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Bovine tuberculosis and its associated risk factors in pastoral and agro-pastoral cattle herds of Afar Region, Northeast Ethiopia

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Bovine tuberculosis (BTB) has a potential public health risk and economic impact in pastoralist community whose livelihood depends on their livestock. A cross-sectional study was carried out from September, 2008 to June, 2011 on 1087 cattle under pastoral and agro-pastoral production system in four districts of Afar Pastoral Region of Ethiopia using comparative intradermal tuberculin skin test to estimate the prevalence of BTB and assess the associated risk factors for infection. The individual animal prevalence of BTB in cattle of Afar pastoralists was 11% (95% confidence interval (CI): 9 to 13%) with ≥ 4 mm cut-off and 18% (95% CI: 16% to 21%) with ≥ 2 mm cut-off. The herd prevalence was 44% (95% CI: 36 to 51%) and 56% (95% CI: 48 to 63%) at ≥ 4 and ≥ 2 mm cut-off points, respectively. In bivariate analysis, the prevalence was significantly associated with study districts, herd size, sex and age, and in multivariable logistic regression analysis, the statistical significance was maintained with study district, age and herd size of the cattle. In conclusion, the present study revealed a moderately high prevalence of BTB in Afar Pastoral Region of Ethiopia and further investigation is recommended to assess the zoonotic significance of the disease to the pastoralist communities of the region.

Key words: Bovine tuberculosis, prevalence, risk factors, comparative intradermal tuberculin test, Afar pastoral region, Ethiopia.

INTRODUCTION

Bovine tuberculosis (BTB) is a chronic, granulomatous mycobacterial infectious disease caused mainly by *Mycobacterium bovis*, which is a member of *Mycobacterium tuberculosis* complex. BTB is a zoonotic disease with a potential health risk to human and has

economic significance to livestock sub-sector (Ayele et al., 2004). Though BTB is controlled in developed countries through test-and-slaughter method, the disease poses a significant problem to the economy of the livestock sub-sector and remains a potential public health

threat in developing countries where controlling programs are lacking. In Africa, approximately 85% of cattle and 82% of human population lives in areas where BTB is partly or not controlled at all in animals (Cosivi et al., 1998) and consumption of raw animal product is a common practice in rural and pastoralist communities of the continent, which creates a potential risk for zoonotic transmission of *M. bovis* (Daborn et al., 1996).

Ethiopia possesses the largest cattle population in Africa with the total of about 51 million of cattle (CSA, 2010). The livestock sub-sector in general contributes about 45% to the gross domestic product of the country's agriculture based-economy (Behnke, 2010). In addition, the sector plays a crucial role in livelihood of the pastoralist communities, who own 42% of the country's livestock in the lowland arid and semi arid regions. In Eastern Africa, Ethiopia has the largest pastoralist population (7 to 8 million) which depends on livestock for their livelihood (Markakis, 2004). The main feature of pastoralist's way of life is that they move from place to place in search of water and pasture for their livestock.

In Ethiopia, BTB is known to be endemic with prevalence ranging from 3.4 to 50% depending on husbandry method, with extensive rural setting showing low prevalence as compared to intensive dairy farms (Ameni et al., 2007; Berg et al., 2009). In spite of large population of livestock, very few studies were carried out in pastoral area of Ethiopia in which prevalence of 0.8% BTB in cattle of Hamer pastoral district (Tschoop et al., 2010) and prevalence of 5.5% of BTB in cattle of Borena pastoral area (Gumi et al., 2011) were reported. However, so far there is no report of BTB in cattle of Afar Pastoral Region of Northeast Ethiopia.

The Afar pastoral communities of Ethiopia are characterized by owing large numbers of livestock with diversity of species of animal. The consumption of raw animal products such as milk and very close physical contact creates a significant risk for transmission of zoonotic diseases like BTB. In addition, the existing epidemiological setting in Afar Pastoral Region is characterized by the presence of large herds of cattle, interspecies mixing of herds of animals at watering point, grazing area, at night in the village and the existence of climatic stress factors in the pastoral regions could suggest the existence of a potential risk factors for infection and transmission of diseases such as BTB in the livestock and pastoral communities of the region. Despite the large livestock population and existence of potential risk factors in the pastoral region, the epidemiology of BTB in the herds of cattle owned by pastoralist has not been well investigated so far.

