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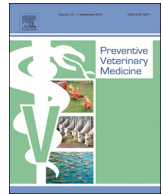
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## Prevalence of bovine tuberculosis and its associated risk factors in the emerging dairy belts of regional cities in Ethiopia

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### ABSTRACT

Bovine tuberculosis (BTB) has become an economically important disease in dairy herds found in and around Addis Ababa City and is emerging in regional cities like Gondar, Hawassa and Mekelle because of the establishment of dairy farms in the milk sheds of these cities. A cross-sectional study to estimate the prevalence of BTB and identify associated risk factors was conducted between February 2016 and March 2017. A total of 174 herds comprising of 2,754 dairy cattle in the cities of Gondar, Hawassa and Mekelle were tested using the Single Intradermal Comparative Cervical Tuberculin (SICCT) test. Data on herd structure, animal origin, body condition, housing condition, farm hygiene, management and biosecurity practices were collected using a pre-tested structured questionnaire. Generalized Linear Models (GLM) and Generalized Linear Mixed Models (GLMM) were used to analyze the herd and animal level risk factors, respectively. The herd prevalence was 22.4% (95% CI: 17–29%) while the animal prevalence was 5.2% (95% CI: 4–6%) at the cut-off > 4 mm. The herd prevalence rose to 65.5% (95% CI: 58–72%) and the animal prevalence rose to 9% (95% CI: 8–10%) when the severe interpretation of > 2 mm cut-off was applied. The mean within-herd prevalence in positive farms at the cut-off > 4 mm was 22.7% (95% CI: 15–31%). At the herd level, the analysis showed that herd size, farm hygiene, feeding condition and biosecurity were significantly associated with BTB status, while new cattle introductions showed only borderline significance and that age of farm, housing condition, farmers' educational status and animal health care practice were not significant. At the animal level, the results showed that age and animal origin were identified as significant predictors for BTB positivity but sex and body condition score were not related to BTB status. Descriptive analysis revealed that herds having 'BTB history' showed slightly higher likelihood of being BTB positive compared to farms having no previous BTB exposure. In conclusion, this study showed relatively lower average prevalence in the emerging dairy regions as compared to the prevalence observed in and around Addis Ababa City, warranting for implementation of control program at this stage to reduce or possibly stop further transmission of BTB.

### 1. Introduction

Bovine tuberculosis (BTB), caused mainly by *Mycobacterium bovis* (*M. bovis*), is a chronic progressive disease characterized by the development of tubercles in different tissues of the infected host. BTB is a zoonotic disease with notable economic significance (WHO et al., 2017), and remains a source of concern for livestock, wildlife and

human health. Economic losses from BTB accrue from its impact on international trade, livestock productivity (e.g., reduced milk yields and meat production, reduced fertility), restrictions to trade, compensation from control programs, and the cost to human health (Cosivi et al., 1998; Olea-Poppelka et al., 2016; WHO et al., 2017).

Geographically, BTB is distributed worldwide. According to the Worldwide Animal Health Information Database of OIE (OIE-WAHID

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Interface, 2018), 91 out of 182 countries reported the presence of BTB infection in cattle during 2015–2017 period. In Africa, 30 of 59 countries reported the presence of the disease in the same period. The disease is also widespread in Central and South America, parts of Asia and Middle East countries. While BTB has been controlled successfully in most developed countries through the implementation of test-and-slaughter schemes, meat inspection at abattoirs and pasteurisation of milk, BTB remains a problem in some developed countries in the face of extensive control programs (e.g., UK, Ireland, New Zealand) (Allen et al., 2018; Humblet et al., 2009), and in most developing countries where practice of control programs are either at early stage or non-existent (Teppawar et al., 2018).

In Ethiopia, BTB is endemic with prevalence varying among regional states. Studies conducted by various investigators in central Ethiopia recorded prevalence ranging between 22% and 47% in intensive dairy herds (Ameni et al., 2007, 2003; Elias et al., 2008; Firdessa et al., 2012; Tsegaye et al., 2009). These dairy herds keep mainly exotic breeds (Jersey and Holstein-Friesian) or their crosses with the indigenous zebu breed, due to the considerably smaller milk yield from zebus. In contrast, in the peripheral areas of the country including pastoral areas where intensive dairy farming is less developed, the reported prevalence has been lower than 9% (Admasu et al., 2014; Ameni et al., 2010; Gumi et al., 2012; Nega et al., 2012; Regassa et al., 2010; Tschopp et al., 2010b, 2009). However, since the start of the new millennium the dairy sector has been expanding from the central part of the country to the different regional states with establishment of many dairy farms in and around regional cities. Given the concentration of dairy cattle around the capital Addis Ababa, the demand for genetically improved cattle in the regional states is likely to be met by purchasing dairy cattle from this central region with high BTB prevalence. As there are no BTB control policies, animal identification and traceability system in Ethiopia, animals can be traded freely without health certification. These centrifugal trades of dairy cattle from areas of higher prevalence are risky for transmission between regions and expected to create new hotspots of BTB breakouts in the newly emerging dairy areas of regional cities. Despite this risk of disease transmission, it is assumed that hundreds of dairy cattle move every year between the central dairy areas and regional cities for dairy development purpose. The objective of this study was therefore, to estimate the prevalence of BTB in the emerging dairy areas and determine associated risk factors for BTB infection at the animal and herd level.

