Prevalence and risk factors associated with bovine tuberculosis in local and crossbred dairy cattle in Debre Berhan milk shed, central Ethiopia

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Abstract

Bovine tuberculosis is a chronic and contagious disease of animals and humans with worldwide distribution. A cross-sectional study was employed to estimate the prevalence of bovine tuberculosis and its associated risk factors in dairy cattle found in the Debre Berhan milk shed. The study covered three districts found in the Debre Berhan milk shed for the duration of six months extending from July to December 2018. A single intradermal comparative cervical tuberculin test was used as a screening test of bovine tuberculosis. Faceto-face interview using a structured questionnaire was also employed to collect data on the risk factors associated with bovine tuberculosis. A binary logistic regression statistical model was used for data analysis. The finding showed that the apparent individual animal level prevalence was 17% (106/625; 95% confidence interval [CI]: 14.2-20.2) at \geq a 4mm cut-off value in 625 heads of dairy cattle tested. The herd prevalence was 16.7% (16/96; 95% CI: 10.1-26) at \geq a 4mm cut-off value in 96 dairy herds tested. Multivariable logistic regression analysis at \geq a 4mm cut-off value revealed that dairy cattle in poor body condition (Adjusted Odd ratio [AOR] = 3.7; 95% CI: 1.6-8.4; p = 0.002), large herd size (AOR = 29.5; 95% CI: 5.6-154.1; p = 0.000) and exotic breed (AOR = 3.7; 95% CI: 1.3-10.7; p = 0.018) had 4, 30 and 4 times the odds of tuberculin positivity with statistical significance, respectively compared to their counterparts. The findings in this study complement the works of other authors who conducted bovine tuberculosis studies elsewhere in Ethiopia. In conclusion, the prevalence of bovine tuberculosis in dairy cattle of Debre Berhan milk shed was found to be moderately high both at animal and herd levels. Moreover, poor body condition, herd size, and breed were important predictors of tuberculin test positivity. A further in-depth study on the prevalence and associated risk factors using a larger sample size are recommended.

Keywords: Bovine tuberculosis; Dairy cattle; Debre Berhan milk shed; Prevalence; Risk factors

Introduction

Bovine tuberculosis (bTB) is distributed worldwide. It is a chronic and contagious disease of animals and humans. It has a wide host range and various routes of transmission. The epidemiology of TB in animals is complex, and is even more so in Africa, given the extreme variation in ecosystems, farming practices that vary from extensive to intensive, and the movement of livestock over long distances (Dibaba *et al.*, 2019). The main risk factors of bTB in African cattle populations include the type of production system (intensive dairy farms and use of upgraded *B. taurus* breeds) and animal movements (Wichatitsky *et al.*, 2013).

Bovine TB is a disease of high economic significance and prevalent in all agroecological zones of Ethiopia mainly affecting cattle, sheep, goats, and camels (Biffa *et al.*, 2019). A pooled prevalence estimate of bTB in Ethiopia was found to be 5.8% (95% CI: 4.5, 7.5). In a multivariable meta-regression analysis, the breed and production system explained 40.9% of the explainable proportion of the in-between study variance computed (Sibhat *et al.*, 2017). Bovine TB is endemic in Ethiopia with prevalence varying from 0.8% to around 10% in an extensive rural farming system. The disease prevalence is particularly high in crossbred dairy cattle in the intensive husbandry system. A study showed an overall bTB herd prevalence of 50% in intensive dairy farms in Addis Ababa and its surroundings (Firdessa *et al.*, 2012).

Bovine TB poses a zoonotic risk to the people of Ethiopia. Impaired immunity, for example, caused by HIV/AIDS and malnutrition is incriminated as the cause of tuberculosis. Despite the high prevalence of bTB in animals and the risk of zoonotic infection, national control and preventive measures for the disease in both human and animal populations are virtually nonexistent (Biffa *et al.*, 2019).

There appears a bidirectional movement of cattle in Ethiopia that serves as a conduit for the dissemination of bTB in the country. Cattle from rural areas are brought to, marketed, and slaughtered in the urban centers. New breeding stock is also distributed throughout the country from the urban areas (Biffa *et al.*, 2019).

Research indicated bTB is endemic in dairy cattle in the Debre Berhan milk shed (Kiros, 1998; Shimeles, 2008; Woldemariyam *et al.*, 2021). These reports are the only available information on bTB in the milk shed and are restricted to Debre Berhan town and its few surroundings. Some of these studies involved a small sample size and had limited geographical coverage to describe the status of bTB in the area. Specifically, the studies did not cover most of the dairy farms included within the Debre Berhan milk shed.

