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Research Article

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Bovine Tuberculosis: A cross-sectional study in dairy farms of Arsi Negele and Wondogenet town, Southern Ethiopia.

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Abstract

Bovine tuberculosis (BTB) is highly prevalent diseases in intensive and semi-intensive dairy farms in Ethiopia. A cross-sectional study was conducted between December 2020 and May 2021to estimate the prevalence of bTB and identify the potential risk factors in Wondogenet and ArsiNegele towns. A total of 755 cattle from 153 herds were tested for bTB reaction using comparative intradermal tuberculin test. Data on individual animal and herd level factors were collected using a semi-structured questionnaire. The finding was summarized both in descriptive and analytical statistics *i.e.*univariable and multivariable logistic regression to identify some potential risk factors. Accordingly, the individual animal level prevalence was 7% (95% CI: 5.4-9.0%) at the cut-off ≥ 4 mm point. These reactors were distributed in 26.1% (95% CI: 19.5–33.3%) of the herds. Prevalence recorded at cut-off >3mm was 11% (95% CI: 8.9 – 13.4) and 33.3% (95% CI: 26.2 - 41.2) at individual and herd level and increased to 40% (95% CI: 32.3 – 47.9) and 16.7% (95% CI: 14.2 - 19.5) at herd and individual level, respectively, when > 2 mm cut-off was used. In a multivariable logistic regression analysis, older age was identified as in association with increasing number reactors significantly ($p<0.05$) at individual animal level. Likewise, at herd level, poor standard of housing, presence of farm animals' contact and larger herd size were the presumed predictors identified to have significant association with bTB reactors. Regarding the level of awareness, among one hundred fifty six respondents interviewed, 46% of respondents consume raw milk. 55% of respondents knew that cattle can be infected with tuberculosis, while, 35.3% were aware of bTB possible transmission from animals to humans. Among these respondents, 25% of them knew that bTB could use raw milk as a vehicle. Therefore, the responsible stakeholders should emphasize farmers' awareness creation on the zoonotic nature of tuberculosis and the need for developing control strategy.

Keywords: *ArsiNegele, Bovine, Dairy Farm, Prevalence, Tuberculosis, Wondogenet.*

Introduction

Ethiopia has large potential for dairy development due to its large livestock population, which accounts 59.5 million cattle, 30.70 million sheep, and 30.20 million goat populations (CSA, 2017) with dairy cattle having potential production of 3.8 billion liters of milk per year (FAO and NZAGRC, 2017). However there is development of dairy production system in urban and peri-urban areas, diseases like bovine tuberculosis was one of hindering effect on the production system in Ethiopia (MoA, 2015).

Tuberculosis (TB) is a chronic granulomatous disease of humans and animals caused by a group of closely related bacteria termed as *Mycobacterium tuberculosis* (*M. tuberculosis*) complex (MTBC) (Brites and Gagneux, 2017). The main pathogenic species of the MTBC members are *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. caprae*, *M. pinnipedii*and *M. microti*(Forrellad*et al*., 2013). The most important types of tuberculosis are bovine TB (bTB) caused by *Mycobacterium bovis*, avian TB caused by *Mycobacterium avium*, and human TB caused by *Mycobacterium tuberculosis*(AFF, 2016). Besides their primary hosts, MTBC species infect other secondary hosts (Smith *et al*., 2006).

The potential factors for transmission of *M. bovis*and other mycobacteria between cattle and humans are the presence of close contact of animal and humans or the rural societies living together with their animals in the same microenvironment and house, and the prevalence of HIV increasing and HIV patient's susceptibility to TB (Olmstead and Rhode, 2004; Mbugi *et al*., 2012; Tekle *et al*., 2020).The Veterinarians, farmers, and abattoir workers are most considered at the risk of this disease occupationally (Cosivi*et al*., 1998).

Tuberculosis in cattle is caused by *M. bovis*and the disease has become an important infectious disease which spread between species by affecting a wide range of animals and humans (Dwight and Yuan, 1999; OIE, 2010; Tenguria*et al*., 2011). It is widely distributed around the world with significant economic impact on the livestock production sector (Ayele *et al*., 2004; Rodriguez-Campos *et al*., 2014).