## 2. Materials and methods

### 2.1. Study sites

The study was conducted in three selected regional administrative cities namely Gondar, Hawassa and Mekelle (Fig. 1). These sites were purposively selected in light of the Ethiopian government plan for dairy expansion and are representative of the modern dairy industry managed under the intensive and semi-intensive systems in the southern, northwestern and northern parts of Ethiopia, respectively. Their respective distances from the capital, Addis Ababa, are 273, 738 and 783 km. The majority of the dairy herds involved in the study were owned by private owners and the remainder was government owned.

### 2.2. Study population

The study population was dairy cattle managed in the selected intensive or semi-intensive herds of the study sites, i.e. cattle in the emerging dairy areas of the country. Dairy cattle in the selected herds were study units and their breed compositions were either of the following: crosses of Holstein Friesian and Zebu, crosses of Jersey and Zebu, or pure Zebu. The husbandry and farm setting differed somewhat from one site to the other depending on the level of awareness, educational status of farmers and access of extension services. All cattle

except calves younger than four weeks, clinically sick cattle with disease not suggestive of BTB and cows in the last month of pregnancy, were included for tuberculin testing and sampling. These inclusion criteria were set to avoid possible interference with the action of tuberculin test (De la Rúa-Domenech et al., 2006; Goodchild and Clifton-Hadley, 2001).

### 2.3. Study design and sampling strategy

A cross-sectional study design with a one-stage cluster sampling strategy was used. This choice was informed by historical testing data collected by the National Animal Health Diagnostic and Investigation Centre (NAHDIC) Ethiopia (Alehegne et al., 2015) that suggested there was considerable variability in prevalence both between regions and between herds in the same region. Herds therefore represent both a unit of convenience for sampling effort and an important source of biological variation that must be included in sample size calculations. In this situation, where the variability between clusters is high while the variability within-cluster is negligible, the required number of groups can be calculated by the formula recommended by Thrusfield (2007) and Ahmed (2009):  $g = Z^2 [nVc + q(1-q)]/nd^2$ , where,  $g$  = number of clusters to be sampled;  $Z = 1.96$  (critical value for 95% confidence level, Z distribution);  $n$  = predicted average number of animals per cluster;  $d$  = desired absolute precision (0.05),  $Vc$  = between-cluster variance and  $q$  = within-herd prevalence. The historical test data from NAHDIC was used as pilot data to estimate the within-herd prevalence and between-herd variance for Gondar, Hawassa and Mekelle as 11% and 0.018, 7% and 0.03, 6.6% and 0.01, respectively.

The number of herds calculated for the respective study sites were 54 for Hawassa, 61 for Mekelle and 59 for Gondar. Herds with number of animals greater than 20 were selected without any prerequisite in all the sites as they were few in number, while herds with fewer than 20 animals were recruited using random selection method among the list of dairy herds obtained from local agricultural agents. Within the selected herds all animals fulfilling the inclusion criteria were subjected to tuberculin testing.

### 2.4. Skin testing

The Single Intradermal Comparative Cervical Tuberculin (SICCT) test method was used to differentiate between animals infected with *M. bovis* and those sensitized to tuberculin due to exposure to other mycobacteria or related genera. Two sites at the middle of the neck were shaved and cleaned 12–15 cm apart on the same side of the neck parallel to the shoulder for larger cattle while for calves the opposite sides of the neck were used because of the limited space. The skin of the neck and nearby lymph nodes were checked for any visible lesion or swelling before measuring the skin fold thickness at the two sites with a digital caliper. Animals were then injected with 0.1 ml (2500 IU/ml) avian PPD and 0.1 ml (3000 IU/ml) bovine PPD (Lelystad B.V., The Netherlands) intradermally using insulin syringes at the respective sites. The injection sites were examined and the skin thicknesses measured 72 h post-injection. The difference in the increase of skin thickness measurements at the bovine and avian sites before and after inoculation was considered for interpretation. A reaction was considered positive if the increase in skin thickness at the bovine site of injection was more than 4 mm greater than the reaction shown at the site of the avian injection. The reaction was interpreted as inconclusive if the increase was from 1 to 4 mm (OIE, 2009), or negative if the increase was less than 1 mm. A severe cut-off value of > 2 mm was also applied to re-estimate the prevalence to compare with that of the standard cut-off (4 mm) (Ameni et al., 2008; Downs et al., 2013; Goodchild et al., 2015).

