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Prevalence of bovine tuberculosis and assessment of Cattle owners' awareness on its public health implication in and around Mekelle, Northern Ethiopia

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A cross-sectional study was conducted from September, 2010 to July, 2011 on 480 cattle in and around Mekelle town, northern Ethiopia, to determine the prevalence of bovine tuberculosis (BTB) using comparative intradermal tuberculin (CIDT) test and to assess cattle owners' awareness on its public health implication using a questionnaire survey. The individual animal and herd bovine tuberculin positivity prevalence were 54/480 (11.3%) (95% CI: 8.4 to 14.1%) and 24/120 (20%) (95% CI: 12.7 to 27.3%) at cut-off > 4 mm, respectively. Cattle kept in intensive type of production (odds ratio (OR) = 3.7), in larger herds with more than 10 cattle (OR = 11.3) and under poor management condition (OR = 4.3), were more likely to be infected with bovine tuberculosis. On the basis of animal characteristics, female (OR = 4.8), exotic (OR = 6.1) and cross bred (OR = 6.6), and cattle with poor body condition (OR = 2.7) were more reactive to tuberculin test than male, Zebu breed and good body conditioned animals, respectively. Out of the 54 CIDT positive cattle, 4 were slaughtered and tuberculous lesions were detected from the organs and carcasses of those cattle. One hundred and twenty household cattle owners or members of these households were interviewed, of these only 37 (30.8%) and 18 (15%) respondents had recognized or had heard about BTB and aware of zoonotic importance of BTB, respectively. The result of this study revealed poor awareness of cattle owners on BTB and its transmission; likewise, the study suggests that the prevalence of BTB in the study area is moderate and strong tuberculoid like lesions found from CIDT tested slaughtered animals. Further molecular characterization of cattle TB isolates present in the area are warranted.

Key words: Bovine tuberculosis, cattle, comparative intradermal tuberculin (CIDT), Ethiopia.

INTRODUCTION

Cattle are considered to be the main hosts of *Mycobacterium bovis* (*M. bovis*) although isolations have been made from many other livestock and wildlife species and transmission to humans poses a public health problem (Ayele et al., 2004; Mamo et al., 2009, 2011; Office International des Epizooties (OIE), 2010; Hiko and Agga, 2011). Bovine tuberculosis (BTB) along with other diseases, become a serious problem in intensive dairy farms (Asseged et al., 2000; Radostits et al., 2007) and seriously affects the productivity of the livestock industry in developing countries (Cosivi et al., 2010; Radostitis et al., 2007).

The standard method for BTB detection in live animal is the comparative intradermal tuberculin (CIDT) test based on delayed hypersensitivity reactions. The CIDT test includes bovine and avian purified protein derivatives (PPD) and is used mainly to differentiate between animals infected with *M. bovis* and those sensitized to tuberculin due to exposure to other mycobacteria or related genera (OIE, 2010). In developing countries, the occurrence of BTB in human is widely distributed in those areas where control measures are not applied or are applied sporadically and pasteurization of milk is rarely practiced (Cosivi et al., 1998).

In Ethiopia, BTB is endemic in cattle; prevalence varies from 0.8 to 50% depending on the geographical location, breed and the husbandry practices (Berg et al., 2009; Demelash et al., 2010; Regassa et al., 2010; Tschopp et al., 2010; Firdessa et al., 2012). However, there is lack of adequate data on the epidemiology and public health implication of this disease since most of the studies conducted in Ethiopia have concentrated around the central part of the country.

As the dairy industry in Ethiopia has expanded in recent years and is expected to continue doing so, significant number of high productive exotic and cross bred animals are likely to be traded from the urban areas around the capital to the rural areas where dairy cattle numbers are still relatively low (Firdessa et al., 2012). Consequently, the communities of the study area were observed with a great tendency to own crossbred (Zebu × Holstein) and exotic (Holstein) breeds with intensive production system to implement the program. However, milk pasteurization has not been practiced in the area; as a result people residing in the study area have been consuming raw milk.