The present study, therefore, was designed to investigate the epidemiology of BTB and assess the associated risk factors in the Afar Pastoral Region of Ethiopia.

MATERIALS AND METHODS

Study area

The study was conducted from September, 2008 to June, 2011 in four districts namely (Amibara, Dubti, Afambo and Chifra districts) of Afar Pastoral Region. The Afar Pastoral Region is located in northeast of Ethiopia between 39° 34' to 42° 28'E longitude and 8° 49' to 14° 30' N latitude (Figure 1). The region shares common international boundaries with Eritrea in the north-east and Djibouti in the east, and it is characterized by an arid and semi-arid climate with low and erratic rainfall. Rainfall is bi-modal throughout the region, with a mean annual rainfall below 500 mm in the semi-arid western escarpments and decreasing to 150 mm in the arid zones to the east. The altitude of the Region ranges from 120 m below sea level in Danakil depression to 1500 m above sea level. Temperatures vary from 20°C in higher elevations to 48°C in lower elevations. The human population of Afar region is 1.5 million in which the majority are pastoralists who largely depend on livestock production for their livelihood (Afar National Regional State (ANRS), 2010).

There are about 1.9 million Afar breed cattle in Afar Region, of which 90% of the cattle are managed under pastoral production system and the rest 10% in agro-pastoral production system (ANRS, 2010). The four districts were selected based on the cattle population, accessibility of their sub districts and presence of potential risk factors. Because of the presence of large pasture land and rivers in the districts, animals from different districts migrate to river banks and vast pasture lands where intermixing of different species (cattle, camel, goat and sheep) and herds of livestock occur. In Amibara and Dubti districts, there are large state-owned and private cotton farms, which after harvesting, become grazing sites where large number of herds of different species of livestock (cattle, camel and small ruminants) from various districts congregate to graze on the leftovers of the harvest, creating a potential risk factor for interspecies (cattle, camel, goat and sheep) and interherd disease transmission. Majority of the grazing land and watering points in Amibara district are shared by wild animals (including oryx, warthog, gazelle and zebra) from the Awash National Park. It was very common to observe cattle grazing in close proximity with wild animals in the pasture land of Amibara district. The sites selected in Amibara, Chifra and Dubti districts were pastoral and that of Afambo were mainly agro-pastoralist in their production system.

Study design

A cross sectional study was conducted in the four districts of Afar Pastoral Region and a total of 17 sub districts were included in the study based on the inclusion criteria (accessibility, security, and willingness of the pastoralists to participate in the research). All settlements (villages) in each sub district were included after obtaining the elder's consent to participate in the study. In our study, cattle owned by one owner and/or his close relatives, in which the animals shared common grazing sites, watering points, kept at night in common site and move together during migration, were considered as a herd to calculate the herd prevalence. In settlements which had super-herd, larger herd composed 500 to 600 animals, herd selection was made proportionally to represent each cluster in the super-herd. A total of 180 herds were tested and the final analysis was carried out on 171 herds, the rest 9 (5%) were drop-outs in which they were not available for reading after 72 h of tuberculin injection. In each herd, individual cattle were selected randomly after recording all the animals in the herd.