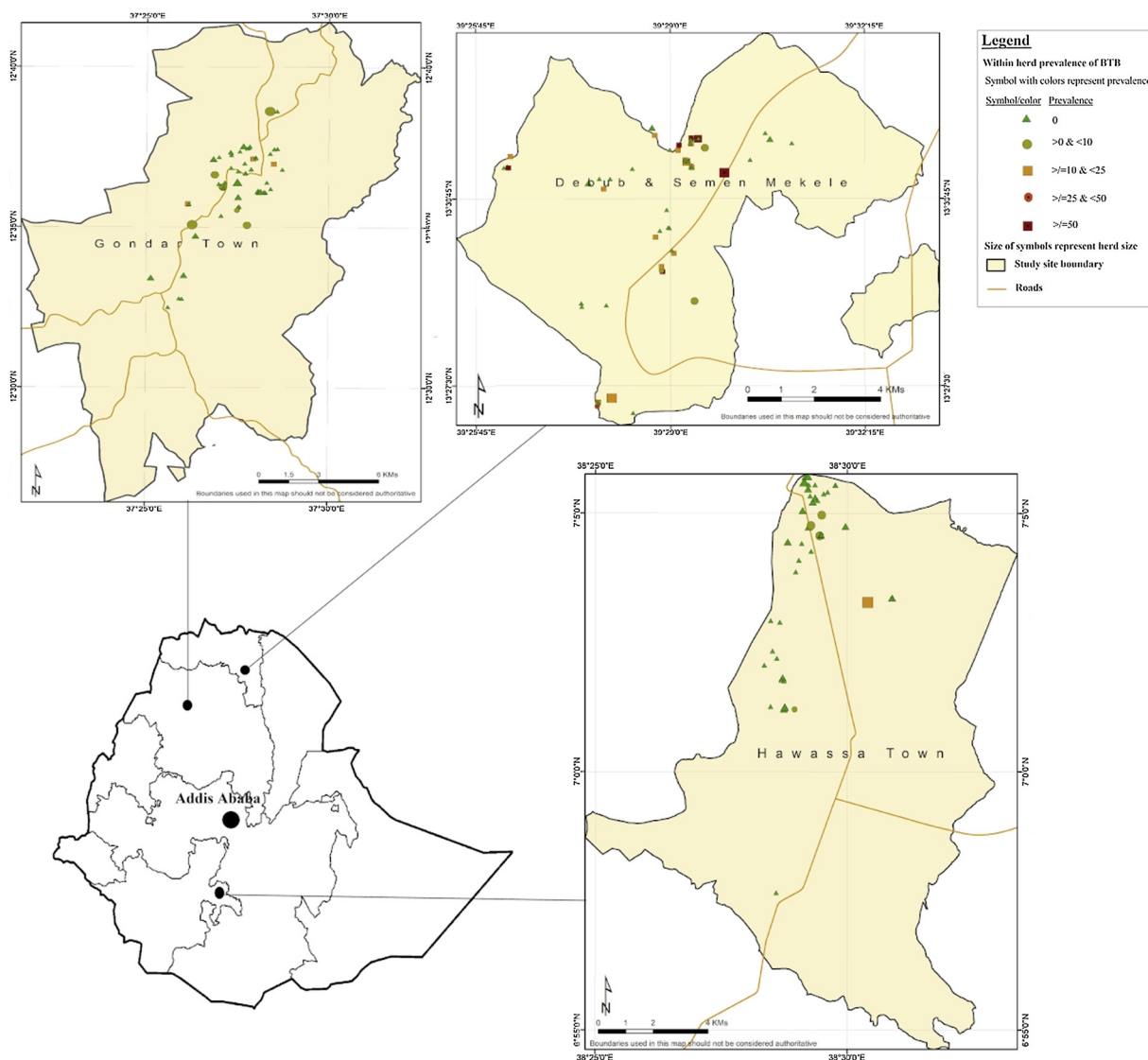


Fig. 1. Study sites and selected farms distributions. Shape of symbols and colours represent within-herd prevalence, while size of symbols represent size of the investigated herds.

2.5. Data collection

Information on herd level risk factors was collected from cattle owners using a pretested questionnaire. The questionnaire used contained open ended and closed questions and was filled in by the researcher on the farm at the same occasion as tuberculin testing. Administration of the questionnaire was based on translation into the local language and in a way that the respondents would feel comfortable. The objective and possible outcomes of the study were described to each respondent and the respondents were told to discontinue responding whenever they felt not to do so.