The disease is one of the seven most neglected endemic diseases in the world, particularly in developing countries including Ethiopia (WHO, 2006) that can spread to humans through aerosols, by the consumption of unpasteurized milk and dairy products and through meat from infected cows (Olmstead and Rhode, 2004; Ameni, 2010). Even though most of Europe and several Caribbean countries (including Cuba) are virtually free of bovine tuberculosis, the disease is endemic to many developing

ArsiNegele is one of potential milk source area from where milk and other dairy products supplied to Yirgalem Agro-industry processing, Shashemene and Hawassa towns and other areas for human consumption. Wondogenet town is also one of potential milk shed area in Sidama regional state from where milk production distributed mainly to Yirgalem Agro-industry processing, Hawassa town, and consumed in different hotels, restaurants and in household level. There was custom of raw milk consumption in the study area and sharing of shelter human with animal in the study areas was also observed. There is also free movement of dairy animals for marketing purpose in the study area. Due to this and other related preconditions, probability of bovine tuberculosis disease distribution was high in the area. However, there was no enough data on the prevalence of bovine tuberculosis in these areas. Thus, the current study was initiated to fill these gaps following a holistic approach. Therefore, the study attempted to address simultaneously the prevalence of the disease in intensive and semi-intensive dairy farms kept under small, medium and large scale farms located in urban areas.

Objective of the Study

General objective: To estimate the prevalence of bovine tuberculosis (bTB) and identify the potential risks factors in dairy farm in ArsiNegele, and, Wondogenet town, and assess zoonotic importance of the disease in the area.

Specific objectives:

- To estimate the prevalence of bTB in dairy farms of in ArsiNegele and Wondogenet towns.
- To identify potentially risk factors associated with the occurrence of bTB in dairy animals.
- To assess knowledge, attitude and practice of the zoonotic importance of bTB among dairy farm owners.

Materials and Methods

Description of Study Area

The study was conducted in ArsiNegele, west Arsi zone, Oromia regional state and Wondogenet towns, Sidama regional state from December 2020 to May 2021. Geographically, Wondogenet town is located at about 265 km South of Addis Ababa and 22 km to Hawassa town at latitude of 7^o 00'N and longitude of 38^o 35'E with altitude of 1756 m.a.s.l at Southern part Ethiopia. Its minimum and maximum temperatures vary from 18^oC to 34^oC, respectively with annual rain fall 230-240 mm which experiences a bimodal rainfall pattern with a long rainy season from June to September and a short rainy season from March to April. Mixed agricultural activities are practiced in the area in common (WDAO, 2020).

Research Design

Study Population

Dairy animals kept in intensive and semi-intensive farms of ArsiNegele and Wondogenet town were considered as the study animals to represent the target population, the Shashamane milk-shed. The majority of these animals are *Holstein Friesian* and their local crosses, yet, some local breeds with no exotic blood were found in few farms. These farms were commercial oriented largely and in few cases, milk was produced for household consumption. Male and female animals above 6 months of age and cows one month before and after parturition were considered for the study.

Sample Size Determination

In this study, the sample size was determined by using a cluster random sampling technique described in Thrusfield (2018). To this end certain pre-determined parameters that include herd level expected prevalence,11% (Abie *et al*., 2019), 95% confidence level, predicted average number of animals per-cluster was five and 5% absolute precision were used.

The relevant formula for a 95% confidence interval is:

Cluster variance (VC) can be calculated by

S.D (standard deviation) = $(P_{exp})^2 = (0.11)^2 =$ 0.0121

Cluster variance (VC) = $(S.D)^2 = (0.0121)^2 =$ 0.00014641

$$g = \frac{1.96^2 \times (nVc + P_{exp}(1-P_{exp}))}{nd^2}$$

Where:
g = number of clusters to be sampled;
n = predicted average number of animals per-cluster;
P_{exp} = expected cluster level prevalence;
d = desired absolute precision;
Vc = between-cluster variance.

Therefore, according to the above formula, 151 dairy farms would have sufficed as the minimum number of herd. However, 153 dairy farms were included in the study. In each farm a minimum of 10 % of the animals were considered. Altogether, 755 animals (391 from Wondogenet and 364 from ArsiNegele) were considered for the study.

