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Prevalence and risk factors analysis of bovine tuberculosis in cattle raised in mixed crop-livestock farming system in Tigray region, Ethiopia

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Summary

Bovine tuberculosis (BTB) is a disease of animal and public health importance in developing countries. In rural Ethiopia, there is potential for a shift in the epidemiologic of this disease driven by transformation of dairy industry. This includes gradual change from the traditional mixed-crop livestock husbandry practice to a semi-intensification system. It is therefore, essential to document This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/tbed.13050

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the prevalence and risk factors of BTB to continuously update the designing and implementation of control and prevention strategies.. Here, we present findings of a cross-sectional study on the prevalence and associated risk factors of BTB among cattle reared under mixed crop-livestock farming system in Tigray region, Ethiopia. A multi-stage purposive sampling approach was used to select districts, villages, herds and individual cattle. A total of 1357 cattle from 310 herds were examined for BTB infection using a comparative intradermal tuberculin skin test (CIDT). Questionnaires were used to gather data on herd structure and herd management practices. A multilevel logistic mixed effect model was used to determine risk factors after accounting for clustering effect at three levels (village, herd and individual animal). Overall prevalence of BTB was 4.3% (95% CI=3.4-5.6), with the highest prevalence recorded in Alamata district (5.6%) and lowest in Korem (1.6%). Multilevel logistic mixed effect model analysis identified exotic breed (OR=3, P=0.014), closed barn (OR=2.6, P=0.018), large herd size (OR=2.6, P=0.05), and purchase of cattle (OR=2.1, P=0.027) as important risk factors for BTB. Taken together, these findings suggest that the current dairy development program centered on the introduction of exotic and or crossed animals could have contributed to changing epidemiological situations of BTB in the study area.

Keywords: Bovine Tuberculosis, Cattle, Ethiopia, Mixed Farming, Tigray, Tuberculin.

Introduction

Ethiopia has the largest cattle resources in Africa with an estimated population of 59.48 million head of cattle (Central Statistical Agency, 2016). This sector is not only critical for the livelihood of people living in rural parts of the country, but also contributes 20 % of the gross domestic product (GDP) as well as generates 37-87% of the household incomes (Central Statistical Agency, 2010; GebreMariam, Amare, Baker, Solomon, & Davies, 2013). The purpose of livestock production varies considerably according to the prevailing system of production, and can be classified as mixed farming, pastoral

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and agro-pastoral settings (Food and Agricultural Organization, 2005). Over 80 % of cattle are reared in the highlands of Ethiopia that includes the Tigray region, where the main agricultural activity is mixed-crop livestock production. In mixed production systems the livestock and crop sectors supplement each other in that oxen are important suppliers of draught power while the residues from crop harvest used as the major feed sources for cattle (Food and Agricultural Organization, 2005).

Despite the huge untapped resource potential, the meagre productivity obtained from this sector is used to provide the much-needed nutritional supplies to the rural population of Ethiopia. Over the past decades, there have been numerous efforts to transform the livestock productivity and profitability through dissemination of improved breeds and awareness education about efficient cattle management. However, such efforts have been severely hampered by pervasive livestock diseases that have crippled the economy of livestock keeping by farmers and pastoralists. For example, in a recent study, it was indicated that the economic cost associated with livestock diseases was estimated to have been \$0.5M and \$385M in 2005 and 2011, respectively with urban settings affected disproportionately (Tschopp et al., 2013).

Bovine tuberculosis is one such infectious diseases. It is primarily caused by *Mycobacterium bovis*, which also affects a wide range of warm-blooded animals. *M. bovis* belongs to a group of pathogenic mycobacteria called *Mycobacterium tuberculosis* complex (MTC) which also includes *M. tuberculosis*, *M. africanum*, *M. microtti*, and *M. canetti* (Coetzer, 2004). The pathogen is also responsible for nearly 150,000 new cases of zoonotic tuberculosis globally, commonly acquired through the consumption of contaminated untreated milk and its products (Muller et al., 2013; Olea-Popelka et al., 2017). The spreading pandemic of HIV/AIDS infection especially in developing countries like Ethiopia where BTB is an endemic disease poses an eminent threat to public health. Furthermore, BTB is known to have a significant socio-economic impact affecting international trade

in livestock and livestock products (Muller et al., 2013). In Ethiopia, the limited small-scale studies mostly carried out in central regions showed the median proportion of tuberculosis cases caused by *M. bovis* in the range of 16.7% to 31.4% (Muller et al., 2013).