Data on animal level risk factors such as sex, age, breed, animal origin, body condition score, pregnancy and lactation were collected during the skin testing. Cattle were categorized as calves ( $\leq 1$  year of age), juvenile ( $> 1$  and  $< 3$  years), young adults ( $\geq 3$  and  $< 5$  years), adults ( $\geq 5$  and  $< 7$  years), mature adults ( $\geq 7$  and  $< 10$  years), and old adults ( $\geq 10$  years). Body condition scoring was categorized into three scales: poor, medium and good, a modification from the five scales described by Kellogg (2010) to better reflect the assessment in field conditions. Necessary training was given to the survey team at the beginning of the study to reduce discrepancy on subjective measures.

2.6. Ethical considerations

Ethical approval to implement the research was granted by the Institutional Review Board (IRB) of Aklilu Lemma Institute of Pathobiology, Addis Ababa University (Reference number IRB/ALIPB/2018). This study was supported by the Ethiopian Ministry of Livestock and Fisheries. Skin testing was based on the international standards (OIE, 2009), and all skin testing and data collections were reliant on the willingness of herd owners and/ or managers following elaboration of the study purpose, adverse effect and benefits of the research. As part of the survey, the BTB testing team also treated sick animals in the herds with antibiotics, anthelmintic drugs and wound spray to incentivise participation and the team also advised owners to seek further advice from the local veterinary clinic for close follow up and further medication of their sick animals.

2.7. Data analysis

Herd, animal and within-herd prevalence both at standard and severe interpretations were calculated using proportion (summarized in Table 1). Herd level predictor variables collected by the questionnaire survey were initially selected after data screening for presence of

outliers, lack of variability, small number of observations in each categories (merged where possible biologically), effect of missing values, and existence of correlation among predictors. The remaining number of variables was further reduced by creating indices through grouping related predictors based on the perceived importance and weight for their contribution according to Dohoo et al. (2003) and Anderson et al. (2007) (Supplementary Table S2). Weights were assigned based on expert opinion and other evidence wherever possible (operational definitions and determination of categories are provided in Supplementary Table S1). The strength of association between herd level risk factors and BTB status (binary response: positive or negative) were analyzed by Generalized Linear Models (GLM, binomial family with, logit link) using 'glm2' package (Marschner and Donoghoe, 2017). Animal level risk factors analysis was conducted using a Generalized Linear Mixed Model approach (GLMM) using maximum likelihood (Adaptive Gauss-Hermite Quadrature) with the logit link of the binomial family using 'lme4' package (Bates et al., 2017). In this study herd ID nested in study site was considered as group level random effect to account for clustering. The random effect was tested by comparing the likelihood ratios of the models with and without the random effects.

In both the herd and animal level risk factor analyses, variable selection for the multivariable analysis was made based on p value in the univariate regression, i.e. variables with p value less than 0.20 were considered in the multivariable regression (Sperandei, 2014). Intra-cluster coefficients were calculated based on Killip et al. (2004). Absence of interactions between variables was tested using 'MASS' package (Ripley et al., 2018). Multi-collinearity among predictor variables was checked using variance inflation factors (VIF) using 'car' package (Fox et al., 2017) and confirmed to be less than 2 for all variables. The Hosmer and Lemeshow goodness-of-fit test was calculated using the 'resourceSelection' package (Lele et al., 2017). The discrimination ability of the model was checked using the receiver operating characteristic curve (ROC) using 'pROC' package (Robin et al., 2017).

Software used for the statistical analysis was R statistical software (version 3.5.1) (R Core Team) with R Studio editor. Package 'aod' (Lesnoff and Lancelot, 2012) and 'questioner' (Barnier et al., 2017) were used to calculate odds ratio (OR) and confidence intervals in the GLMM and GLM, respectively. Confidence intervals for prevalence were calculated using 'EpiTools epidemiological calculators' with Wilson methods (Sergeant, 2019). In all cases, 95% confidence level and significance level of 5% were used to determine statistical significance.

### 3. Results

#### 3.1. Animal and herd level prevalence

The prevalence of BTB at herd and individual animal levels are stratified by study sites and shown in. The overall herd and animal prevalence were 22.4% and 5.2%, respectively, as determined by SICCT at > 4 mm cut-off. This prevalence increased to 65.5% and 9%, respectively, when > 2 mm cut-off (severe interpretation) was used. Variation in prevalence was observed among the study sites. Compared to Gondar and Hawassa, Mekelle showed higher herd and animal prevalence at both the standard and severe interpretations. Considering only positive farms in both standard and severe interpretations, higher within-herd prevalence was observed in Mekelle, while the findings in Hawassa and Gondar were comparable to each other. Overlap of BTB and *Mycobacterium avium* Complex (MAC) positivity was observed in 2% of the tested cattle based on the standard interpretations of SICCT for BTB and > 4 mm cut-off for MAC (considering only the reaction to avian PPD); however, 3.2% and 12.7% of them reacted to BTB and MAC alone, respectively Table 1.

#### 3.2. Herd level risk factors

A questionnaire survey with twelve potential herd level risk factors (defined in Supplementary Table S1) were summarized. Outputs of the univariate and multivariable final model GLM analysis for the selected herd level risk factors are summarized in Table 2.