There are currently emerging dairy farms in the Debre Berhan milk shed because of the encouraging privatization policy of the government and to satisfy the growing demand for milk due to the establishment of new towns and increase in population size. Therefore, this study was initiated to estimate the prevalence and risk factors associated with bTB in the Debre Berhan milk shed which would contribute its share towards the launching of practical and cost-effective control methods of bTB in Ethiopia.

Materials and methods

Study areas

The study *Woredas* were purposively selected based on their potential of dairy cattle which consist of Zebu (Z), Holstein Friesian (HF), crosses of Z with HF, and Jersey breeds. *Angolelana-Tera, Basona-Werana, and Debre Berhan Woredas* (*Woreda* here means District in English) were the study areas (Figure 1). These three *Woredas* are representing a milk shed of the North Shoa Administrative Zone of Amhara Region in central Ethiopia and collectively called the Debre Berhan milk shed. Next to *Woreda*, there are *Kebele*/peasant and sub-peasant associations in decreasing order of administrative importance.

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Kebele/peasant association here refers to the smallest administrative unit in Ethiopia. Debre Berhan is the capital, administrative, and economic center of the North Shoa Administrative Zone. Dairy farms included in the study originated from 11 *Kebeles*: two *Kebeles* from *Angolelana-Tera*, two *Kebeles* from *Basona-Werana*, and seven *Kebeles* from *Debre Berhan*. The systems of dairy farm management were intensive and semi-intensive. The study duration was six months extending from July to December 2018.

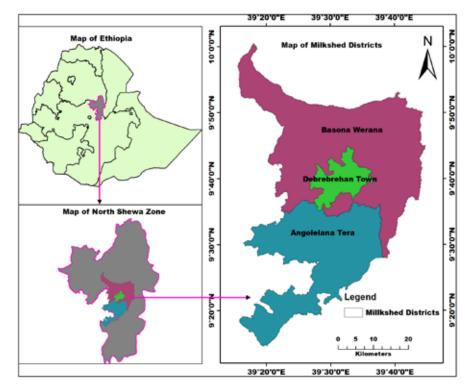


Figure 1. Map of the study areas (ArcGIS, 2021)

Study animals and risk factors

The recruitment of dairy herds within the study areas was based on the existence of high numbers of Holstein-Friesian and their crossbred cattle as well as variation in herd size with the latter was being categorized into three groups: small [1–10 cattle], medium [11–50 cattle], and large [>50 cattle] herds based on the method followed by Firdessa *et al.* (2012). Hence, the sampled dairy farms were categorized into small (85%: 82/96), medium (13%: 12/96), and large (2%: 2/96) based on the herd size they possessed. The study was carried out on 625 heads of dairy cattle originating from 96 herds.

The working definition for farm characteristics (sub-categorization):

Households in the context of this study refer to dairy farmers managing their dairy cattle within their residential compound.

Dairy farms in the context of this study refer to those farms managed on the land allocated for dairy cattle farming purposes.

Households (n=78) resided in three sub-peasant associations (PAs) namely Angolella_Seminesh (Basona-Werana), Kormargefiya (Angolelana-Tera) and Zanjira (Debre Berhan) participated in this study. Dairy cattle involved in this study were 256 heads categorized into small (99%: 77/78) and medium (1%: 1/78) herd sizes.

Dairy farms (n=18) included in the present study were found in Debre Berhan and Angolelan-Tera districts. Dairy cattle involved in this study were 369 heads categorized into small (28%: 5/18), medium (61%: 11/18), and large (11%: 2/18) herd sizes.

Keen observation and discussion (using a structured questionnaire) with each farm owner/manager were carried out during tuberculin testing to know basic dairy cattle related risk factors such as sex, age, body condition score (BCS), breed (local or exotic), physiological state (open or pregnant), lactation status (dry or lactating) and parity (no parity or number of calving), management (intensive or semi-intensive), herd size (small, medium, or large), and farm hygiene. The data collected from farm records/owners were recorded in a Log-Book.

The hygienic status of each farm was judged as poor, medium, or good based on aspects such as odour, waste drainage, cleanness of floor and the animals, barn ventilation and light source, and animal stocking density (herd size) based on the objective criteria adopted by FAO and IDF (2011). Data on animal feed sources and feeding practices were also collected and recorded.

The ages of cattle were categorized into ≤ 5 and >5 years based on the dentition formula adopted by Mississippi State University (MSU, 2013). BCS of local and exotic cattle was categorized into poor, medium, or good following the methods adopted by Nicholson and Butterworth (1986) and Kellogg (2009), respectively.