Despite the existence of potential risk factors in the study area, the occurrence of BTB has not yet been investigated.

The present study therefore was designed to determine the prevalence of BTB using CIDT test and to assess cattle owners' awareness on its public health implication in and around Mekelle town, northern Ethiopia.

MATERIALS AND METHODS

Study area and population

The study was conducted in and around Mekelle town, northern Ethiopia. Mekelle is the capital of Tigray regional state and located at 783 km north of Addis Ababa, at a latitude and longitude of 13° 29′N and 39° 28′E, respectively with an elevation of 2,084 m above sea level. Climatic condition of the area is characterized by semi-arid weather with bimodal rainfall patterns, with an average annual rainfall of 479 to 650 mm. The annual average temperature is 20.9°C, with an annual mean humidity 75.4% (Bureau of Planning and Economic Development of Tigray Region, 1998). Cattle belonging to Mekelle town and its surroundings were the study animals. Extensive and intensive types of cattle production systems practiced in the study area and the cattle breeds reared in the area are zebu, exotic (Holstein) and cross-bred (Zebu × Holstein).

Design, sample size and sampling method

A cross-sectional study was conducted from September, 2010 to July, 2011 on 480 cattle in and around Mekelle town, northern Ethiopia, to determine the prevalence of BTB using CIDT test and to assess cattle owners' awareness on its public health implication using a questionnaire survey. The sample size for tuberculin testing was calculated using the cluster sampling formula described by Bennett et al. (1991). We assumed an intra-class correlation coefficient (rho) of 0.2, an expected prevalence of 46.8% (Ameni et al., 2003) and standard error of 2.9%. The total sample size calculated for 120 households/farms was n = 480 cattle.

A list of 138 households/farms owning dairy cattle was obtained from Mekelle town Urban Agricultural unit and these households/farms were used as sampling frames. The sampling method involved was a two-stage cluster sampling where households/farms and individual animals were considered as primary and secondary units, respectively and both the primary and secondary units were selected using the simple random sampling. Four animals above 6 months of age were randomly selected per household/farm and the selected risk factors (Table 1) considered for data collection at animal and herd levels were recorded before PPD injection. Temporary unique identification numbers were given for each tested animal. Body condition scoring was done according to Nicholson and Butterworth (1986). The management of the farm was categorized as described by Ameni et al. (2003) on the basis of housing condition (neatness, waste disposal, nature of the floor, presence of confinement), feeding (concentrate plus hay), possession of an exercise yard, contact with other herds and provision with clean water.

Study methodology

Tuberculin skin testing

The CIDT test was performed using both bovine and avian mycobacterium purified protein derivative (PPD) obtained from the Veterinary Laboratories Agency, Addlestone, Surrey, UK. Two injection sites were chosen in the middle third of the side of the neck, one above the other, separated at least 12 cm. The hair was

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Table 1. Risk factors considered for data collection during tuberculin testing/PPD injection in cattle of the study area.

No.	Risk factor	Category
1	Sex	Male, female
2	Age	<2, 2≤x ≤ 5, 5<x≤ 9, > 9
3	Breed	Local, cross bred, exotic
4	Body condition score	Good, medium, poor
5	Lactation status	Lactating, Non lactating
6	Parity class	Heifer, Parity 1 and 2, Parity 3-5, Parity ≥ 6
7	Pregnancy status	Pregnant, non pregnant
8	Farming system	Intensive, extensive
9	Management condition	Good, medium, poor
10	Owners' health(TB) status	Sick with TB, Not sick with TB
11	Herd size	≤5, 6 -10, >10
12	Presence of other livestock	Present, absent
13	Presence of wildlife	Present, absent