In the univariate analysis, seven out of eleven variables (namely herd size, hygiene, feeding conditions, new cattle introduction, biosecurity, study site and stress condition) demonstrated statistical significance ( $p < 0.05$ ). However, age of farms, housing conditions, educational level and animal health care practices were not significant. The remaining variable from the survey, 'BTB history', was not considered in the regression analysis due to lower sample size within their classes. However, descriptive analysis of this variable showed that 41 herds among the recruited herds for the study were previously tested for BTB, of which 11 herds had infection history while 30 herds had not. The odds of BTB positivity in herds with infection history was higher compared to herds with no BTB history (OR 1.6, 95% CI; 0.4–6.4) although the difference was not significant. Cumulative stress conditions of a herd, assessed based on various indicators (Supplementary Table S2) and defined in Supplementary Table S1, was significantly related with herd BTB positivity ( $p < 0.05$ ) in the univariate regression. Herds in stressful conditions showed higher odds of BTB positivity than herds with less stressed conditions.

Multivariable final model showed that herd size, hygiene, feeding condition and biosecurity were significant predictors ( $p < 0.05$ ) for herd BTB positivity, while new cattle introduction was only borderline significant ( $p = 0.054$ ) (Table 2). Fifty one percent of the investigated herds introduced at least one new cattle in the duration of the last two to three years and the prevalence of BTB in these herds was 29% while, on the other hand, the prevalence was only 14.3% in the remaining 49% that did not introduce new cattle.

#### 3.3. Animal level risk factors

The breed compositions of cattle involved in the study were 96% Holstein-Friesian – Zebu crosses (HZ), 2% Jersey – Zebu crosses (JZ) and 2% Zebu. Skin test data by the standard interpretation revealed 5% BTB positivity in the HZ crosses, 2% in the JZ crosses and 0% in Zebu breeds. Due to fewer numbers of JZ and Zebu cattle, we excluded breed composition in the GLMM regression analysis.

Intra-cluster coefficients (ICC) demonstrated a higher inter-cluster variability than within-cluster variability. The ICC calculated using the pooled dataset was 0.78 while site specific ICC values were 0.91, 0.77 and 0.69 for Gondar, Hawassa and Mekelle, respectively.

The results of GLMM univariate and multivariable final model outputs for the other animal level risk factors are presented in Table 3.

Age group and animal origin were identified in the multivariable final model as significant predictors for BTB positivity ( $p < 0.05$ ). A non-linear relationship between BTB positivity and age was observed, with positivity increasing with age until mature adults and then declining for older animals. We also found that about 3% (20/674) BTB reactors among calves ( $\leq 1$  year old), and 25% of them were younger than six months.

### 4. Discussion

In the present study, the prevalence of BTB was estimated among dairy farms in three Ethiopian cities with an emerging dairy sector, namely in Gondar, Hawassa and Mekelle. A total of 174 herds comprising of 2,754 dairy cattle were sampled from these three emerging dairy centers, and tested for BTB using the SICCT test. Additionally, the study explored risk factors at herd and animal levels to inform possible control policies of the disease. Our results demonstrated that herd prevalence was high but with considerable differences between the study sites. At the standard interpretation and in order of magnitude,

**Table 1**  
Herd, animal and within-positive herd animal prevalence of BTB using SICCT test at the standard and severe interpretations.

Site	Level	n	> 4 mm cut-off		> 2 mm cut-off	
			n positive	Prevalence (95% CI)	n positive	Prevalence (95% CI)
Gondar	Herd	59	10	17 (9, 28)	23	73 (60, 83)
	animal	976	14	1.4 (0.9, 2)	42	4.3 (3, 6)
Hawassa	Animal within-positive herd <sup>§</sup>	302 (549) <sup>*</sup>	14	8.2 (4, 12)	42	11 (7, 14)
	Herd	54	6	11 (5, 22)	24	67 (53, 78)
	animal	960	29	3 (2, 4)	66	7.4 (5, 9)
Mekelle	Animal within-positive herd	212 (600) <sup>*</sup>	29	8.7 (2, 15)	66	9.2 (7, 12)
	Herd	61	23	38 (27, 50)	32	57 (45, 69)
	animal	818	100	12 (10, 15)	141	17.2 (15, 20)
Total	Animal within-positive herd	393 (581) <sup>*</sup>	100	32.7 (20, 45)	141	30.5 (20, 41)
	Herd	174	39	22.4 (17, 29)	79	65.5 (58, 72)
	animal	2754	142	5.2 (4, 6)	248	9 (8, 10)
	Animal within-positive herd	903 (1730) <sup>*</sup>	142	22.7(15, 31)	248	18.3 (13, 23)

\* Numbers outside brackets shows number of animals within positive herds by standard interpretation while numbers inside brackets are number of animals within positive herds by severe interpretation.