Sample size determination

The sample size for dairy cattle was determined by **o**ne-stage cluster sampling considering an individual cluster as a unit of sampling. A cluster in this study had an equivalent contextual meaning to a farm, a herd, or a household. The first step in determining sample size in one-stage cluster sampling is a prediction of the average number of animals per cluster. The appropriate formula to calculate the sample size for a 95% confidence interval and 5% precision by Thrusfield (2007) was adopted:

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

Where: g = number of clusters to be sampled; 1.96 = multiplier of the 95% confidence interval (CI); n = predicted average number of animals per cluster; Vc = between-cluster variance; Pexp = expected prevalence and d = desired absolute precision

Values: n = the average number of dairy cattle per cluster = 4; Pexp= expected prevalence = 2.7% (Shimeles, 2008); d= desired absolute precision = 5% and Vc = assumed between-cluster variance = 0.02 (previous experience in bTB studies of central Ethiopia). In total, 96 dairy herds representing 625 heads of dairy cattle were included in the study.

Study design and sampling strategy

A cross-sectional study was employed to estimate the prevalence of bTB in dairy cattle with associated risk factors. Multistage purposive sampling was employed to select the study areas up to the *Kebele* level. The sampling frame containing lists of clusters was established for each study area with the help of local livestock development agents. Clusters were selected by simple random sampling using random numbers. All animals in all herds that fulfilled the inclusion criteria were then included in the study. Animals were identified by their tags or their names on each of the farms before the skin test. Temporary ID numbers were written on the back of animals without ear tags using indelible ink that could not be erased for at least a week. All data were recorded in a LogBook before the skin test.

The following inclusion and exclusion criteria were employed before dairy cattle were tuberculin tested (Radostits *et al.*, 2007; Strain *et al.*, 2011): *Inclusion criteria*: Cattle in all herds that were seven weeks of prepartum

and postpartum (due to immunological hyporeactivity occurring in association with birth), and calves with the age of above 3 months (calves drinking colostrum from infected cows give positive reactions for up to 3 weeks after birth even though they may not be infected) were included in the skin test.

Exclusion criteria: Animals younger than 3 months of age and within six weeks before and after calving (periparturient cows) were excluded from the study.

Skin testing

Skin testing of dairy cattle was carried out by a single intradermal comparative cervical tuberculin (SICCT) test following an already established global protocol (OIE, 2009). All animals were restrained. Two sites on the lateral side of the mid-neck at 12-15cm approximate distance were delineated, shaved, and cleaned. The injection sites were positioned on the same side of the neck in a horizontal direction. A fold of skin was picked up, measured with 0.01 graduated calipers, and recorded before any purified protein derivative (PPD) injection (B0: at time 0). The site was marked with indelible ink before injection. Here, tuberculin and PPD had a similar meaning. Aliquots of 0.1ml of 3000 IU/mL bovine PPD and 0.1ml of 2500 IU/mL avian PPD (PRiONiCS, Lelystad, The Netherlands) were injected intradermally into the respective shaved sites. The area near to the head of the animal was injected with avian PPD and the other near to prescapular lymph node was injected with bovine PPD using 1mL separate preset sterile insulin syringes fitted with short sterile needles separately. A correct injection was confirmed by palpating the intradermally injected tuberculin into the raised fold, which was lodged so that a small pealike swelling (induration) at each site of injection was palpable. The skin-fold thickness of each injection site was re-measured 72 hours after injection (B72: at 72h). The skin test responses to bovine PPD were interpreted following two cut-off values namely the standard (OIE, 2009) and severe (Ameni et al., 2008): **Standard:** The interpretation of the result was made based on the difference in skin thickness at the bovine and avian PPD injection sites. The animal was considered as positive for bTB if the skin thickness at bovine PPD injection site minus the skin thickness at avian PPD injection site was $\Delta B - \Delta A \ge 4$ mm, inconclusive/doubtful if $\Delta B - \Delta A \ge 2$ mm -< 4mm, and negative if $\Delta B - \Delta A < 2$ mm. A positive reaction to *M. avium* was interpreted if $\Delta A > 4$ mm and negative if $\Delta A < 4$ mm.

Severe: The same skin test results after injection of bovine PPD were reinterpreted. Accordingly, the skin test result was considered positive if $\Delta B \cdot \Delta A > 2mm$ and negative if $\Delta B \cdot \Delta A < 2mm$.

All animals in the selected clusters were tuberculin tested. A herd with at least one positive reactor to tuberculin in a farm was considered positive (OIE, 2009).