shaved around the sites to a radius of about 2 cm. Skin folds at both sites were measured with a caliper and the measurements were recorded. An aliquot of tuberculin containing 2,500 IU/0.1 ml bovine PPD was injected into the skin intradermally at the lower injection site and similarly, tuberculin containing 2,500 IU/0.1 ml avian PPD was injected at the upper site. After 72 h, the thickness of the same skin fold at both sites was measured and recorded. Bovine and avian positive reactors were obtained using the formula: $[(Bov_{72}-Bov_0) - (Av_{72}-Av_0)]$ and $[Av_{72}-Av_0) - (Bov_{72}-Bov_0)]$, respectively. Bov_0 and Av_0 indicated skin thickness before injecting bovine and avian tuberculin, and Av_{72} and Bov_{72} were the corresponding skin fold thickness 72 h post-injection. The tuberculin test results were interpreted based on OIE (2010) recommended cutoff, > 4mm. Increase in skin fold thickness of > 4 mm was regarded as positive reactor and negative if the increase in skin thickness at the bovine site of injection was less than the increase in the skin fold thickness at the avian site of injection. Increase in skin fold thickness of > 1 mm with visible reaction at avian site than at the bovine site was considered as positive for *Mycobacterium avium* spp.

Questionnaire survey

All herd owners of tuberculin tested animals were interviewed using pre-tested structured questionnaires to assess the knowledge and awareness of the communities of the study area regarding BTB and its transmission associated with feeding habits and other factors such as education level.

Data entry and analysis

The data collected from the study areas were entered into MS excel spread sheets and analyzed using STATA (11.0) statistical software. Individual animal prevalence was defined as the number of positive reactors per 100 animals tested. The farm level prevalence was calculated as the number of herds with at least one-reactor animal per 100 herds tested. The effects of different potential risk factors were computed using univariate analysis. A statistically significant association between the result and the risk factors was said to exist if the calculated P < 0.05 and the 95% confidence interval (CI) for odds ratio (OR) does not include 1.

RESULTS

Herd level prevalence and risk factors

From a total of 120 households/farms tested, 24 were positive; each one exhibited at least one tuberculin reactor animal with a herd prevalence rate of 20% (95% CI: 12.7 to 27.3%). The effect of the risk factors (herd size, farming system, management conditions, owners health status, presence of other livestock and presence of wildlife nearby the farm) on the herd prevalence are presented in Table 2.

Animal level prevalence and risk factors

The prevalence of bovine tuberculin positivity at animal level was 54/480 (11.3%) (95% CI: 8.4 to 14.1). On univariate analysis, female (OR = 4.8; 95% CI: 1.5 to 15.8), exotic (OR = 6.1; 95% CI: 2.2 to 17.4) and cross-bred (OR = 6.6; 95% CI: 1.8 to 24.8) breeds and poor body condition (OR = 2.7; 95% CI: 1.2 to 5.9) cattle were five, six and three times more at risk for BTB infection than male, Zebu breed and good body conditioned animals, respectively (Table 3). Seven of the 480 cattle were positive for avian tuberculin, with a prevalence rate of 1.5% (95% CI: 0.4 to 2.5).

Necropsy findings of tuberculin reactor cattle

Out of the 54 CIDT reactor cattle, four animals (three dairy

Table 2. Univariate analyses of risk factors for CIDT test result at farm/herd level.

Risk factor	No. of herd		OR (95% CI)	P-value
	Tested	Positive (%)		
Type of farming				
Intensive	84	21 (25.0)	3.67 (1.0-13.2)	0.036
Extensive	36	3 (8.3)	1	
Herd size				
≤5	49	4 (8.2)	1	0.000
6-10	45	7 (15.6)	2.1 (0.6-7.6)	
>10	26	13 (50.0)	11.3 (3.1- 40.4)	
Owners' health status				
Sick with TB	13	5 (38.4)	2.8 (0.9-9.8)	0.078
Not sick with TB	107	19 (17.7)	1	
Management				
Good	6	1 (16.7)	1	0.030
Medium	75	5 (6.7)	0.4 (0.03-3.7)	
Poor	39	18 (46.2)	4.3 (1.5-40.2)	
Presence of other livestock				
Present	82	20 (24.4)	2.7 (0.9-8.7)	0.077
Absent	38	4 (10.5)	1	
Presence of wildlife				
Present	57	13 (22.8)	1.4 (0.57-3.4)	0.465
Absent	63	11 (17.4)	1	

cows and one bull) with strong bovine tuberculin positivity were slaughtered for postmortem examination.