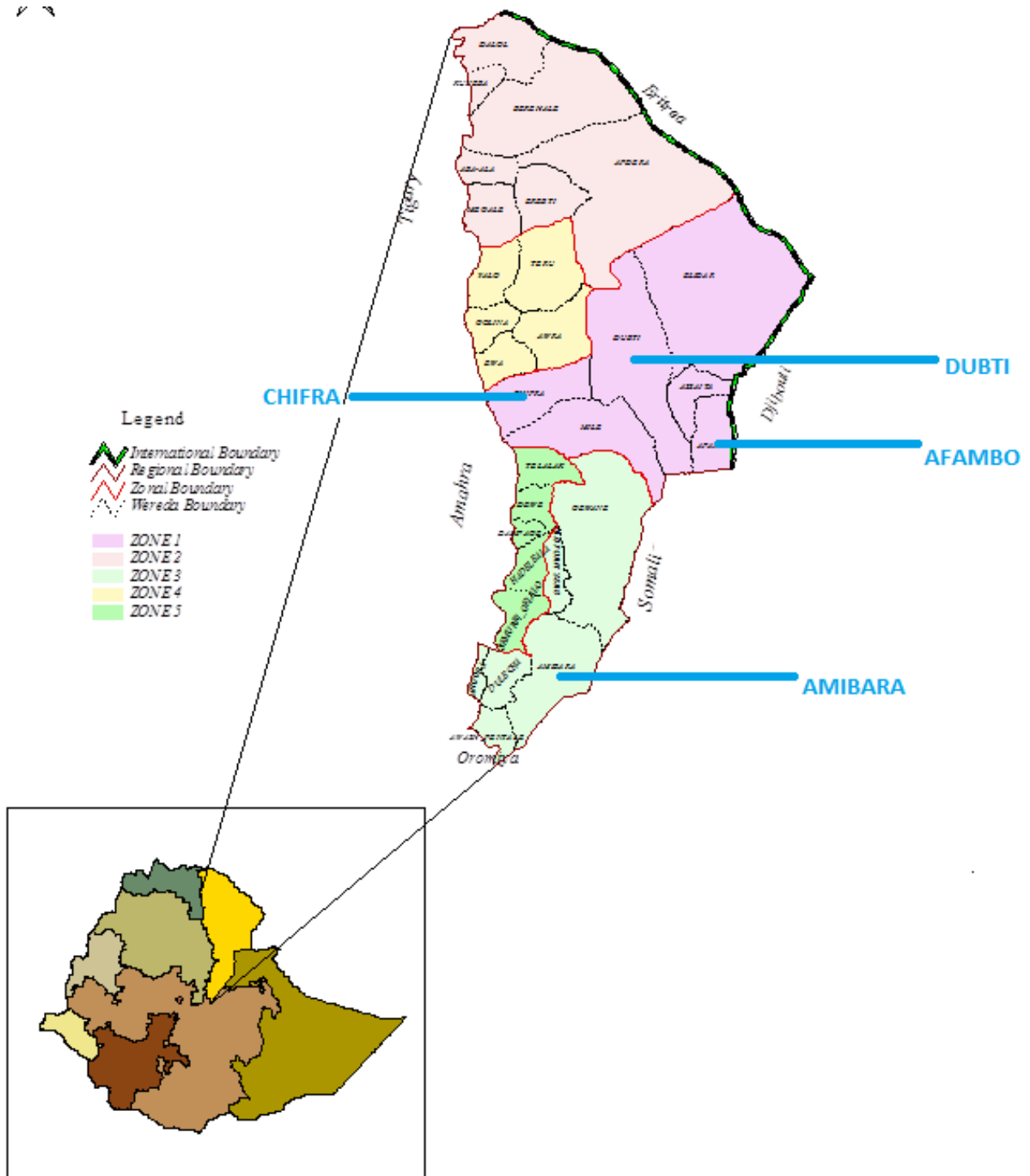


Figure 1. Map of Afar Pastoral Region indicating the study districts.

Study animals

For the Comparative intradermal tuberculin skin test (CIDT), cattle above the age of six months having no clinical symptom of any disease were included. Study animal related information on each

tested cattle (such as sex, age, body condition score, lactation and reproductive status, parity number (number of calving)) were collected and recorded at the time of the test. All the cattle in this study were Afar (Danakil) breed of cattle which are categorized under Sanga breed group. Each animal was de-wormed with antihelminthic drug after testing and collecting fecal sample. Sample

size was determined according to Thrusfield (1995) considering the recommendation for sample size estimation involving three or more cluster stages (Thrusfield, 1995). Based on this estimation, the estimated sample size was 1,152. Hence, a total of 1,147 cattle were tested, although 5% were not available for the reading after 72 h of tuberculin injection, and hence were considered as drop-outs. Thus, the final analysis of the data was based on the results of 1087 cattle tested.