Ethical clearance

The present study was carried out in strict accordance with the recommendations in the guide for the care and use of animals for research. The protocol was approved by the Animal Research Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University (CVMA, AAU: Ethical Clearance Certificate Ref. No.: VM/ERC/02/08/11/2018). The research proposal was submitted, evaluated, and approved by the Ethical Review Committee. The objective of the study and its non-invasive nature were told to dairy farm owners/managers. All owners of dairy cattle gave their informed oral consent to have their animals tuberculin tested. Moreover, they had the liberty either to proceed with the study or discontinue at any stage of the research. Free medication, consultation, and follow-up were provided by members of the study team and local veterinarians. After permission was obtained from dairy farm owners, all efforts were made to ameliorate the suffering of dairy cattle by gentle restraining, avoidance of disturbing noise, and unnecessary movement.

Data analysis

The data collected and recorded during the field tuberculin test were entered and stored in a separate MS-Excel 2010 spreadsheet, thoroughly screened for errors, coded, imported, and analyzed in Stata Version 13.0 for Windows (Stata, 2013). The individual animal level prevalence of bTB was calculated as the number of tuberculin tested positive cattle to the total number of tuberculin inoculated expressed in percent. Similarly, the herd prevalence of bTB in cattle was calculated as the number of herd/s tested positive to the total number of herds tested expressed in percent. Doubtful reactors were included in positive reactors in statistical analysis. Descriptive statistics were used to summarize and present data. The association of putative risk factors to bTB was determined using odds ratio (OR) in univariable logistic regression. The strength of association of the potential risk factors to bTB was indicated by the OR in multivariable logistic regression. Statistical significance was set at p < 0.05. Cattle herd was considered as a random effect and risk factors were considered as fixed effects. Variables in the univariable logistic regression analysis were selected for multivariable logistic regression analysis when the p-value was <0.25. Variables were fitted separately to the final multivariable logistic regression model. A variable was considered to be a confounder and included in the model if its inclusion altered the OR of the estimated risk by 25% or more (Stata, 2013).

Results

Prevalence

The purpose of keeping dairy cattle in the Debre Berhan milk shed is predominantly indispensable for milk production for sale. The apparent individual animal level prevalence of bTB in dairy cattle was 17% (106/625; 95% CI: 14.2-20.2) at \geq a 4mm cut-off value and 18.4% (115/625; 95% CI: 15.5-21.7) at > a 2mm cut-off value. The herd prevalence was 16.7% (16/96; 95% CI: 10.1-26) at \geq a 4mm cut-off value and 22.9% (22/96; 95% CI: 15-33) at > a 2mm cut-off value. Of the tested dairy cattle, 11.4% (71/625; 95% CI: 9-14) reacted to only avian PPD and 5.6% (35/625; 95% CI: 4-8) to both avian and bovine PPDs. Also, 1.1% (7/625; 95% CI: 0.5-2.4) of dairy cattle were doubtful reactors and included with positive reactors because they are zoonotic risks to humans. All the results were interpreted at \geq a 4mm cut-off value.

Risk factors

Univariable logistic regression analysis to see the association of the risk factors with tuberculin test positivity of dairy cattle to bovine PPD in the Debre Berhan milk shed was carried out on data generated at \geq a 4mm cut-off value.

Hence, the association of sex with bovine tuberculin positivity was statistically marginally significant (p = 0.060). The association of poor body condition, pregnant physiological state, large herd size, poor farm hygiene, exotic breed, and intensive management with tuberculin test positivity was also statistically highly significant (p<0.05). However, the association of age, lactation and parity with tuberculin test positivity was not statistically significant (p>0.05) (Table 1).