In regions like Tigray, livestock play an important socio-economic role. These include draught power for traction and transport, household cash income from sale of animals and animal products, food such as milk for household consumption and manure to maintain soil fertility (Gebremedhin, Pender, & Tesfay, 2004). On the other hand, productivity obtained from livestock sub-sector, both qualitatively and quantitatively, is below standard level, due to, among other factors, a widely prevailing diseases, BTB being one of them. Therefore, the need for implementing efficient and sustainable disease control program in the region is evident and this requires prior and adequate understanding of the disease epidemiology.

In Tigray region, major changes in land use either due to climate change and national land use programs or increasing human population along with changing patterns of settlement may have influenced the epidemiology of diseases like BTB whose impact disproportionately affect large communities. Designing evidence-based disease control strategies coupled with robust epidemiological studies is essential to limit the impact of BTB. Here, we reported findings from a study conducted to determine prevalence of BTB and associated risk factors in cattle maintained under mixed crop-livestock farming system in Tigray region, Northern Ethiopia.

Materials and methods

Study area

The study was conducted from July 2008 to December 2008 in three districts of Tigray region, northern Ethiopia namely: Enderta, Ofla and Alamata. Alamata is the south most district and located at 600 km north of the capital city, Addis Ababa. The altitude ranges from 1,178 – 3,148 meters

above sea level (m.a.s.l), with 75 % of the area lying in the low altitude (<1500 m.a.s.l). Mean annual temperature and rainfall is 22.3 and 727 mm, respectively. Ofla (Korem) is south district and is located 620 km north of Addis Ababa with an altitude range of 1700 to 2800 m.a.s.l. Mean annual temperature and rainfall is 22 °C and 950 mm, respectively. It has bi-modal rainfall featured by erratic yet high intensity rains similar to most highland areas of the country. Enderta is south-east district and is located 778 km north of Addis Ababa. The altitude ranges from 1,500 to 2,100 m.a.s.l. In Enderta district, 96% of the agro-climatic condition is mid-altitude with the mean annual rainfall and temperature of 500 mm and 26 °C, respectively.

The main agricultural economic activity in the study districts is crop-livestock mixed farming system. Cattle are mainly kept for draught power. According to the recent livestock census (Bureau of Agriculture and Natural Resource Development, 2008), the districts consist of 1,131,614 cattle, 80,249 sheep, 52,396 goats, 48,998 equines, and 136,108 poultry.

Study Animals

The study population consisted of 1,357 heads of cattle (47.8% male; 52.2% female) selected from 310 herds managed under mixed crop-livestock farming practice. The breeds were predominantly zebu cattle constituting 89.9% of studied animals, with few exotic breed (6.9%) (Holstein) and their cross with local cattle (3.1%). Holstein breed is kept by small-holder farmers for milk production for household consumption and sale to local market for income generation. Zebu cattle are mainly kept for draught power, subsistent milk and meat production as well as cash income generation through live animal sale. Livestock are managed in typical traditional husbandry system with no extra feed supplement other than communal field grazing and supplementation with crop residues. The zebu breed has gone through significant population size reductions as a result of famine and man-made problems over the past 20 to 30 years (Zerabruk, Bennewitz, Kantanen, Olsaker, & Vangen, 2007). Furthermore, diseases and chronic feed shortage in the region have been major constraints to

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livestock productivity.. Prevailing livestock diseases in the region range from chronic parasitism to highly acute infectious disease like Contagious Bovine Pleuropneumonia (CBPP). Veterinary care is inadequate and is often provided by the state-owned clinics run under the regional agricultural bureau.

