimize the risk of delay in detecting infected animals. Likewise, dairy herds kept for milk production should be subject to have an official tuberculosis control plan that addresses public health concerns in terms of food safety.

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