Variable	Category	Number Examined	Number (%) Positive	95% CI	COR (95% CI)	p-value
Sex	Male	76	7 (9.2)	(4.1, 18.6)	1	
	Female	549	99 (18.0)	(15.0, 21.6)	2.2 (1.0, 4.9)	0.060
Age	$\leq 5 \text{ yrs}$	378	58 (15.3)	(12.0, 19.5)	1	
	>5 yrs	247	48 (19.4)	(14.8, 25.0)	1.3 (0.9, 2.0)	0.184
BCS	Good	218	33 (15.1)	(10.8, 20.8)	1	
	Medium	352	52 (14.8)	(11.4, 19.0)	1.0 (0.6, 1.6)	0.905
	Poor	55	21 (38.2)	(25.7, 52.3)	3.5 (1.8, 6.7)	0.000
Physiol. state	Open	313	68 (21.7)	(17.4, 26.8)	1	
	Pregnant	236	31 (13.1)	(9.2, 18.3)	1.8 (1.2, 2.9)	0.011
Lactation	Dry	254	47 (18.5)	(14.1, 24.0)	1	
	Lactating	295	52 (17.6)	(13.6, 22.6)	0.9 (0.6, 1.5)	0.807
Parity	Heifer	182	32 (17.6)	(12.5, 24.1)	1	
	[1-3]	275	53 (19.3)	(14.9, 24.5)	1.1 (0.7, 1.8)	0.630
	≥ 4	92	14 (15.2)	(8.9, 24.6)	0.8 (0.4, 1.7)	0.635
Herd size	Small	273	7 (2.6)	(1.2, 5.4)	1	
	Medium	95	5 (5.3)	(2.0, 12.4)	2.1 (0.7, 6.8)	0.212
	Large	257	94 (36.6)	(30.7, 42.8)	21.9 (9.9, 48.4)	0.000
F. hygiene	Good	192	66 (34.4)	(27.8, 41.6)	1	
	Medium	101	7 (6.9)	(3.1, 14.2)	0.7 (0.3, 1.6)	0.363
	Poor	332	33 (9.9)	(7.0, 13.8)	4.7 (3.0, 7.6)	0.000
Breed	Local	230	6 (2.6)	(1.1, 5.9)	1	
	Exotic	395	100 (25.3)	(21.2, 30.0)	12.7 (5.5, 29.4)	0.000
Management	S. intensive	263	8 (3.0)	(1.4, 6.1)	1	
	Intensive	362	98 (27.1)	(22.6, 32.0)	11.8 (5.6, 24.8)	0.000

Table 1. Univariable logistic regression analysis of predictors with the outcome (n = 625)

BCS: Body condition score; Physiol. State: Physiological state; F. hygiene: Farm hygiene; COR: Crude Odds Ratio; CI: Confidence interval; Number 1: Refers to reference; S. intensive: Semi-intensive Multivariable logistic regression analysis at \geq a 4mm cut-off value revealed dairy cattle with poor body condition (AOR= 3.7; 95% CI: 1.6-8.4; p = 0.002), large herd size (AOR = 29.5; 95% CI: 5.6-154.1; p = 0.000) and exotic breed (AOR = 3.7; 95% CI: 1.3-10.7; p = 0.018) had 4, 30, and 4 times the odds of being tuberculin reactors compared to their counterparts with statistical significance, respectively. However, the association of age, sex, physiological state and management with tuberculin test positivity was not statistically significant (p>0.05) (Table 2).

Variable	Category	Number Examined	Number (%) Positive	95% CI	AOR (95% CI)	p-value
Sex	Male	76	7 (9.2)	(4.4, 18.2)	1	
	Female	549	99 (18.3)	(15.0, 21.5)	1.0 (0.3, 3.1)	0.983
Age	$\leq 5 \ { m yrs}$	378	58 (15.3)	(12.0, 19.4)	1	
	>5 yrs	247	48 (19.4)	(14.9, 24.9)	1.4 (0.8, 2.4)	0.215
BCS	Good	218	33 (15.1)	(11.0, 20.6)	1	
	Medium	352	52 (14.8)	(11.4, 18.9)	1.2 (0.7, 2.2)	0.908
	Poor	55	21 (38.2)	(26.3, 51.7)	3.7 (1.6, 8.4)	0.002
Physiol. state	Open	313	68 (21.7)	(17.4, 26.6)	1	
	Pregnant	236	31 (13.1)	(9.2, 18.3)	1.8 (1.0, 3.4)	0.696
Herd size	Small	273	7 (2.6)	(1.2, 5.3)	1	
	Medium	95	5 (5.3)	(2.2, 12.1)	2.5 (0.6, 10.1)	0.278
	Large	257	94 (36.6)	(30.9, 42.7)	29.5 (5.6,154.1)	0.000
Breed	Local	230	6 (2.6)	(1.2, 5.7)	1	
	Exotic	395	100 (25.3)	(21.3, 30.0)	3.7 (1.3, 10.7)	0.018
Management	S. intensive	263	8 (3.0)	(1.5, 6.0)	1	
	Intensive	362	98 (27.1)	(22.7, 31.9)	0.3 (0.1, 1.7)	0.191

Table 2. Multivariable logistic regression analysis of predictors with the outcome (n = 625)

Farm hygiene was not considered in the multivariable logistic regression model due to collinearity with the breed, thus not presented in the table; AOR: Adjusted Odds Ratio

Discussion

Prevalence

The apparent animal level prevalence of bTB obtained in this study at standard (17%) and severe (18.4%) cut-off values was moderately high. Some of the tuberculin tested dairy cattle were reactive to both avian and bovine PPDs (5.6%) indicating the presence of mixed infection.