<sup>§</sup> Within-herd prevalence was average of animal level prevalence in the positive herds based on the respective cutoff.

**Table 2**  
GLM point estimates of the herd univariate and multivariable models for BTB positivity at standard interpretations (n = 170, with missing values from four herds).

Risk factor	Class	% positive	Univariate		Multivariable final model	
			Crude OR (95% CI)	P value	Adjusted OR(95% CI)	P value
Herd size	≤ 10 animal/herd	16.2 (12/74)	–	–	–	–
	> 10 & ≤ 20 animal/ herd	15.8 (9/57)	0.8 (0.3, 2)	0.645	1.3 (0.5,3.7)	0.627
	> 20 animal/herd	41 (16/39)	2.8 (1.2, 6.6)	0.016	10.5 (3.5, 36)	< 0.001
Farm age	≥ 20 years	20.5 (9/44)	–	–	–	–
	10-20 years	27.5 (14/51)8	1.5 (0.6,4)	0.425	–	–
	< 10 years	18.7 (14/75)	0.9 (0.4,2)	0.804	–	–
Housing condition	Poor	19.8 (17/86)	–	–	–	–
	Good	23.8 (20/84)	1.1 (0.5,2.2)	0.779	–	–
Educational level	Schooling ≥ 15yrs	29.4 (10/34)	–	–	–	–
	Schooling 11-15yrs	16 (11/71)	0.49 (0.2,1.3)	0.150	–	–
	Schooling for ≤ 10	25 (16/65)	0.9 (0.4,2.3)	0.853	–	–
Hygiene	Modest	17 (15/88)	–	–	–	–
	Meager	26.8 (22/82)	2.1 (1.4,3)	0.048	3.3 (1.3,9)	0.014
Animal health care	Case based	18.7 (17/91)	–	–	–	–
	Regular	25.3 (20/79)	1.2 (0.6,2.5)	0.541	–	–
	Excellent	11.8 (8/68)	–	–	–	–
Feeding condition	Moderate	30 (8/27)	3.3 (1,10)	0.034	5.5 (1.6, 21)	0.010
	Deprived	28 (21/75)	3 (1.3,8)	0.014	3.3 (1.3,10)	0.021
	No	14.3 (12/84)	–	–	–	–
New cattle introduction	Yes	29 (25/86)	2.3 (1.1, 5)	0.023	2.3 (1, 6)	0.054
	No	12.5 (10/80)	–	–	–	–
Biosecurity	Modest	30 (27/90)	2.4 (1.1, 5.3)	0.03	3.3 (1.3, 9)	0.015
	Poor	11 (6/54)	–	–	–	–
	Good	17 (10/59)	1.6 (0.6, 5)	0.370	–	–
Study site <sup>*</sup>	Mekelle	38 (23/61)	4.8 (2, 14)	0.001	–	–
	Gondar	13.6 (14/103)	–	–	–	–
	Hawassa	34 (26/76)	3.3 (1.6, 7)	0.002	–	–
Stress conditions <sup>‡</sup>	Stressed	–	–	–	–	–
	Less stressed	–	–	–	–	–

\* Study site was not significant in the global model thus not presented in the table.

<sup>‡</sup> Stress condition was not considered for multivariable models due to collinearity issues.

Mekelle showed the highest prevalence followed by Gondar and then Hawassa. The overall animal prevalence was 5.2% at the standard cut-off > 4 mm of SICCT and was comparable with the pooled prevalence estimate (5.8%) from a recent meta-analysis of national level data from Ethiopia (Sibhat et al., 2017). Our estimated animal level prevalence in Mekelle was higher than published in previous reports (Romha et al., 2013; Zeru et al., 2014; Zeweld, 2014; Zeweld et al., 2013), in Gondar it was similar to estimates by Mengistu et al. (2015) and Nuru et al. (2015), while in Hawassa it was slightly lower than what previous reports had found in that area (Alehegne et al., 2015; Romha et al., 2014). However, estimates from all of our ‘emerging’ study sites are much lower than the average prevalence of 16.6% estimated in intensive and/or semi intensive herds of the central areas of the country (Sibhat et al., 2017) where the dairy sector has been established for much

longer. The difference of prevalence between this study and previous reports could be associated with differences in sampling design, environment, breed compositions, husbandry practices, subject measuring the skin test and nature of the tuberculin itself.

Since Ethiopia has not implemented a control strategy for BTB, the disease has established endemicity in most part of the country with higher prevalence in the central and some peripheral areas (Ameni et al., 2007, 2003; Elias et al., 2008; Firdessa et al., 2012; Tsegaye et al., 2009). The Ethiopian Government has initiated various dairy programs to expand the dairy production to the peripheral areas sourcing cattle mainly from the central part of the country to respond to the increasing demand of milk related to the rapidly growing human population in these peripheral ‘emerging’ areas, ranging between 1.7–3.3% (Central Statistical Agency (CSA), 2013). However, the risk of BTB transmission,

**Table 3**

GLMM analysis for animal level risk factors of BTB based on standard interpretations; herd ID nested in study site was considered as random effect (*p* value for the difference between likelihood ratios was less than 0.001 by chi-square test); *n* total = 2715 with 39 missing values, *n* matured female = 1607.