Study Design and Sampling Technique

This study was a cross-sectional study where herds were considered as primary sampling unit followed by animals representing secondary sampling unit. In each district, list of dairy farms was developed with the help of respective districts Livestock and Fisheries Department. Once the issue of developing sampling frame was finished, half the calculated herd number was allotted for each district. In each district farms were selected randomly however; when the owner disagree the next adjacent farm was considered after discussing on the purpose of the study. In each farm a minimum of 10% of animals were selected randomly after developing the second sampling frame in line with the inclusion criteria. In each farm data on individual animal and management related issues were collected using a semi structured questionnaire.

Method of Data Collection

Associated risk factors considered for data collection at animal and herd levels were recorded before purified protein derivatives (PPD) were injected. Impermanent unique identification number was given for each PPD injected animal which has no permanent identity number.

Body condition scoring (BCS) of the animals was determined according to (Ivanković, 1998; Nicholson and Butterworth, 1986) as poor, medium or good. Animal body scoring condition mentioned by Ivankovic which classified in to 9 stages was sub-merged in to three groups in this study. Body scoring condition described by above author 1 to 3 were classified as poor, score-4 to 6 as medium and score-7 to 9 were classified as animals having good body condition. Poor body condition score 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 were categorized as a medium body condition score and a good body condition score were articulated for the animals when fat cover is easily observed in critical areas and the transverse processes were not visible.

Comparative Intradermal Tuberculin Test

Both avian and bovine mycobacterium antigens were used to perform the comparative tuberculin skin test. Intradermal injection was made approximately between 12-15 cm range apart from each other in the skin of the middle neck of selected cattle (Manual, 2016; OIE, 2010). Prior to injection, the two injection sites around the middle of neck region was carefully cleaned and shaved by using surgical blade and disinfectants. Then a fold of skin thickness of shaved area measured with caliper and recorded. Animals were then injected with 0.1 ml (3000 IU/ml) bovine and 0.1 ml (2500 IU/ml) avian PPD antigens (Veterinary Laboratories Agency, Addlestone, Surrey, United Kingdom)by using graduated tuberculin syringe which inserted obliquely into the deeper layers of the skin intradermally in both parts. The skin-fold thickness of each injection site was measured 72 hours after injection. If the difference between skin fold thickness at the bovine site of injection and the avian site of injection after 72 hours is 2 mm, between 2 mm and ≤4 mm, or >4 mm, the animal will be classified as negative, doubtful (inconclusive), or positive for BTB, respectively. The skin thickness difference between bovine and avian site (B-A) results were interpreted based on OIE standard. Based on OIE standard, antigen-antibody reaction was considered as positive. A herd with at least one positive reactor was considered as 'PPD positive' (OIE, 2012).

Ethical Considerations

Ethical approval to implement the research was obtained from the College of Natural science, Hawassa University. Permission was obtained from the Sidama region livestock and fishery resource agency, Wondogenet town agriculture department and as well as from ArsiNegele town agriculture department. Skin testing was based on the international standards (OIE, 2012), and all skin testing and data collection according to the willingness of herd owners and/ or managers following elaboration of the study purpose, adverse effect and benefits of the research. All participants were given general consideration about bovine tuberculosis and on safe animal product consumption. Prior to interviewing herd owners, willingness to interview and sample collection was asked and all the voluntary owners were included in the sample collection. During the study period discussion and advice was given to those households identified as bovine TB positive or doubtful result. Anthelmintic drugs were given as incentive to animals which having poor body condition and after taking record of treatment time and advised owners who needs further advice from the local veterinary clinic follow up closely and further medication of their sick animals.