The endemic nature of bTB has been elucidated in Ethiopia since 1967 (Ameni et al., 2007). A pooled prevalence estimate of bTB in Ethiopia based on systematic review and meta-analysis of research results published from January 2000 to December 2016 was 5.8% (Sibhat et al., 2017). SICCT test based studies in cattle in Ethiopia reported at an animal level prevalence of 13.5% (732/5424) in the central highlands of Ethiopia (Ameni et al., 2007), 2.7% (14/524) in Debre Berhan and Basona Werana (Shimeles, 2008), 6.4% (27/425) in Arsi-Negele (Amenu et al., 2010), 1.8% (36/2033) in Sellale (Ameni et al., 2013), 11.4% (98/858) in Sululta (Biru et al., 2014), 2.1% (10/481) in North Gondar and Wollo (Mengistu et al., 2015), 1.3% (10/788) in and around Bahir Dar (Nuru et al., 2015), 4.4% (22/501) in and around Adama (Woldemariam et al., 2016), and 5.2% (143/2654) in emerging milk sheds of regional cities such as Gondar, Hawassa and Mekelle (Mekonnen *et al.*, 2019) at \geq a 4mm cut-off value are lower than the current finding. On the other hand, an animal level prevalence of 34.1% (386/1132) in five sub-cities of Addis Ababa City Administration (Tsegaye et al., 2010), and 30% (887/2956) in six selected sites in major dairying areas of central Ethiopia (Firdessa *et al.*, 2012) at \geq a 4mm cut-off value are higher than the finding in this study, and 17% (95/558) in Bishoftu (Bekele *et al.*, 2016) at \geq a 4mm cut-off value is similar to the present finding.

The sources of variation in the tuberculin test result might be related to differences in the extent of study area coverage and duration of cattle to stay in the households (Mengistu *et al.*, 2015). In addition, Ameni *et al.* (2000) described that early tuberculous infections cannot be detected by traditional tuberculin testing but can be identified by the interferon-gamma (IFN-Y) test. Moreover, the major epidemiological factors which might favour transmission among animals in this study were communal watering and grazing points, herd contact, and incompatible spacing to the number of dairy animals (overstocking). Poorly ventilated housing to animals predisposes to bTB in that, the closer the animals are in contact the greater is the chance that bTB will be transmitted. Inadequate ventilation results in insufficient dilution of bacilli or insufficient removal of infectious droplet nuclei (Radostits *et al.*, 2007).

Risk factors

The risk factors discussed here are those factors facilitating the occurrence of bTB in dairy cattle at the animal level. Stress caused by poor nutrition in animals could be subject to severe diseases like TB (Ameni *et al.*, 2006). Higher prevalence of bTB was reported in animals with poor body condition compared to those with good body condition scores which conforms well to the fact that animals' resistance to TB is reduced by a shortage of feed and/or unbalanced diet attributable to a deficiency of proteins, minerals, and vitamins in the diet (Elias *et al.*, 2008). Poor body conditioned animals in this study were associated with the increased risk of bovine tuberculin positivity as compared to medium and good body conditioned cattle which is congruent to Ameni *et al.* (2006) and Elias *et al.* (2008). Nuru *et al.* (2015) also indicated poorly conditioned animals to be susceptible to bTB infection due to weak immunological responses.

The present study demonstrated more tuberculin reactivity in exotic dairy cattle breeds. This finding is in agreement with the findings of many researchers (Cosivi *et al.*, 1998; Ameni *et al.*, 2006; Radostits *et al.*, 2007; Elias *et al.*, 2008) who concluded that exotic breeds of cattle may be less resistant to bTB compared with the autochthonous cattle breeds in the tropics. However, the exact cause of susceptibility differences to bTB between these breeds is a major subject of future genomic research.

Large herds in this study were observed to be more tuberculin reactors like to the finding of Elias *et al.* (2008) who indicated an increment in herd positivity parallel to increasing herd size. Factors significantly associated with an increased risk of tuberculin reaction are herd size, poor housing condition, age, and poor body condition score (Elias *et al.*, 2008). Increased inter-herd and intra-herd contacts in larger herds favour the lateral spread of infection making the prevalence of the disease greater than in small herds (Ameni *et al.*, 2006; Elias *et al.*, 2008).

Limitations

Test positive cattle would have been purchased and slaughtered for mycobacterial culturing and molecular typing. However, it was not done due to the absence of a budget.