Sample size determination

There was no published data on BTB in Tigray region and mean national prevalence estimate of 5% was considered to estimate the required sample size. Therefore, with 5% expected prevalence, 90% confidence level and 1% desired precision, the minimum sample size was calculated to be 1,286. Considering possible dropouts, the final sample size was adjusted to 1,357 cattle by including 5% of the calculated sample.

Study design and sampling strategy

A cross-sectional multi-stage sampling strategy (district, village and herd level) was used in selecting the study population. Herd was considered as primary sampling unit and individual animals as unit of concern. The three districts were selected based on their potentials for livestock production in the region and climate diversity. Four villages were selected per district except for Korem district where only two villages were selected because of logistic problems encountered in the field. The sampling frame consisted list of herds in the village associations, which in turn consisted of 70-80 herds per village. On average, about 20 to 30 herds were selected randomly from each village, with herd size ranging from 3 to 60 cattle. Considering the variation in the outcome, which tended to be greater between herds than within the herds, we included more number of herds, and cattle were sampled randomly proportional to the herd size. For herds with size 5-10 cattle, five animals were sampled; for herds containing less than 5 cattle, all animals were sampled; and for herd size more than 10 animals, 5-10 cattle were sampled. A total of 310 herds were tested (207 farms with < 10 cattle and

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103 farms with ≥ 10 cattle). Exclusion criteria for study animals were age less than 1 year, cows at late stage of pregnancy and clinically sick animals. These group of animals can have immunocompromised and anergic status and are unable to react to the skin test, thus, may contribute to false BTB test negative results.

Tuberculin testing

A comparative cervical intradermal tuberculin test (CIDT) was performed using Bovine Tuberculin PPD 3000 (30,000 IU/ml) and Avian Tuberculin PPD 2500 (25,000 IU/ml) (Animal Science Group, Wageningen UR, Netherlands) in the neck skin. Two injection sites were marked on the left side of the mid-neck region, 12 cm apart, and shaved. The initial skin thickness at each site was measured with an electronic calliper device (Preco Machine Tool Co., Ltd Qingdao/Shandong, China). One site was injected with 0.1ml of bovine PPD and the other with similar amount of avian PPD, into dermis using insulin syringe (Shanghai care life International Trading Co., Ltd). After 72 hours, the increase in skin thickness at the injection sites was re-measured. The results were interpreted according to the recommendations of the Office International des Epizooties (OIE, 2009). The skin reaction was classified as positive when there was a tendency towards bovine reaction of 4 mm greater than the avian reaction; inconclusive when the reaction at the bovine site was 1-4 mm greater than the avian site. The result was recorded as negative, if the increase in skin thickness at the bovine site of injection is less than or equal to the increase in the skin reaction at the avian site of injection. Herds were classified as tuberculosis positive if at least one positive reactor animal was reported (Oloya et al., 2007; Tschopp, Schelling, Hattendorf, Aseffa, & Zinsstag, 2009).

Data collection and management

Data obtained from the farms were first recorded on data collecting sheets. Structured questionnaires were administered to the livestock owners to generate data on herd size, hygiene and management of animals. Questionnaires were delivered to the farmers by the local languages (Tigrinya) and translated back to English upon filling the forms. Putative exposure variables were given specific codes and dataset established in an Excel® spreadsheet for appropriate storage. Age of an animal was determined based on dentition characteristics (Pace & Wakeman, 2003) combined with owner's recall information on birth date. Body condition score (BCS) was recorded as lean, moderate and fat based on guidelines described in the manual for body condition scoring of zebu cattle (Nicolson, 1986).

Data analysis

Dataset was read in R, a statistical computing and graphics software (R 3.5.0 for windows), for appropriate statistical analysis. Prior to analysis, data were verified for any missing observations and incorrect entries. Categories with many missing values (>15) and fewer observations (<10) were excluded. BTB prevalence was determined as the proportion of skin test positive animals out of the total tested cattle. Herd level prevalence was defined as the number of herds with at least one-reactor cattle divided by the total number of herds tested.