Risk factor	Class	% positive	Univariate Crude OR (95% CI)	<i>P</i> value	Multivariable final model Adjusted OR (95% CI)	<i>P</i> value
Sex <sup>*</sup>	Male	2.9 (9/315)	–	–	–	–
	Female	5.6 (134/2415)	2.4 (1, 6.4)	0.05	–	–
Age group <sup>§</sup>	Calves	3.0 (20/674)	–	–	–	–
	Juvenile	4.4 (27/617)	2 (0.9, 4.6)	0.085	2 (1,4.8)	0.096
	Young adults	6.4 (32/497)	4.1 (1.9, 9)	< 0.001	3.7 (1.7, 8.8)	0.002
	Adults	7.7 (30/391)	4.9 (2.3, 11)	< 0.001	3.7 (1.6, 9)	0.002
	Matured adults	7.3 (30/409)	3.8 (1.7, 8.8)	0.001	3 (1.3, 7.2)	0.010
	Old adults	3.1 (4/127)	1.5 (0.3, 6)	0.56	1.3 (0.2, 5)	0.760
Animal origin	Same herd	4.9 (116/2375)	–	–	–	–
	Other herd	7.6 (26/340)	2.6 (1.3, 5)	0.005	2.4 (1.2, 4.8)	0.017
BCS	Poor	4.6 (12/261)	–	–	–	–
	Moderate	6.2 (122/1975)	0.8 (0.4, 1.8)	0.624	–	–
Pregnancy <sup>§</sup>	Good	1.9 (9/479)	0.5 (0.2, 1.4)	0.199	–	–
	No	6.9 (71/1033)	–	–	–	–
Lactation <sup>§</sup>	Yes	4.4 (25/574)	0.9 (0.5, 1.6)	0.70	–	–
	No	5 (28/556)	–	–	–	–
Lactation <sup>§</sup>	Yes	6.5 (68/1051)	1.6 (0.9,3)	0.147	–	–

<sup>§</sup> analysis was made using data specific to matured females.

\* sex as a variable did not show significance in the global model, thus not presented in the table.

<sup>§</sup> age category: calves: ≤ 1 year of age, juvenile: > 1 and < 3 years, young adults: ≥ 3 and < 5 years, adults: ≥ 5 and < 7 years, matured adults: ≥ 7 and < 10 years, old adults: ≥ 10 years.

as can be deduced from the comparison of our findings with previous report, in particular that of Mekelle, has increased because the dairy development plans implemented so far have not considered the need for a control strategy for BTB.

Results presented in this study suggest that introduction of new cattle into apparently BTB free herds is one of the major risk factors for transmission of BTB. As of the time of writing, the country has no legislation or regulation in place for BTB control including traceability, accountability and animal movement. Therefore, animals moved from one region to another without any regulatory checks such as animal health certification, might insidiously promote the BTB dissemination into wider areas. The present data demonstrate that movement of animals onto herds is associated with increased risk of BTB positivity at both herd (OR 2.3, 95% CI: 1–6) and animal (OR 2.4, 95% CI: 1.2–4.8) levels. Continuing to allow the free movement of animals without any precaution could increase the risk of BTB spreading into wider areas. Similar findings have been reported in UK, Italy, Tanzania and Michigan, USA (Dejene et al., 2016; Gopal et al., 2006; Johnston et al., 2011; Kaneene et al., 2002; Marangon et al., 1998; Shirima et al., 2003). Sourcing animals from BTB free herds, reducing cattle trade in general, and prioritizing trade of young animals before adults have previously been suggested as effective steps to reduce the spread of BTB (Reilly and Courtenay, 2007). Our data supports the prompt implementation of regulation on the trade of animals in Ethiopia, should arresting the spread of BTB truly be a priority for the nation.

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; Firdessa et al., 2012; Griffin et al., 1996; Inangolet et al., 2008; Kaneene et al., 2002; Munroe et al., 1999; 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; Reilly and Courtenay, 2007; van Arendonk and Liinamo, 2003). 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).