Conclusions

The prevalence of bTB in dairy cattle in the Debre Berhan milk shed was moderately high in both animal and herd levels. Poor body condition, large herd size, and exotic dairy cattle breeds were important predictors of bovine tuberculin test positivity.

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Conflict of interest

The authors declared that there is no conflict of interest.

References

- Ameni, G., Miorner, H., Roger, F., and Tibbo, M., 2000. Comparison between comparative tuberculin and gamma-interferon tests for the diagnosis of bovine tuberculosis in Ethiopia. *Trop. Anim. Hlth. Prod.*, 32, 267-276.
- Ameni, G., Aseffa, A., Engers, H., Young, D., Hewinson, G., and Vordermeier, M., 2006. Cattle husbandry in Ethiopia is a predominant factor affecting the pathology of bovine tuberculosis and gamma-interferon responses to mycobacterial antigens. *Clin. Vaccine Immunol.*, 13, 1030–1036.
- Ameni, G., Aseffa, A., Engers, H., Young, D., Gordon, S., Hewinson, G., et al., 2007. High prevalence and increased severity of pathology of bovine tuberculosis in Holsteins compared to Zebu breeds under field cattle husbandry in central Ethiopia. *Clin. Vaccine Immunol.*, 14, 1356-1361.

- Ameni, G., Hewinson, G., Aseffa, A., Young, D., and Vordermeier, M., 2008. Appraisal of interpretation criteria for the comparative intradermal tuberculin test for diagnosis of tuberculosis in cattle in central Ethiopia. *Clin. Vaccine Immunol.*, 15, 1272–1276.
- Ameni, G., Tadesse, K., Hailu, E., Deresse, Y., Medhin, G., Aseffa, A., et al., 2013. Transmission of *M. tuberculosis* between farmers and cattle in central Ethiopia. *PLoS ONE*, 8, e76891.
- Amenu, K., Thys, E., Regassa, A., and Marcotty, T., 2010. Brucellosis and tuberculosis in Arsi-Negele District, Ethiopia: prevalence in ruminants and peoples' behaviour towards zoonoses. *Tropicultura*, 28, 205-210.
- ArcGIS software, 2021. Basic tool for mapping and spatial data, the latest version of 2021.
- Bekele, M., Mamo, G., Mulat, S., Ameni, G., Beyene, G., and Tekeba, E., 2016. Epidemiology of bovine tuberculosis and its public health significance in Debre Zeit intensive dairy farms, Ethiopia. *Biomed. Nursing*, 2, 8-18.
- Biffa, D. A., Muwonge, A., and Dibaba, A. B., 2019. Status of bovine tuberculosis in Ethiopia: challenges and opportunities for future control and prevention. In: Dibaba, A. B., Kriek, N. P. J., and Thoen, C. O., 2019. Tuberculosis in animals: an African perspective, Springer Nature Switzerland AG 2019, Switzerland, eBook, Pp. 317-334.
- Biru, A., Ameni, G., Sori, T., Desissa, F., Teklu, A., and Tafess, K., 2014. Epidemiology and public health significance of bovine tuberculosis in and around Sululta district, central Ethiopia. *Afr. J. Microbiol. Res.*, 8, 2352-2358.
- Cosivi, O., Grange, J. M., Daborn, C. J., et al., 1998. Zoonotic tuberculosis due to M. bovis in developing countries. Emerg. Infect. Dis., 4, 59-70.
- Dibaba, A. B., Kriek, N. P. J., and Thoen, C. O., 2019. Tuberculosis in animals: an African perspective. Springer Nature Switzerland AG 2019, Switzerland, eBook, Pp 3-15.

https://doi.org/10.1007/978-3-030-18690-6

- Elias, K., Hussein, D., Asseged, B., Wondwossen, T., and Gebeyehu, M., 2008. Status of bovine tuberculosis in Addis Ababa dairy farms. *Rev. Sci. tech. Off. Int. Epiz.*, 27, 915-923.
- FAO and IDF, 2011. Guide to good dairy farming practice. Animal production and health guidelines, No. 8, Rome, Italy, Pp. 1-34.