Method of Data Analysis

Data from PPD skin test, and questionnaire survey analyzed by using STATA Version 14 for different Statistical analysis of variables. Initially, a descriptive analysis of mean, frequencies and percentages was performed to describe the results while tables and graphs are used to present the findings. Univariable logistic regression analysis was run to determine the unconditional association of bTB at individual animal and herd level. Considering p- value (p=0.25) potential predictors were selected for multicollinearity assessment using Goodman and Kruskal's Gamma values. All the non-collinear predictors (gamma values between -0.6 to +0.6) were subjected to multivariable logistic regression. The final model was developed by using backward elimination technique, based on likelihood ratio test and Wald's statistics ($p < 0.05$). The possible interactions between predictors were also tested for statistically significant variables, forcing them into the model and examining changes in OR and p-values of the main effects. Association of exposure variables with in group difference of bovine tuberculosis was also assessed by measuring odds ratio. Confounding effect of predictors were also checked by using the changes in the proportion of OR. Then, a covariate variable was considered as a confounder and added in the model if its inclusion changed the OR of an estimated risk at least by 10%. Finally, Hosmer and Lemeshow statistics used to assess the goodness-of-fit and the receiver operating curve (ROC) for reliability of the model developed (Dohoo*et al*., 2003).

Results

General data about Study Animals

Among a total of 755 study animals, 391 were from Wondogenet town and 364 animals were from ArsiNegele town. The sex of majority (95%) of the study animals was female. The breed composition included in study animal was mostly cross-breed (92%) and local breed (8%). Of the total animals included in the study, 31.1% were from dairy farm with small herd size, 48.5% from medium herd size which comprises 5-8 animals and 20.4% animals included from dairy farm with large herd size. Among total herd included in the study 56.2% were from Wondogenet town and 43.8% dairy farms were selected from ArsiNegele town (Table 1).

Table 1: general information about study animals

Variables	Categories	No. of animals examined	Percent (%)
Town	Wondogenet	391	56.2
	ArsiNegele	364	43.8
Sex	Male	39	5
	Female	716	95
Breed	Cross	697	92
	Local	58	8
Herd size	Small	232	31.1
	Medium	366	48.5
	Large	157	20.3
Farming system	Intensive	684	90.6
	Semi-intensive	71	9.4

Individual Animal and Herd Level bTB Prevalence

The overall prevalence of bovine tuberculosis with comparative intra-dermal tuberculin test in individual level was 7% (95% CI: 5.7 -10) and 26.1% (95%CI: 25 -51) at herd level in dairy cattle at study areas at cut-off point >4mm. The prevalence recorded at cut-off >3mm was 11% (95% CI: 8.9 – 13.4) and 33.3% (95% CI: 26.2 - 41.2) at individual and herd level, respectively.

The prevalence also increased to 40% (95% CI: 32.3 – 47.9) and 16.7% (95% CI: 14.2 – 19.5) at herd and individual level, respectively, when > 2 mm cut-off (severe interpretation) was used. Prevalence of bovine tuberculosis at Wondogenet town was 8.2% (95%CI: 5.8 – 11.4) and 5.8% (95%CI: 3.8 – 8.7) at ArsiNegele town at individual animal level in which there was no statistically significant difference between two study areas.

Individual Animal Level Risk Factors

The prevalence of bovine tuberculosis in study areas at individual animal level was 7% at cut-off > 4 mm and different risk factors were observed for the occurrence and transmission. In univariable logistic regression analysis, age and body scoring condition were identified as statistically significantly (p<0.05) associated with prevalence of bovine tuberculosis in the study areas. However, sex, breed, reproductive status and parity had no significant association with bovine tuberculosis prevalence in the study area

Table 2: Evaluation of the association of individual animal level risk factors in univariable logistic regression model with prevalence to bovine tuberculosis**.**