Given multi-stage sampling design of the study that led to hierarchical structure of the data, as well as binary outcome of the response variable, use of multilevel logistic mixed effect model was deemed appropriate. A multilevel logistic mixed effect regression model incorporating cluster-specific random effects to account for within-cluster correlation of BTB infection was used. Use of such model allows accurate estimation of coefficients and standard errors by incorporating cluster-specific random variables to account for correlation of the data.

Multilevel logistic mixed model was fitted with maximum likelihood estimation (adaptive Gauss-Hermite Quadrature). An introduction of random effect terms (village and herd-size) allowed an estimation of the variation in the level of association between BTB and individual animals at village and herd level, respectively, thus accounting for within village and within herd correlation of the infection. Such approach gives a better estimate of measures of association and underlying heterogeneity at individual levels.

Significance of clustering effects due to village and herd was calculated by computing likelihood ratio test statistic (LR) as two times the difference in the log likelihood values between three-level model (that incorporated, in an hierarchical way, village, herd, and individual animals). The LR was also calculated for two-level model (that incorporated herd as level 2 cluster variable and individual animals), as well as two-level model (that incorporated herd and individual animals) and single level model (individual cows level). A higher value of the difference in the log-likelihood ratio of any of two models compared (in relation to reference value from χ^2 table, $p < 0.05$) was considered statistically significant (between-village or between herd variance is non-zero). Intra-cluster coefficient (ICC), an estimate which allows comparison of heterogeneity in the risk of exposure of individual cows to BTB within villages compared to between villages, as well as within herds compared to between herds was estimated according to method described by Austin (Austin & Merlo, 2017). Large value of ICC indicates similarity in risk of exposure to BTB for cows within a village or a herd. For all statistical tests, $p < 0.05$ was considered statistically significant.

Ethical considerations

Ethical approval was obtained from the Research Ethics Committee of Tigray National Regional State Science and Technology Agency, after critically reviewing the research proposal. The purpose of this study was explained to cattle owners by the local veterinary officers to solicit consent to participate in the study.

Results

Prevalence of BTB

Of the 1357 cattle tested, 59 (4.3%; 95% CI=3.4-5.6) were positive for BTB; with 38 cattle (2.8%) giving doubtful reactions to the test. The distribution of skin test reactions (as the frequency and proportion of non-reactors, doubtful, or positive) with respect to categories of different exposure variables are shown in Table 1. The prevalence was significantly higher in Alamata (5.6 %; 95% CI= 3.9-8.0) compared to Korem (1.6%; 95% CI= 0.7-3.8). The prevalence was significantly higher in exotic breeds (12.8%; 95% CI= 7.4-21.2) compared to indigenous cattle (3.5%; 95% CI= 2.6-4.7). Animals acquired through purchase had insignificantly higher tuberculosis prevalence (6.2%; 95% CI= 4.3-8.9) than in-born cattle (3.5%; 95% CI= 2.5-4.9).

Risk factors associated with BTB

Table 2 presents the results from univariate analysis of the association between individual level exposure factors and cattle positive reactivity to tuberculin test. The results showed that the positive tuberculin test in the study population was significantly associated with breed and animal source at $p < 0.05$. BTB positivity was higher in exotic (OR= 3.8) than zebu cattle; while purchased cattle had higher BTB prevalence (OR=2.5) compared to in-born cattle. On the other hand, there wasn't a significant difference in BTB prevalence in age (<10 years or \geq 10 years old), sex, body condition, function and animal type.

Results from the univariate analysis on the association between herd level prevalence and exposure variables are shown on Table 3. Herd prevalence was 16.1% (95% CI= 12.4-20.7). Except a lower prevalence of BTB in Korem district, no other tested variables showed any significant association with herd level BTB positivity. The herd prevalence was higher in Alamata (19.6%; 95% CI=13.3-28.1; $p=0.018$) and Enderta districts (18.3%; 95% CI=12.6- 25.9; $p=0.025$) compared to Korem (6.0%; 95% CI= 2.2- 14.9).