After detailed postmortem examination, gross pathological tuberculous lesions were detected from organs and carcasses of all (100%) the slaughtered cattle (Figure 1).

About 57% of the lesions were found in the thoracic cavity, followed by retropharyngeal (14.3%) and mesentric (14.3%) lymph nodes (Table 4).

Cattle owner awareness on public health importance of BTB

One hundred and twenty household cattle owners or members of these households were interviewed. Of these, 37 (30.8%) of the respondents reported that they had the knowledge of or had heard about BTB and only 18 (15%) respondents became aware of zoonotic importance of BTB (Table 5). Out of the total interviewed households (120), 13 (10.8%) had TB cases in their families or farm workers and in five of the thirteen households, both PPD-positive cattle and human tuberculosis cases were found but were statistically non significant (P > 0.05) (Table 2). Moreover, the 120 household cattle owners were interviewed regarding their milk drinking and meat eating habits and house sharing with their animals (Table 6). Several demographic characteristics and other factors were considered to investigate their possible association with BTB recognition of the respondents. The awareness of the respondents regarding cattle infection with BTB and the transmission of BTB from cattle to man improved as the educational background of the respondents increased (data not shown).

DISCUSSION

In the present study, the prevalence of bovine tuberculin positivity at animal and herd levels were 11.3 and 20%, respectively. The finding was in agreement with the findings of Ameni and Erkihun (2007) who reported 11

Table 3. Univariate analysis of host risk factors for CIDT test result at animal level in cattle rose in and around Mekelle town.

Risk factor	No. (%) of animals		OR (95% CI)	P-value
	Examined	Positive		
Sex				
Male	97	3 (3.1)	1	0.004
Female	383	51 (13.3)	4.8 (1.5-15.8)	
Age (years)				
<2	52	6 (11.5)	1	0.292
2≤x≤ 5	207	26 (12.5)	1.1 (0.4-2.8)	
5<x≤ 9	120	16 (13.3)	1.2 (0.4-3.2)	
>9	101	6 (5.9)	0.5 (0.2-1.6)	
Breed				
Local	145	4 (2.7)	1	0.001
Cross bred	38	6 (15.8)	6.6 (1.8-24.8)	
Exotic	297	44 (14.8)	6.1 (2.2-17.4)	
Body condition				
Good	134	9 (6.7)	1	0.036
Medium	184	19 (10.3)	1.6 (0.7-3.7)	
Poor	162	26 (16.0)	2.7 (1.2-5.9)	
Lactation				
Lactating	212	31 (14.6)	1	0.321
Non lactating	170	19 (11.2)	0.7 (0.4-1.4)	
Pregnancy				
Pregnant	205	28 (13.6)	1	0.722
Non pregnant	177	22 (12.4)	0.9 (0.5-1.6)	
Parity class				
Heifer	118	16 (13.9)	1	0.094
Parity 1 and 2	116	9 (7.8)	0.5 (0.2-1.2)	
Parity 3-5	119	22 (18.5)	1.4 (0.7-2.8)	
Parity ≥6	32	3 (9.4)	0.6 (0.2-2.4)	