Comparative intradermal tuberculin skin test (CIDT)

CIDT was carried out by injecting both bovine purified protein derivative (PPD) and avian PPD (Observe™ bovine and avian tuberculin,ASURE Quality Company, Mt. Wellington, Auckland, New Zealand). Two sites on the skin of the mid-neck of the cattle, 12 cm apart, were shaved, and skin thickness was measured with a caliper. One site was injected with an aliquot of 0.1 ml of 2,500 IU/ml bovine PPD into the dermis, and the other was similarly injected with 0.1 ml of 2,500 IU/ml avian PPD. After 72 h, the skin thickness at the injection sites was measured and recorded. Results were interpreted according to the recommendations of the Office International des Epizooties (OIE, 2009) at ≥ 4 mm cut-off and also at ≥ 2 mm cut-off (Ameni et al., 2008). Thus, at cut-off ≥ 4 mm, if the increase in skin thickness at the injection site for bovine PPD (PPD-B) was greater than the increase in skin thickness at the injection site for avian PPD (PPD-A) and PPD-B minus PPD-A was less than 2 mm, between 2 and 4 mm, or 4 mm and above, the animal was classified as negative, doubtful, or positive for BTB, respectively. At cut-off ≥ 2 mm, if the difference between B and A was greater or equal to 2 mm, the animal was considered as positive, while if the difference is less than 2 mm, the animal was considered as negative. When the change in skin thickness was greater at PPD-A injection site, the animal was considered positive for mycobacterial species other than *Mycobacterium tuberculosis* complex. A herd was considered as positive if it had at least one tuberculin reactor animal.

Body condition scoring

The body condition of each of the study animal was scored using the guidelines established by Nicholson and Butterworth (1986). Accordingly, on the basis of observation of anatomical parts such as vertebral column, ribs, and spines, the study animals were classified as lean (score, 1 to 2), medium (3 to 4), or fat (greater than 5).

Fecal sample examination

Fecal samples from CIDT tested cattle were collected during tuberculin injection directly from the rectum of each animal using sterile glove and placed in labeled vials containing 10% formalin solution and then transported to the laboratory for microscopic examination using floatation technique (Soulsby, 1982), and eggs of the parasites were classified based on their morphology and size.

Data management and analysis

Data were classified, filtered, coded using Epidata software and Microsoft Excel sheet, and was transferred and analyzed using STATA version 11 (Stata Corp., Collage station, TX). Pearson chi-

square was used to evaluate the statistical significance of the associations of different categorical variables with skin test results and McNemar's chi-square was used to assess the association of PPD-A and PPD-B results. Bivariate and multivariable logistic regression analyses were performed to quantify crude and adjusted effects of pre-specified risk factors on tuberculin reactivity. P-value less than 5% was considered statistically significant. In cases of estimating the effect of different risk factors in terms of odds ratio (OR) with corresponding 95% confidence interval, statistical significance was assumed if the confidence interval did not include one among its values.

RESULTS

Individual animal prevalence

On the basis of CIDT, the animal prevalence of BTB was 11% (119/1087) with 4 mm cut-off point and 18.4% (200/1087) with 2 mm cut-off point. At 4 mm cut-off point, there were statistically significant differences in proportions of bovine positive reactor animals between the four districts ($\chi^2 = 21.7$, $P = 0.000$), herd size category ($\chi^2 = 8.72$, $P = 0.013$), sex ($\chi^2 = 6.96$, $P = 0.008$), age category ($\chi^2 = 21.12$, $P = 0.000$) (Table 1). At 2 mm cut-off point, in addition to the factors indicted above, there was a statistically significant difference in proportion of bovine positive reactors between the pastoral and agro-pastoral production system ($\chi^2 = 3.8$, $P = 0.05$) where a higher proportion of positive reactors in cattle under pastoral production system than those in agro-pastoral production system. Multivariable logistic regression analysis (Table 2) showed that older cattle (9 years and above) had 2.66 times the odds of being tuberculin reactors compared with those cattle less than 2 years old (adjusted OR = 2.66; CI = 1.21-5.84). Cattle found in Amibara district had also the higher odds of being tuberculin positivity in relative to those cattle in Chifra district (adjusted OR = 6.56; CI = 1.63 to 28.73). At both cut-off points, there was no statistical significance difference in the proportion of bovine tuberculin positivity between groups in relation to body condition score, breed, gastrointestinal parasite infestation status, lactation status, reproductive status, and number of parity. The gastrointestinal parasite infestation status in general was low both in tuberculin nonreactors and reactor cattle. In majority of the tested animals eggs of *Trichostrongylus* species were the most common parasite eggs identified in this study.