- Firdessa, R., Tschopp, R., Wubete, A., Sombo, M., Hailu, E., Erenso, G., et al., 2012. High prevalence of bovine tuberculosis in dairy cattle in central Ethiopia: implications for the dairy industry and public health. PLoS ONE, 7, e52851.
- Kellogg, W., 2009. Body condition scoring in dairy cattle. The University of Arkansas, United States Department of Agriculture, Agriculture and Natural Resources, Elanco Animal Health, USA, Pp. 1-6.
- Kiros, T., 1998. Epidemiology and zoonotic importance of bovine tuberculosis in selected sites of Eastern Shoa, Ethiopia. MSc. Thesis, Faculty of Veterinary Medicine, Addis Ababa University and Freie Universitat, Berlin, Germany.
- Mekonnen, G. A., Conlanb, A. J. K., Berg, S., Teshome, B. A., Alemua, A., Guta, S., et al., 2019. Prevalence of bovine tuberculosis and its associated risk factors in the emerging dairy belts of regional cities in Ethiopia. Prev. Vet. Med., 168, 81–89.
- Mengistu, A., Enqueselasie, F., Aseffa, A., and Beyene, D., 2015. Bovine tuberculosis in rural Ethiopia: a comparative cross-sectional study on cattle owned by households with and without tuberculosis. J. Mycobac. Dis., 5, 191.
- MSU, 2013. Estimating cattle age using dentition. Extension service of Mississippi State University (MSU), cooperating with U.S. Department of Agriculture, 8 May and 30 June 1914, published in furtherance of acts of congress, USA, Pp. 1-8.
- Nicholson, M. J., and Butterworth, M. A., 1986. A guide to condition scoring of Zebu cattle. International Livestock Center for Africa (ILCA). Addis Ababa, Ethiopia, Pp. 72-74.
- Nuru, A., Mamo, G., Teshome, L., Zewude, A., Medhin, G., Pieper, R., et al., 2015. Bovine tuberculosis and its risk factors among dairy cattle herds in and around Bahr Dar City, Northwest Ethiopia. Ethiop. Vet. J., 19, 27-40.
- OIE, 2009. Bovine tuberculosis. OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. World Organization for Animal Health (OIE), Paris, France, Pp. 1-12.
- PRIONICS, 2018. PRIONICS Lelystad B. V., Tuberculin PPD Kit solution for injection, The Netherlands.
- Radostits, O. M., Gay, C. C., Hinchcliff, K. W., and Constable, P. D., 2007. Tuberculosis associated with *M. bovis*. Veterinary Medicine, A textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats, 10th edition, W.B. Saunders Elsevier, London, UK, Pp. 1007-1014.
- Sibhat, B., Asmare, K., Demissie, K., Ayelet, G., Mamo, G., and Ameni, G., 2017. Bovine tuberculosis in Ethiopia: a systematic review and meta-analysis. *Prev. Vet. Med.*, 147, 149–157.

- Shimeles, S., 2008. Bovine tuberculosis: epidemiologic aspects and public health implications in and around Debre Berhan, Ethiopia. MSc Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.
- Stata, 2013. Stata Corporation. Stata statistical software, Release 13.0. 4905 Lakeway Drive, College Station, Texas, USA.
- Strain, S. A. J., McNair, J., and McDowell, S. W. J., 2011. Bovine tuberculosis: a review of diagnostic tests for *M. bovis* infection in cattle. Bacteriology Branch, Veterinary Sciences Division, Agri-Food, and Biosciences Institute, Great Britain, UK, Pp. 1-33.
- Thrusfield, M., 2007. Surveys. Veterinary Epidemiology, 3rd edition, Blackwell Science Ltd, Oxford, UK, Pp. 233-245.
- Tsegaye, W., Aseffa, A., Mache, A., Mengistu, Y., Berg, S., and Ameni, G., 2010. Conventional and molecular epidemiology of bovine tuberculosis in dairy farms in Addis Ababa City, the capital of Ethiopia. *Int. J. Appl. Res. Vet. Med.*, 8, 143-151.
- Wichatitsky, M. D. G., Caron, A., Kock, R., Tschopp, R., Munyeme, M., Hofmeyr, M., et al., 2013. A review of bovine tuberculosis at the wildlife–livestock–human interface in sub-Saharan Africa. Cambridge University Press, UK. Epidemiol. Infect., 141, 1342–1356.
- Woldemariam, T., Mamo, G., Mohammed, T., and Ameni, G., 2016. Prevalence of bovine tuberculosis in feedlot of Borena cattle by using a comparative intradermal skin test, Adama, Ethiopia. *Ethiop. Vet. J.*, 20, 17-29.
- Woldemariyam, F. T., Markos, T., Shegu, D., Demissie, K., and Paeshuyse, J., 2021. Evaluation of postmortem inspection procedures to diagnose bovine tuberculosis at Debre Berhan municipal abattoir. *Animals*, 11, 1-10. <u>https://doi.org/10.3390/</u> ani11092620.