and 15% prevalence of BTB at animal and herd level, respectively in Adama town. Similarly, Regassa et al. (2010) had reported 11.6% BTB prevalence at animal level in Hawassa town and its surroundings, Southern Ethiopia. This agreement could be due to the similarity in study subjects, herd size and production systems. In the present as well as in the aforementioned studies, majority of the tested herds were smallholders found in and around urban areas with smaller herd size and with similar herd compositions. However, the herd and animal prevalence recorded in this study were lower than that of the previous study carried out by Tsegaye et al. (2010) who reported herd and individual animal bovine tuberculin positivity prevalence rates 53.6 and 34.1%, respectively. Moreover, Elias et al. (2008) has reported higher prevalence of bovine tuberculin positivity than the present study with animal and herd prevalence of 23.7 and 43.4%, respectively. This difference could be because the studies conducted by Elias et al. (2008) and Tsegaye et al. (2010) had been on large herd sizes, Holstein breeds and intensive production system.

Several studies have indicated that as herd size increases, the risk of cattle within the herd showing a positive reaction also increases (O'Reilly and Daborn, 1995;

Table 4. Distribution and frequency of tuberculous lesions in four slaughtered CIDT test positive cattle.

Infected organ	Frequency	Percent (%)
Retropharyngeal LN	2	14.3
Bronchial LN	3	21.4
Mediastinal LN	3	21.4
Lungs	1	7.1
Visceral surface of rib	1	7.1
Mesenteric LN	2	14.3
Prescapular LN	1	7.1
Supramammary LN	1	7.1
Total	14	100

LN=lymph nodes.

Table 5. Knowledge of cattle owners about BTB and its transmission to humans.

Statement	Number of interviewed	Owners who know (%)
Know BTB can affect animals	120	37 (30.8)
Know BTB is Zoonotic	120	18 (15)
Know milk is vehicle for *M. bovis*	120	18 (15.0)
Know meat is vehicle for *M. bovis*	120	14 (11.7)
Know BTB can transmit by inhalation of cough spray	120	18 (15)
Know close-contact can facilitate BTB transmission	120	4 (3.3)

Table 6. Summary on milk consumption and meat eating habits, and housing status of the respondents.

Habit of respondents	Number of respondents	Present (%)
Milk drinking		
Raw milk	11	9.2
Boiled milk	43	35.8
Both raw and boiled milk	66	55
Yoghurt milk consumption		
Consume	113	94.2
Do not consume	7	5.8
Meat eating habit		
Cooked meat	96	80.0
Both cooked and row meat	24	20.0
House sharing		
Sharing	65	54.2
Not sharing	55	45.8