Results from multilevel logistic mixed effect model showing risk factors for BTB are presented in Table 4. Likelihood ratio test (LR) test statistics for cluster effect due to village was 4.5 indicating significant evidence that within-village variance was non-zero ($p < 0.05$). Similarly, within herd variation of BTB prevalence was significant (LR=6.2; $p < 0.05$). Estimated value of ICC for village was 0.257 (25.7% variability in BTB between individual cows was due to difference in village). Similarly, ICC for herd was 0.392 (39.2% variability in BTB between individual cows was due to BTB herd variability). The model showed that breed and animal source were the main predictive variables of importance for a higher risk of tuberculin positivity in individual cattle.

Accordingly, the risk of exotic cattle having tuberculin positive reaction was three times higher (OR= 3.0; 95% CI= 1.2-7.4; $P = 0.014$) compared to indigenous breed. Likewise, the odds of purchased cattle becoming BTB positive was twice higher than inborn cattle (OR=2.1; 95%CI= 1.1-4.1; $P=0.027$). Similarly, barn type, herd size and barn hygiene were important predictors of herd tuberculin positivity. Herds maintained in closed confinement were at a greater risk of becoming tuberculosis positive (OR=2.8, 95% CI=1.2-6-8; $p=0.018$) than those maintained in open-air barn. Herds with ≥ 20 heads of cattle were more likely to become tuberculin positive compared to those herds with ≤ 10 heads (OR=2.6, 95%CI= 1.0- 6.9; $p=0.05$). Poor hygienic condition of animal barn appeared to be only marginally linked to high risk of positive reactivity to skin test (OR=95%CI=1.0-4.2; $p=0.055$).

Discussion

Prevalence of BTB

We reported an overall BTB individual animal prevalence of 4.3% in cattle. This is in agreement with findings from previous studies in Ethiopia that reported prevalence ranges between 3.5% and 4.7% (Berg et al., 2009; Shitaye et al., 2006; Tschopp et al., 2009). However, the prevalence reported in the present study was much lower than compared to the 24-30% prevalence reported in central

Ethiopia (Elias, Hussein, Asseged, Wondwossen, & Gebeyehu, 2008; Firdessa et al., 2012). A higher prevalence was reported in small holder farms (13.5%) (Ameni et al., 2007) and in dairy farms (19%) in Addis Ababa (Shitaye et al., 2006). This suggests that animals kept in peri-urban farms in Ethiopia tend to have higher prevalence of BTB. It is important to notice that animal breeding programs in the area tend to follow some patterns of distribution of improved breeds from the urban to rural Ethiopia. This is likely to have profound impacts in the evolution of epidemiology of BTB.. It is worthy mentioning that East African countries like Tanzania have reported a much lower (0.9 – 2.4%) individual animal prevalence (Cleaveland et al., 2007; Katale et al., 2013). In our study, the herd level prevalence was 16.1%, which is much lower than 50- 60% reported by others (Ameni, Amenu, & Tibbo, 2003 (a); Firdessa et al., 2012; Tschopp et al., 2009), this too support the eminent shift in epidemiological landscape in rural settings.

The variation in BTB prevalence in Ethiopia may be attributed to the difference in cattle husbandry practice, breeds and possibly some climatic factors. For example, in the central Ethiopia and most peri-urban areas, more exotic breeds of cattle are reared in small scale and intensive dairy production where as in the countryside, above 90% of cattle are indigenous zebu breeds managed under traditional extensive system. Therefore, introduction of the exotic breeds of cattle and increasing intensification of animal husbandry without test and control program of tuberculosis might have been a factor for increased infection in the susceptible cattle population in central and urban areas in Ethiopia, and similar pattern is likely to happen in the rural settings. The relative variation in the prevalence of BTB among the study districts (Table 1) suggests that high altitude areas are likely to have low prevalence of BTB compared to low altitude areas. This could be attributed to difference in agro-ecology as well as breed, which may possibly be attributed to high level of immunity among indigenous breeds in the high land cattle and the long horn breeds in the low lands.

Risk factors for BTB

Multivariable analysis revealed that breed (exotic cattle) ($p=0.014$) and purchase of new cattle into the herd ($p=0.027$) were important risk factors for tuberculin positive reactivity in the study area.