Risk factor	Categories	No. of animals		OR (95% CI)	P-value
		Examined	Positive (%)		
Sex	Female	715	51 (7.1)	1	-
	Male	40	2 (5)	0.3 (0.04-2.5)	0.287
Age	Calf	61	1 (1.6)	1	-
	Juvenile	72	2 (2.8)	1.7 (0.15-19.4)	0.663
	Young adults	392	28 (7.1)	4.6 (0.6-34.5)	0.137
	Adults	201	17 (8.5)	5.5 (0.7-42.5)	0.100
	Old adults	29	5 (17.2)	12.5 (1.4-112.6)	0.024
Breed	Local	58	2 (3.5)	1	-
	Cross	697	51 (7.3)	2.2 (0.5-9.3)	0.280
Parity	Non-reproductive	13	13 (7.7)	1	-
	1 to 2	428	34 (7.7)	1 (0.13-8)	0.998
	>3	89	6 (7.9)	1 (0.12-9)	0.983
	Heifers	163	11 (6.7)	0.9 (0.1-7.3)	0.897
Reproductive status	Dry	31	1(3.2)	1	-
	Lactating	437	34 (7.8)	2.5 (0.3-19)	0.368
	Pregnant	114	12 (10.5)	3.5 (0.4-28)	0.235
	Heifer	111	5 (4.5)	1.4 (0.16-12.6)	0.755
Body scoring condition	Good	556	26 (4.7)	1	-
	Medium	154	17 (11)	2.5 (1.3-4.8)	0.004
	Poor	45	10 (22.2)	5.8 (2.6-13)	0.000

Herd Level Risk Factors

Ten herd level potential risk factors were identified during questionnaire survey and farm observation. Univariable and multivariable logistic regression and prevalence in association to each risk factor were summarized in table 3.

In multivariable final regression model, management standard of farm, farms having history of contact with other farms and herd size were identified as statistically significant risk factors ($p < 0.05$) at herd level bTB prevalence while, farming system, housing condition, ventilation and separate farm house had no statistical significant association with bTB positivity.

Table 3: Univariable logistic regression analysis of herd level bovine tuberculosis status with associated potential risk factors

Risk factor	Categories	No. of animals		OR (95% CI)	P-value
		Examined	Positive (%)		
Herd size	Small	71	16 (22.5)	1	
	Medium	66	12 (18.2)	0.8 (0.4-1.9)	0.695
	Large	16	12 (75)	12.4 (3.5-43.4)	0.000
Management condition	Good	60	7(11.7)	1	
	Medium	64	17 (26.6)	7.2(2-25.8)	0.002
	Poor	29	16 (55.2)	23(5.9-90.8)	0.000
Farming system	Intensive	136	35 (25.7)	1	
	Semi-intensive	17	5 (29.4)	1 (0.3-3)	0.957
Housing condition	Good	44	10 (22.7)	1	
	Medium	74	15 (20.3)	0.8(0.4-2)	0.698
	Poor	35	15 (43)	2.55 (1-6.7)	0.059
Ventilation	Good	57	12 (21.1)	1	
	Medium	63	16 (25.4)	4.8 (1.5-15)	0.007
	Poor	33	12 (36.4)	8 (2.4-24.2)	0.001
Share common feed/water trough	No	44	4(9.1)	1	
	Yes	109	36 (33)	4.8(1.6-14.4)	0.005

Discussion

In present study, the prevalence of bovine tuberculosis was estimated among dairy farms in Wondogenet and ArsiNegele towns. The result of bovine tuberculosis prevalence indicated in the present study was comparable with the previous report of pooled prevalence estimate of 5.8% from a recent meta-analysis of national level data from Ethiopia, 20% herd level prevalence of Mekelle town, 5.2% individual and 22.4% herd level prevalence in regional cities of Gondar, Hawassa and Mekele, 13.7% individual and 26.6% herd level prevalence of in and around Nekemte town, 11.4% herd and 20% individual level at sululta, 23.6% herd and 8.7% individual level at north Gondar and North Wollo zones (Sibhat *et al*., 2017; Zeru *et al*., 2014; Abie *et al*., 2019; Bedaso and Kitila, 2017; Birue *t al*., 2014; Mengistu*et al*., 2015), respectively.

Prevalence of BTB at herd level of current study was lower than previous studies held at Guji zone (41.9%),central Ethiopia (54.4%), Eastern Ethiopia 51.2%, Hawassa (48.7%), Addis Ababa (58.5%), Shashamane (48.9%) while lower herd level prevalence was reported at Eritrea (17.3 %) (Gumi, 2013; Almaw *et al*., 2021; Kemal *et al*., 2019; Regassa *et al*., 2009; Tulu *et al*., 2021; Lemu *et al*., 2020; Ghebremariam*et al*., 2016), respectively. The difference between previous and current result could be due to the current study occurred farms with smaller herd size, difference in farm system, level of contact with other farm animals, breed composition, sampling design, management system, socio-economic aspect and subject measuring the skin test and nature of the tuberculin itself. The reports of high bTB prevalence in different areas also indicated that the endemicity of the disease in Ethiopia was due to weakness of strict control strategy.