Herd prevalence

The herd prevalence was 44% (95% CI = 36 to 51%) and 56% (95% CI = 48 to 63%) at ≥ 4 mm and ≥ 2 mm cut-off points, respectively. In multivariable logistic regression analysis, herds found in Amibara district had the higher

Table 1. Association of different risk factors to skin test positivity at 4 mm cut-off point for bovine tuberculosis in Afar Pastoral Region of Ethiopia.

Variable	Number of cattle examined	Number of positive (%)	χ^2	p-value
Districts				
Chifra	106	2 (1.9)	21.768	0.000
Dubti	151	10 (6.6)		
Afambo	137	9 (6.6)		
Amibara	693	98 (14.1)		
Herd size				
<11	330	50 (15.2)	8.720	0.013
11≤X<31	533	50 (9.4)		
≥31	224	19 (8.5)		
Sex				
Male	112	4(3.6)	6.968	0.008
Female	975	115(11.8)		
Age*				
<2	183	9(4.9)	21.123	0.000
2-5	220	13(5.9)		
5-9	419	55(13.1)		
>9	265	42(15.9)		
BCS				
Poor	298	31(10.4)	0.140	0.932
Good	596	66(11.1)		
Fat	193	22(11.4)		
Production system				
Pastoral	937	109 (11.6)	3.271	0.071
Agro-pastoral	150	12 (6.7)		
GIT Parasite				
Absence	246	16(6.5)	2.811	0.094
Present	131	15(11.5)		
Lactation status				
Lactating	274	23(8.4)	2.453	0.117
Non-lactating I	277	14(5.1)		
Reproductive status				
Pregnant	174	12(6.9)	0.280	0.597
Non-pregnant	299	17(5.7)		
Parity (Calving) number				
<2	83	3(3.6)	3.776	0.151
2≤X<5	87	7(8.1)		
X≥5	47	6(12.8)		

*A given age range includes its lower bound and excludes its upper bound. BCS: Body condition score; GIT: gastrointestinal tract.

Table 2. Multivariable logistic regression analysis of tuberculin reactors with various host-related risk factors at 4 mm cut-off point.

Variable	Number of cattle examined	Number of positive in CIDT	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Districts				
Chifra	106	2	1	1
Dubti	151	10	3.68 (0.79-17.18)	2.79 (0.58-13.45)
Afambo	137	9	3.65 (0.77-17.29)	2.49 (0.16-37.49)
Amibara	693	98	8.56 (2.07-35.27)	6.84 (1.63-28.73)
Herd size				
<11	330	50	1	1
11≤X<31	533	50	0.57 (0.38-0.88)	0.54 (0.35-0.85)
≥31	224	19	0.51 (0.29-0.90)	0.42 (0.23-0.77)
Sex				
Male	112	4	1	1
Female	975	115	3.61 (1.30-9.98)	1.66 (0.55-4.98)
Age*				
<2	183	9	1	1
2-5	220	13	1.21 (0.50-2.90)	1.05 (0.42-2.56)
5-9	419	55	2.92 (1.41-6.04)	2.11 (0.98-4.56)
>9	265	42	3.64 (1.72-7.68)	2.66 (1.21-5.84)
BCS				
Poor	298	31	1	1
Good	596	66	1.07 (0.68-1.68)	1.06 (0.66-1.70)
Fat	193	22	1.10 (0.62-1.97)	1.17 (0.62-2.18)
Production system				
Pastoral	937	109	1	1
Agro-pastoral	150	12	0.54 (0.27-1.06)	1.45 (0.15-13.41)

CI: Confidence interval; BCS: body condition scoring. *A given age range includes its lower bound and excludes its upper bound.

odds of showing tuberculin positivity in relation to those cattle in Chifra district (adjusted OR = 8.15; 95% CI = 1.77 to 37.59), and no significant association was found between herd positivity, herd size and production system (Table 3).