Previous studies (Romha, Gebrezgabiher, & Ameni, 2014; Sibhat et al., 2017) also indicated the importance of breed difference for increased risk of BTB in cattle. Genetic composition of host animal, among other factors, could influence the outcome of mycobacterium infection. For example, African zebu cattle are known to be more resistant to BTB than exotic breeds of cattle (Vordermeier et al., 2012). The importance of introducing newly purchased animals into herds as an important risk factor is consistent with a previous finding (Tschopp et al., 2009).

An increased risk of BTB infection in cattle kept in closed enclosures is similar to previous findings (Ameni et al., 2006), suggesting the importance of management practice in influencing the spread of tuberculosis in cattle. Poor ventilation and humid conditions are known factors associated with the spread of diseases that are transmitted through aerosol, including BTB.

An increase in tuberculin reactivity observed in large herd size (OR= 2.6; $p=0.05$) agrees with findings from previous studies (Cleaveland et al., 2007; Dejene et al., 2017; Tschopp et al., 2009). This could be attributed to an increase in the rate of contact that favors transmission of infection in the herd, indicating animal husbandry practice as an important risk factor for contracting BTB.

The finding that age wasn't a risk factor for BTB contradicts findings from previous studies (Ameni et al., 2003 (a); Ameni et al., 2007; Cleaveland et al., 2007; Firdessa et al., 2012; Moiane et al., 2014).

Biologically, it is believed that older animals have a higher probability of encountering infection over time and reduced immunity with old age, while this was not suggestive in our case. It would be interesting to investigate if age contributed to the low BTB prevalence among old animals. The other possible explanation could be due to the low number of old animals (>10 years) included in the present study (318 out of 1357).

On the other hand, the finding that sex and body condition weren't risk factor for BTB agrees with previous studies (Ameni et al., 2003 (a); Demelash et al., 2009; Katale et al., 2013; Oloya et al., 2007) #21).

The finding of lower prevalence (4.3%) of BTB in rural cattle production system in which the majority of cattle breeds are local zebu animals indicates that tuberculosis isn't a major animal health problem for mixed crop-livestock production system of Tigray region, at least compared to other infectious diseases with high and acute mortality and morbidity. It is worth noting that actual scenario of BTB could be affected by the ability of tuberculin skin test in detecting chronically infected cattle. In the study area, cattle are managed poorly under chronic malnutrition, high burden of gastrointestinal parasites and concurrent infections with other diseases. These conditions could lead to a decline in the cell mediated immune responses of the animal making them less sensitive (anergic state) to tuberculin test (de la Rua-Domenech et al., 2006).

Zoonotic and socioeconomic implications of BTB

Despite the low prevalence BTB finding, the present study shows that BTB is endemic and widespread in rural cattle production system in Tigray and other parts of Ethiopia with similar ecosystem. Even though the occurrence of clinically active tuberculosis is apparently low in local zebu breeds in the locality, maintenance of subclinical infection and the existence of aggravating risk factors for its transmission could be a potential threat for animal health and productivity. The endemic situation in local zebu breeds of cattle could act as reservoir of *M. bovis* and source of infection for the relatively susceptible exotic breeds. Especially, with introduction of the exotic breeds of cattle and an increase in intensification of animal husbandry without BTB test and slaughter policy leads to an increase in susceptible cattle population and spread of the disease in the area.

Moreover, *M. bovis* in cattle remains to be a threat to public health because of pervasive ideal conditions for zoonotic transmission and human infections. Lack of awareness about the disease, close intimacy between human and cattle such as sharing of the same house, poor hygienic conditions and habit of raw milk and meat consumption among the rural communities are contributing factors for human transmission and vice versa.

Although the findings of this study may not accurately represent current status of BTB in Tigray, the study may raise pertinent questions regarding changing husbandry system of livestock in the region.

Majority of the dairy development projects have been built around the dissemination of exotic or improved cattle breeds, which require housing and intensification. These animals are often originated from central regions of Ethiopia where prevalence of BTB is known to be high. It is therefore, important to continuously revise disease control programs including BTB control to avoid un intended epidemiological consequences. We also recommend that molecular genetic analysis be carried out to identify predominant genotypes in the area and establish possibility of epidemiological links with genotypes from highland areas.