This report was higher when compared study reported at Eastern Ethiopia, sululta, Bahir Dar and Mekele (Kemal *et al*., 2019; Tadesse, 2020; Biru *et al*., 2014; Nuru *et al*., 2015), respectively and lower when compared to study of central Ethiopia (Ambaw *et al*., 2017).Similarly, around 46.2% of the respondents were consuming raw milk and 53.8% boil milk before consumption. As most of respondents replayed raw milk preferred due to taste, cultural value, high availability with lower price and lack of understanding regarding the zoonotic effect of bTB which is in similar with previous reports (Radostits *et al*., 2007; Ameni *et al*., 2008; Ambaw *et al*., 2017; Kemal *et al*., 2019; Tulu *et al*., 2021). Food consumption behavior and poor sanitary measures are among the potential risk factors of bTB to public health (SNV, 2008). Only 25% of the interviewed farm owners and/or farm attendants know bTBcan be transmitted by drinking raw and unpasteurized milk and meat. This is in line with study of Kemal *et al* (Kemal *et al*., 2019). This result was lower when compared to the result from central Ethiopia (Ambaw *et al*., 2017). The present result also revealed that only 39.1% of respondents only consider about bTB when buying and selling dairy animals which implicates one of predisposing factor for distribution of the disease in addition with animals having no health certificate. In this study all the practices and awareness regarding bTB; Consumption of raw milk, awareness of bTB as cattle disease, awareness of. zoonotic importance, awareness on mode of transmission and awareness of clinical signs were not significant with or influenced by both sex and education status of respondents which is agree with previous report (Ambaw*et al*., 2017). However, respondents of illiterate ones had better awareness regarding to zoonotic importance of bovine tuberculosis, clinical signs of bovine tuberculosis and transmission of bovine tuberculosis by drinking raw milk when compared to educated ones which might be due long exposure and practices in the farm. This result also disagrees with the previous result (Tadesse, 2020) in which educated ones had better awareness.

Limitations of the studies were not conducting pathological examinations of CIDT positive animals due to compensation problem, lack of retesting animals with inconclusive results after two months, lack of getting result of milk sample culture sent to Aklilu Lemma Institute of pathobiology and isolation of the strain and some of an unwillingness of farm owners to become voluntary when sample collection due to fear of the result.

Conclusion and Recommendation

The current result showed the presence of bTB in Wondogenet and ArsiNegeletown, Accordingly, the estimated animal level prevalence at Wondogenet was 8.2% while 5.8% for ArsiNegele. Likewise, the corresponding herd level prevalence at Wondogenet was 30.2 %while for ArsiNegele was 21%. Among the potential risk factors considered both at individual and animal level, presence of animal contact with other farms, poor/medium management standards, large herd size, and older age categories were recorded as potential risk factors for bTB positivity in the area. There is no correlation between awareness and practices concerning with bTB and educational status in this study. In general, contact between animals with other herds and poor management condition in addition to lack of awareness of farm owners regarding bTB were a major source for bovine bTB transmission in the area. The report in this finding regarding knowledge, attitude and practice indicates;

- To give due attention through integrated work of educational, medical and veterinary personnel to build awareness with regard to bTB and its public health aspects in the study area.
- Isolation of strains which circulate in the area should be confirmed further
- Training and routine follow up of herd health should be improved regarding management .condition of dairy farms

- Regular testing/surveillance, segregation of infected animals should be applied
- Drinking boiled or pasteurized milk should be recommended in the area
- Applying animal health certification when buying and selling animals
- Creation of awareness among livestock owners as well as including livestock diseases in educational curriculum in schools
- Designing cost effective preventive and control measures at national level is recommended

Finally, investigation of the result on bTB situation in humans in bTB positive households should be given attention to evaluate the possible cross infections or reverse zoonosis verification.

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