Association of tuberculin reaction to bovine and avian PPD

Comparative result of skin reaction to PPD-A and PPD-B is summarized in Table 4. Based on the ≥ 4 mm cut-off point, a statistically significant association was observed

between the skin reaction to PPD-A (avian) and PPD-B (bovine) ($\chi^2 = 75.98$; p-value = 0.000). As indicated in Table 4, 0.5% of the tested cattle responded positively to both PPD-A and PPD-B. On the other hand, 10.5% of them reacted only to PPD-B, while 1.8% reacted only to PPD-A.

DISCUSSION

BTB is known to be endemic in Ethiopia (Hailemariam, 1975), and in spite of a good deal of studies carried out in Ethiopia in the last decade, very few addressed the

Table 3. Multivariable logistic regression analysis of herd positivity with selected herd risk factors at 4 mm cut-off point.

Variable	Number of herds examined	Number of positive herds (%)	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
District				
Chifra	17	2 (11.8)	1	1
Dubti	32	8 (25)	2.5 (0.47-13.39)	2.39 (0.44-12.93)
Afambo	13	6 (46.2)	6.42 (1.03-40.26)	5.71 (0.89-36.31)
Amibara	109	59 (54.1)	8.85 (1.93-40.58)	8.15 (1.77-37.59)
Herd size				
<11	99	39 (39.4)	1	1
11≤X<31	57	26 (45.6)	1.29 (0.67-2.49)	1.23 (0.61-2.48)
≥31	15	10 (66.7)	3.08 (0.98-9.69)	2.44 (0.75-7.94)
Production system				
Pastoral	158	87 (55.1)	1	1
Agro-pastoral	13	8 (61.5)	1.11 (0.36-3.44)	-

CI: Confidence interval; BCS: body condition scoring.

Table 4. Response of PPD-A and PPD-B* at 4 mm cut-off point.

PPD A result	Number (%) of animals with PPD-B result		Total number (%)
	Positive	Negative	
Positive	5 (0.46)	15 (1.4)	20 (1.84)
Negative	114 (10.48)	953 (87.67)	1067 (98.16)
Total	119 (10.94)	968 (89.05)	1087 (100)

*Positive and negative reactions were according to OIE guideline with skin indurations ≥4 mm and <4 mm, respectively, McNemar's chi-square=75.98; p-value=0.000.

epidemiology of BTB in pastoral cattle of the country, which owns 42% of the country's cattle population and occupied 61% of the landmass of the country (PFE et al., 2010). In the present study, a moderately high animal prevalence was recorded at ≥ 4 mm cut-off point. The highest was reported in Amibara (14.1%) and lowest in Chifra district (1.9%), indicating a variation in prevalence within the region. The overall prevalence obtained, in general, was higher than the previous reports from other pastoral area of Ethiopia and Uganda. Thus, in Ethiopia, from Hamer and Borna, 0.8 and 5.5% prevalence were reported by Tschopp et al. (2010) and Gumi et al. (2011), respectively, while in Uganda a prevalence of 1.3% was reported by Inangolet et al. (2008). It was also higher than the 4.1% prevalence in cattle under traditional extensive grazing system of Boji [western Ethiopia] which were reported by Laval and Ameni (2004). The difference might be related to the epidemiological factors

that favors the transmission of BTB in the Afar Region, which include large herd sizes, communal grazing and watering of diverse species of animals including camel, cattle, goat and sheep, and an extensive seasonal mobility within and outside the districts, which creates favorable condition for wide range of interspecies contact (cattle, camel, goat, sheep and some wild animals such as Oryx, antelope and warthog).

In addition, the herds owned by individual pastoralist congregate together, forming a larger herd of the village and this larger herd composed of 500 to 600 of cattle and moves together to grazing and watering site. Such type of herd structure was observed particularly in Amibara district where conflict between Afar and Issa Somali tribes has been common and this intermixing of the herds might have increased the chance of contracting the infection, as it is demonstrated by higher prevalence in the district (14.1%) as compared to the others.