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Author Contributions

Conceived and designed the experiments: HT, BD, and ES. Analyzed the data: HT, BD, ES and AM.

Contributed to the writing of the manuscript: HT, BD, ES, AM and GT.

Competing interests

The authors declare that they have no competing interests.

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Table 1. Distribution of cattle reactivity to tuberculin test in cattle raised in mixed crop-livestock system in Tigray, Ethiopia

Exposure variable	Label	Reactivity to tuberculin test			Total
		No reaction (%)	Doubtful (%)	Positive (%)	
Sex	Male	597 (92.1)	22 (3.4)	29 (4.5)	648
	Female	663 (93.5)	16 (2.3)	30 (4.2)	709
Age group	<10 year	966 (92.97)	29 (2.8)	44 (4.2)	1039
	≥ 10 year	294 (92.45)	9 (2.8)	14 (4.7)	318
Breed	Local	1144 (93.7)	34 (2.8)	43 (3.5)	1221
	Cross-breed	37 (88.1)	1 (2.4)	4 (9.5)	42
	Exotic	79 (84.0)	3 (3.2)	12 (12.8)	94
Body condition	Lean	329 (94.0)	9 (2.6)	12 (3.4)	350
	Mediun	863 (92.5)	25 (2.7)	45 (4.8)	933
	Fat	68 (91.9)	4 (5.4)	2 (2.7)	74
Animal type	Milking	423 (93.8)	10 (2.2)	18 (4.0)	451
	Dry cow	215 (92.7)	6 (2.6)	11 (4.7)	232
	Heifer	29 (93.6)	1 (3.2)	1 (3.2)	31
	Bull	67 (93.1)	3 (4.2)	2 (2.8)	72
	Steer	525 (92.1)	18 (3.2)	27 (4.7)	570
Purpose	Milking cow	663 (93.5)	12 (2.4)	219 (4.1)	709
	Fattening	51 (85)	4 (6.7)	4 (6.7)	60
	Draught animal	546 (92.9)	26 (4.4)	26 (4.4)	588
Animal source	In-Born	864 (93.6)	27 (2.9)	32 (3.5)	923
	Purchase	396 (91.2)	11 (2.5)	27 (6.2)	434
Barn/house/	Open	1094 (93.5)	32 (2.7)	44 (3.8)	1170
	Closed [166 (88.8)	6 (3.2)	15 (8.0)	187
Hygiene	Good	727 (93.8)	19 (2.5)	29 (3.7)	775

	Poor	533 (91.6)	19 (3.3)	30 (5.2)	582
Herd mixing	Yes	1087 (93.2)	33 (2.8)	46 (3.95)	1166
	No	173 (90.6)	5 (2.6)	13 (6.8)	191
Contact with wild animals	Yes	604 (92.9)	19 (2.7)	27 (4.2)	650
	No	656 (92.8)	19 (2.7)	32 (4.5)	707
Feeding provided	Individually	130 (90.3)	3 (2.1)	11 (7.6)	144
	All together	1130 (93.2)	35 (2.9)	48 (3.96)	1213
District	Korem	306 (97.1)	4 (1.3)	5 (1.6)	315
	Alamata	452 (90.8)	18 (3.6)	28 (5.6)	498
	Enderta	502 (92.3)	16 (2.9)	26 (4.8)	544
Total		1260 (92.9)	38 (2.8)	59 (4.3)	1357

Table 2. Results from univariate standard logistic regression analysis showing the association between BTB and individual animal level exposure variables in Tigray, Ethiopia.