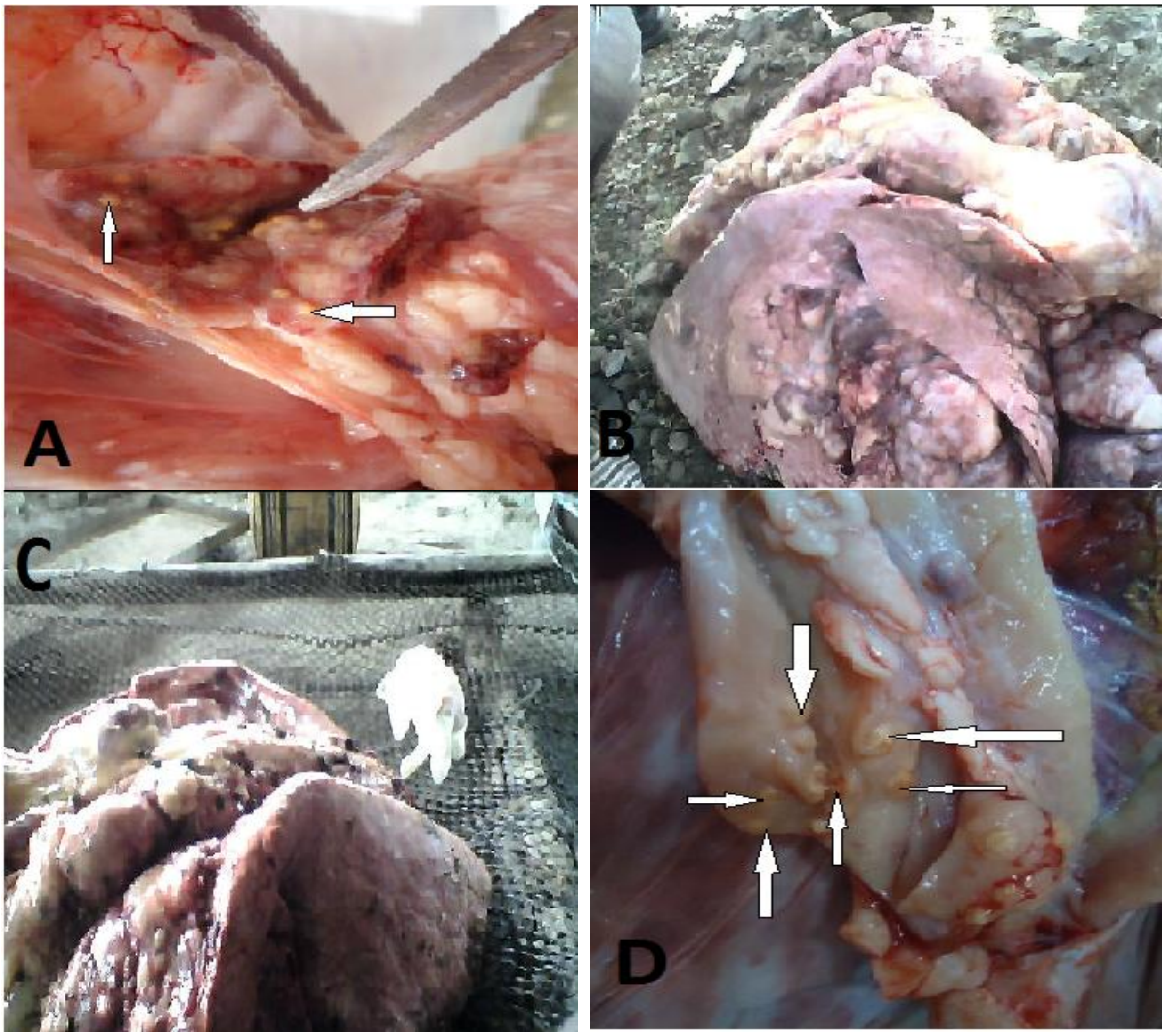

Figure 1. Tuberculous lesions in bronchial lymph nodes and Diaphragmatic view of lung in the sacrificed CIDT test reactor cattle; (A) Tuberculous granulomas in bronchial lymph node (indicated by arrow), (B) Lateral view of lung: extensively damaged with coalesced tubercle lesions and swollen associated lymph nodes, (C) Diaphragmatic view of lung; extensively damaged with coalesced tubercle lesions and swollen associated lymph nodes and (D) Mediastinal lymph node with tuberculous granuloma.

Barwinek and Taylor, 1996; Cook et al., 1996; Asseged et al., 2000). In the present study, the herd tuberculin test results showed a statistically significant association (OR = 11.3) with herd size; also, the proportion of reactors increased parallel to an increasing herd size. This finding is consistent with previous reports (Ameni and Erkihun, 2007; Elias et al., 2008; Tsegaye et al., 2010) and might be due to the risk that an individual animal may introduce tuberculosis infection into a herd and the lateral spread of infection within the herd may also be favored in large herd sizes. Moreover, this study revealed that intensive production system (OR = 3.7) had strong association with prevalence of bovine tuberculin positivity which was in line with the previous reports of Ayele et al. (2004) and Elias et al. (2008). This could be due to the fact that intensive farming system promotes close contact between animals, thereby favoring the spread of the infection.

Similarly, herd tuberculin results showed a statistically significant association with herd management conditions, signifying that poor managerial inputs increase the risk of tuberculosis (Griffin et al., 1993). Previous studies (Ameni et al., 2003; Elias et al., 2008) had similarly documented higher infection rates in farms under poor management conditions. It can therefore be generalized that the status of BTB could be improved by adopting sanitary measures that improve hygiene conditions on farms.