Moreover, during months of November to February, large number of livestock congregate in the cotton irrigation farms (2 to 4 weeks) to graze on the leftover of the cotton farm and different species of livestock (camel, cattle, and small ruminants) coming from neighboring zones and districts interact at the specific point, which create a favorable condition for close contact between animals and potential risk for transmission of diseases such as BTB among the animals. Such epidemiologically conducive conditions could lead to higher prevalence of BTB in the Afar Region as compared to the prevalence in other pastoral regions in Ethiopia. In addition, because of the presence of extensive range land in the districts, particularly in Amibara district, wild animals including oryx, gazelle, warthog and zebras (in and around Awash National Park) were observed grazing in close proximity with cattle which suggests a possible exposure for potential risk of disease transmission either way. The possibility of transmission of *M. bovis* between wildlife and cattle has been reported from other part of Africa and Europe (Woodford, 1982; Phillips et al., 2003; Cleaveland et al., 2005).

On the other hand, the result of the present study was much lower than the higher prevalence of BTB reported in urban intensive dairy farms of Ethiopia, where Holstein and crossbreeds cattle predominantly form the composition of the farms under intensive management system (Ameni et al., 2003, 2007). This difference might be mainly related to the intensive husbandry system practiced and the breed susceptibility (Ameni et al., 2007; Tsegaye et al., 2010). In our study, the animals tested were zebu Afar breed of cattle managed under extensive pastoral husbandry system which might be one reason for the differences in result, as the zebu breeds are known to be relatively resistant to BTB as compared to Holstein and other cross breeds managed under intensive system (Ameni et al., 2007, Cadmus et al., 2010).

The prevalence of BTB showed an increase with age and this finding was in agreement with previous reports by others (Kazwala et al., 2001; Oloya et al., 2006; Ameni et al., 2007; Inangolet et al., 2008; Regassa et al., 2010, Cadmus et al., 2010; Biffa et al., 2011). As indicated by these authors, the possible reasons could be the fact that older animals had longer and repeated chance of exposure to mycobacterial infection during their life time.

Furthermore, it has been observed that cows were more positive reactor than bulls, which is in agreement with other studies (Inangolet et al., 2008; Cadmus et al., 2010). In the Afar pastoral system, the majority (90%) of their herds is composed of cows kept exclusively for milk production and kept for longer time than the bulls, which form less than 10% of the herd, as bulls are sold or slaughtered in their early age. This condition might be the reason for higher tuberculin positivity in cows than bulls. As milk is consumed raw in Afar communities, the high

prevalence in cows might create a potential risk for public health in the pastoralist community and need further investigation to identify its zoonotic significance and further design a control strategy in the region.

Similar to other studies in Ethiopia (Ameni et al., 2007; Tschopp et al., 2010; Gumi et al., 2011; Biffa et al., 2011), there was no association between body condition score and tuberculin skin test positivity. In addition, no statistical significant association was observed between tuberculin positivity and gastrointestinal parasite infestation which was different from previous finding by Ameni and Medihn (2000), which could be due to the difference in geographic locations and climatic condition which determines the existence and load of parasite in the area. In the present study with arid and semi arid climatic condition, the parasite infestation load was low, and immune compromising parasite such as *Fasciola hepatica* (Flynn et al., 2007, 2009) were not abundant as that of the highland area and hence might not have affected the overall tuberculin reactivity of the animals, while Ameni and Medhin (2000) did their study in highland where fasciolosis was highly prevailing.

Finally, because of the poor infrastructure facilities such as road accessibility and insecurity to the remote sites in the pastoral setting, part of the study was carried out with some level of convenient sampling method which can be taken as the limitation of this study.

Conclusion

To the best of our knowledge, this is the first BTB study done in Afar Pastoral Region of Ethiopia. The study revealed a moderately high prevalence of bovine tuberculosis in cattle and the presence of epidemiological risk factors for infection and transmission among cattle of the Region. Considering the fact that the Afar pastoral communities have very close contact with their animals and depend entirely on their livestock for subsistence through consumption of raw milk and other animal products, the findings of this study emphasizes the need for further investigation on isolation of the specific *Mycobacterium* species causing BTB in livestock, and their zoonotic significance in the Afar pastoral community, in order to design control options of the disease both in livestock and humans living in pastoral setting.

Abbreviations

BTB, Bovine tuberculosis; **CIDT**, comparative intradermal tuberculin skin test; **PPD**, purified protein derivative.

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