Exposure variable	Label	No. tested	positive	Prevalence (95% CI)	OR (95%CI)	P -value	
Sex	Male	648	29	4.5 (3.1-6.5)	1.0	-	
	Female	709	30	4.2 (3.0-6.0)	0.8	0.4 - 1.4	0.415
Age group	<10 year	1039	44	4.2 (3.2-5.6)	1.0	-	-
	≥10 year	318	15	4.7 (2.9-7.7)	1.1	0.6 - 2.2	0.759
Breed	Local	1221	43	3.5 (2.6-4.7)	1.0	-	-
	Cross-breed	42	4	9.5 (3.6-22.8)	2.1	0.7 - 6.3	0.186
	Exotic	94	12	12.8 (7.4-21.2)	3.8	1.6 - 9.0	0.003 [†]
Body condition	Lean	350	12	3.4 (2.0-5.9)	1.0	-	-
	Medium	933	45	4.8 (3.6-6.4)	1.2	0.5 - 2.7	0.71
	Fat	74	2	2.7 (0.7-10.2)	1.1	0.2 - 6.3	0.903
Animal type	Milking	451	18	4.0 (2.5-6.2)	1.0	-	-
	Dry cow	232	11	4.7 (2.6-8.4)	0.9	0.4 - 2.0	0.726
	Heifer	31	1	3.2 (0.5-19.7)	0.6	0.1 - 4.8	0.607
	Bull	72	2	2.8 (0.7-10.5)	0.8	0.2 - 3.7	0.768
	Steer	570	27	44.7 (3.4-5.6)	1.2	0.6-2.6	0.573
Function	Milking cow	709	29	4.1 (2.9-5.8)	1.0	-	-
	Fattening	60	4	6.7 (2.5-16.5)	1.6	0.7 - 3.7	0.241
	Draught Animal	588	26	4.4 (3.0-6.4)	1.3	0.7 - 2.4	0.499
Animal source	In-Born	923	32	3.5 (2.5-4.9)	1.0	-	-
	Purchase	434	27	6.2 (4.3-8.9)	2.5	1.4 - 4.7	0.003 [†]

[†]=Significant

Table 3. Results from univariate standard logistic regression analysis showing the association between BTB and herd level exposure variables in Tigray, Ethiopia.

Factors	Label	Herds tested	Positive	Prevalence (95% CI)	OR	P-value
All herds	Herd number	310	50	16.1 (12.4- 20.7)	--	-
District	Korem	67	4	6.0 (2.2- 14.9)	1.0	-
	Alamata	112	22	19.6 (13.3- 28.1)	3.9	0.018
	Enderta	131	24	18.3 (12.6- 25.9)	3.5	0.025
Herd size	< 10	207	32	15.5 (11.1- 21.1)	1.0	-
	10- 20	80	11	13.8 (7.8- 23.2)	0.9	0.716
	>= 20	23	7	30.4 (15.2- 51.7)	2.4	0.076
Hygiene	Good	169	24	14.2 (9.7- 20.4)	1.0	-
	Poor	141	26	18.4 (12.8- 25.8)	1.4	0.313
Herd mixing	Yes	270	41	15.2 (11.5- 20.0)	1.0	-
	No	40	9	22.5 (12.1- 38.0)	1.6	0.244
Wildlife contact	Yes	139	22	15.8 (10.6- 22.9)	1.0	-
	No	171	28	16.4 (11.5- 22.7)	1.0	0.896
Feeding style	Individually	36	9	25.0 (13.5- 41.6)	1.0	-
	Communal	274	41	15.0 (11.2- 19.7)	0.5	0.129
Barn/ house	Open	261	38	14.6 (10.8- 19.4)	1.0	-
	Closed	49	12	24.5 (14.4- 38.5)	1.9	0.087

Table 4. Result from multilevel logistic mixed effect model showing risk factor for BTB in cattle raised in mixed crop-livestock system in Tigray, Ethiopia

Risk factor	Variable	Adjusted OR	(95% CI)	P-value
Breed	Cross-breed vs. Local cattle	1.7	0.54 - 5.4	0.364
	Exotic vs. Local	3.0	1.2 - 7.4	0.014
Animal source	Purchased vs. in-Born	2.1	1.1 – 4.1	0.027
Herd size category	≥20 vs. <10 years	2.6	1.0- 6.9	0.050
Barn/ house	Closed vs. Open	2.8	1.2- 6.8	0.018
Hygiene	Poor vs. Good	2.0	1.0- 4.2	0.055