In this study, the prevalence of bovine tuberculin positivity had significant association with sex, as female animals (OR = 4.8) had a higher odds for tuberculin reactivity when compared to male animals, which was in agreement with the findings of Inangolet et al. (2008). This could be because cows are confined in a barn and kept long for production purpose which may facilitate infection and acquisition of the disease. Moreover, dairy cows experience greater production stress and gathering of cattle during milking increases the risk of transmission as shown by bovine TB transmission modeling in New Zealand (Barlow, 1997).

The finding of lower prevalence in Zebu breed is in line with previous reports (Kiros, 1998; Kazwala et al., 2001; Regassa et al., 2008). The reason could be that genetically improved cattle may suffer more severely from deficient housing and malnutrition and thus be more prone to infection than Zebu breeds. In contrast to earlier reports of Ameni and Erkuhin (2007) and Regassa et al. (2008), poor body conditioned (OR = 2.7) animals were more at risk of getting infected with BTB than good body condition animals. This matches to the established fact that an animal's resistance to tuberculosis is reduced by a shortage of feed and/or unbalanced diet, attributable to a deficiency of proteins, minerals and vitamins in the diet (Griffin et al., 1993). Previous studies (Collins, 1994; Elias et al., 2008) had similarly reported higher bovine tuberculin reactivity prevalence in animals with poor body condition compared to those with good body condition scores.

In agreement with the reports of Ameni et al. (2001, 2003), this study confirmed the presence of human tuberculosis case in households owned PPD reactor cattle. According to Seifert (1996), tubercle bacilli can survive in acidic milk for 15 days and in milk products such as cheese and butter for weeks. In this survey, more than half (55%) of the respondents consume both raw (butter milk) and boiled milk and 94.2% of the interviewed persons had the habit of yoghurt consumption.

The zoonotic risk of BTB is often associated with consumption (ingestion) of dairy products based on unpasteurized milk infected with *M. bovis*. Also, aerosol transmission from cattle-to-human should also be considered as a potential risk factor. Ethiopian milk consumers generally prefer raw milk (as compared to treated milk) because of its taste, availability and lower price (SNV, 2008). The disease transmission may be cyclical: cow-to-man-to-cow (Cosivi et al., 1998), underlying the existence of higher risk of dissemination of mycobacteria among the cattle and human populations.

Further, there is a need to use novel diagnostic techniques to monitor the spread of infection in large areas for developing nations (Liu et al., 2011; Cui et al., 2013; Li et al., 2014). Wadhwa et al. 2012 have described a bead based microfluidic assay for mycobacterial infections. Similarly, an ethanol votex ELISA for bovine tuberculosis in wild animals showing very high diagnostic sensitivity and specificity has been developed (Wadhwa et al., 2012). Such techniques should be tested to diagnose bovine tuberculosis in livestock. Molecular epidemiological, phylogenetic analysis and mathematical modeling should be carried out at the time of new outbreaks to understand the origin, efficacy of current vaccines and design control strategies (Kumar et al., 2014).

CONCLUSION AND RECOMMENDATION

The result of this study revealed that the communities of the study area lack awareness regarding BTB and its routes of transmission and this may allow circulation of the agent between the communities and animals. A test-and-segregation policy of tuberculin positive animals can be suggested and pursued since no compensation scheme for elimination of infected animals is currently in practice in Ethiopia.

Accordingly, government authorities should encourage farmers to regularly test their animals to keep their herds free from BTB. Likewise, milk should be strictly pasteurized before selling and consumption. In general, the communities of the study area should be aware regarding BTB and its routes of transmission. Moreover, the study suggests that the prevalence of BTB in the study area is moderate and the strong tuberculoid lesions found from CIDT tested slaughtered animals call for further molecular characterization of cattle TB isolates present in the area.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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