Cumulative stress conditions happening as the result of

overcrowding due to disproportionately housed herds; recurrence of immunosuppressive diseases; under fed conditions, have all been shown to contribute to BTB positivity of a herd (Humblet et al., 2009). Here we show that herds managed under stressful conditions showed higher odds of BTB positivity (OR 3.3, 95% CI: 1.6–7), implicating the deleterious effect of stress on the animals' resistance for the disease. Our data on feeding practice, as assessed by type and frequency of supplement feeding, indicated that herds fed with poor to moderate level were at higher risk of being BTB positive compared to well-nourished herds. In line with this, a study conducted in the UK on feeding practice revealed that supplement feeding could diminish the risk of transient BTB outbreaks in UK (Reilly and Courtenay, 2007). Stressful conditions created as the result of poor housing conditions and overcrowding have been reported as a potential cause for the increased BTB positivity (Ameni et al., 2006; Costello et al., 1998; Elias et al., 2008). When the housing/barn is poorly ventilated, aerosols carrying the *M. bovis* bacilli remain for long time within the congested air so that cattle can inhale concentrated dose of the pathogen, which will be worsened when close contact between animals is high due to overcrowding (Ameni et al., 2006).

Hygiene - as assessed in terms of the method in use for manure disposal, drainage conditions of the floor and frequency of waste cleaning of the house in a day - has been found to be important for the health of dairy cattle. The present data revealed that herds managed in farms with poor hygiene have a higher risk of being BTB positive compared to herds managed in farms with modest hygiene. Poor hygiene condition in a farm may allow *M. bovis* to remain for a longer period and potentially to proliferate (Humblet et al., 2009).

Biosecurity practices in farm management are important to prevent BTB introduction into a herd. In the present study we assessed biosecurity practices by evaluating relevant indicators such as awareness level, access to wildlife, mixing with neighboring herds, sharing of bulls with neighboring herds, farm enclosure and sharing of livestock extension services. Linear combination of values obtained for these indicators were considered to estimate the biosecurity level. Accordingly, farms practicing poor biosecurity measures showed higher likelihood of herd BTB positivity (OR 3.3, 95% CI: 1.3–9) compared to those which practiced modest levels of biosecurity. Potential contacts of dairy cattle with other species of domestic animals and wildlife have been demonstrated to breach the biosecurity of dairy farms. Of the domestic

animals, goats have been demonstrated to be susceptible to *M. bovis* infection and can maintain infection in the absence of cattle though reported only occasionally (Alvarez et al., 2008; Crawshaw et al., 2008); while sheep are less susceptible and thus reported only rarely (Houlihan et al., 2008a; Malone et al., 2003). Previous studies revealed that wild life plays major role in the transmission of *M. bovis* (Munyeme et al., 2008; Smith et al., 2009). In the present study certain studied herds had frequent contacts with one or more species of wildlife, namely, hyenas, bush duiker [Midaqua], common warthog, and honey badger. Studies in other countries showed that *M. bovis* has been isolated from spotted hyenas (Vathsala et al., 2007), warthog (Miller et al., 2016) and badger (Fitzgerald and Kaneene, 2013). A study from Ethiopia revealed that no *M. bovis* was detected in wildlife species from national parks and sanctuaries (Tschopp et al., 2010a); however, wildlife species that have close contact with dairy cattle managed in the intensive management system may play role in BTB transmission requiring further study.

Previous studies suggest that older cattle are more likely to be positive for BTB than young ones (Cleaveland et al., 2007; Griffin et al., 1996; Inangolet et al., 2008; Munyeme et al., 2008). Our study also concurs with these observed patterns, with the proportion of BTB reactors increasing to a peak at five to seven years of age. The increase with age could be linked to longer exposure time to the *M. bovis*. The apparent decay at later stage in life might be due to development of an anergic state or excess mortality of infected animals. It has been reported that chronically infected animals with severe pathology may be unresponsive to the tuberculin test (Houlihan et al., 2008b; OIE, 2009), thus might be more likely to give a false negative result. However, at what level of the infection or period of time after infection such potential anergic state might develop has not been well characterised.

It is not uncommon to observe overlap of BTB and MAC positivity in cattle due to either antigenic cross reactivity and/ or co-infection (Mamo et al., 2013; Tschopp et al., 2010b). There exists no clear evidence on the immunological relationship between these two but studies have shown that co-infection with MAC compromises BTB skin test results by negatively influencing the sensitivity of the tuberculin test (Alvarez et al., 2008; Aranaz et al., 2006; Walravens et al., 2002). Further evidence (Amadori et al., 2002) suggests that cattle sensitized by MAC might conceal *M. bovis* for a period of time. However, it is not clear to what extent this disease could jeopardize the detection of BTB with SICCT test, thus requiring future research.

## 5. Conclusions

This study showed relatively low average prevalence in the emerging dairy regions as compared to the prevalence observed in the established dairy belt in the central parts of Ethiopia, especially in and around Addis Ababa City. Herd size, feeding, biosecurity, introduction of new cattle from other herd, age and animal origin are important risk factors identified here. Implementation of control program in these cities at this stage could be effective to reduce or possibly stop further BTB transmission between cattle and to reduce the likely zoonotic impact.

## Author contributions

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 Analyzed the data: GAM, AC, BT  
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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.prevetmed.2019.04.010>.

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