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Bovine Tuberculosis in Rural Ethiopia: A Comparative Cross-Sectional Study on Cattle Owned by Households with and without Tuberculosis

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Abstract

Background: *Bovine tuberculosis* (TB) is endemic in Ethiopia. Intimacy of cattle and humans in rural farming communities may transmit *Mycobacterium bovis* to humans. However, there is little information about the possible transmission of pulmonary tuberculosis between humans and cattle. The current study aimed at investigating the likelihood of bovine TB among livestock in households with a confirmed pulmonary TB case.

Method: The households studied were those where TB had been diagnosed in any household member (35 pulmonary TB case households) in comparison to households without a person diagnosed with pulmonary TB (105 comparison households). Cattle owned by both households were tested with the comparative intradermal skin test (CIDT) to collect primary data. Logistic regression was used to assess the factors associated with CIDT reactivity in both households.

Results: The study revealed 23.6% (n=140) of an overall household/herd and 8.7% (n=481) an individual cattle apparent prevalence. The odds of bovine TB among cattle owned by households with pulmonary TB was about three times (2.90, 95% CI: 1.50-5.54) higher compared to those cattle owned by households without pulmonary TB.

Conclusion: The study implicated possible transmission of TB between humans and cattle. It is necessary to investigate whether the pathogen responsible is *M. tuberculosis* or *M. bovis*.

Keywords: *Bovine tuberculosis*; Cattle; Household; Tuberculosis

Background

Tuberculosis (TB), one of the oldest and among the most devastating of human diseases, is a bacterial infection mainly caused by *Mycobacterium tuberculosis*. In 2012, 8.6 million people were infected and 1.3 million died from TB1 Nine million people become ill with TB each year and around 5000 people die in a single day [1-4].

Ethiopia ranks 3rd in Africa and 8th amongst the 22 highest TB burden countries in the world. The prevalence of all forms of TB is estimated at 224 per 100 000 population and an annual mortality rate of 18 per 100 000 population. The incidence rate of all forms of TB is estimated at 247 per 100 000 population, while the smear positivity rate is about 32.0%. TB case detection rate, the treatment success rate and TB cure rate are 64%, 90% and 89%, respectively [5].

Although *M. tuberculosis* is the main cause of human pulmonary TB (PTB), the main causative agent of bovine TB, *M. bovis*, is well described to infect humans, primarily through close contact with infected cattle or consumption of contaminated animal products such as unpasteurized milk. Globally, most cases of zoonotic TB are caused by *M. bovis*, and cattle are the major sources [5,6]. Infection to humans can also occur through wound contamination during slaughtering or inhalation of bacteria in the air exhaled by infected animals. Direct transmission from animals to humans through the air is thought to be rare, but *M. bovis* can be spread directly from person to person when people with the disease cough or sneeze [7]. Likewise, transmission of *Mycobacterium tuberculosis* from human TB patients to cattle could also be possible [8].

Bovine TB is endemic in Ethiopia with a prevalence of 10% to 54% [9,10]. In humans, *M. bovis* causes TB disease that can affect the lungs, lymph nodes, and other parts of the body. However, the relationship of human pulmonary tuberculosis mainly caused by *Mycobacterium tuberculosis* to cattle skin test positivity owned by households with pulmonary TB is not well understood. Still pulmonary TB is a problem in the country. *Mycobacterium tuberculosis* has been isolated from cattle [11]; they may serve as a source of *Mycobacterium tuberculosis* infections to humans, indicating the possibility of reverse zoonosis and therefore, this study was carried out to assess prevalence of bovine TB status in cattle owned by individuals with confirmed human pulmonary TB cases compared to those owned by pulmonary TB free households in the rural north western and north-eastern Ethiopia.

Methodology

Study setting and design

This study used a comparative cross-sectional design and was conducted in North Gondar and North Wollo Zones of Amhara National Regional State, North Western and North Eastern Ethiopia, where the livelihood is mainly mixed farming. Dembia, Chilga, Dabat, Debark, Adarkay and Wegera, and Meket, Gubalafto, Habru and Raya Kobo were the districts used to conduct tuberculin skin test on cattle in North Gondar and North Wollo Zones, respectively. The study area covered an estimated area of 58,117.13 square kilometers

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with a total human population of 4,429,931. Rural dwellers accounted for about 90% and 85% in North Wollo and North Gondar Zones, respectively [12]. The data collection was carried out between September and November 2013. Health institutions of the two Zones were used as a source to identify individuals diagnosed with pulmonary TB (Figure 1).

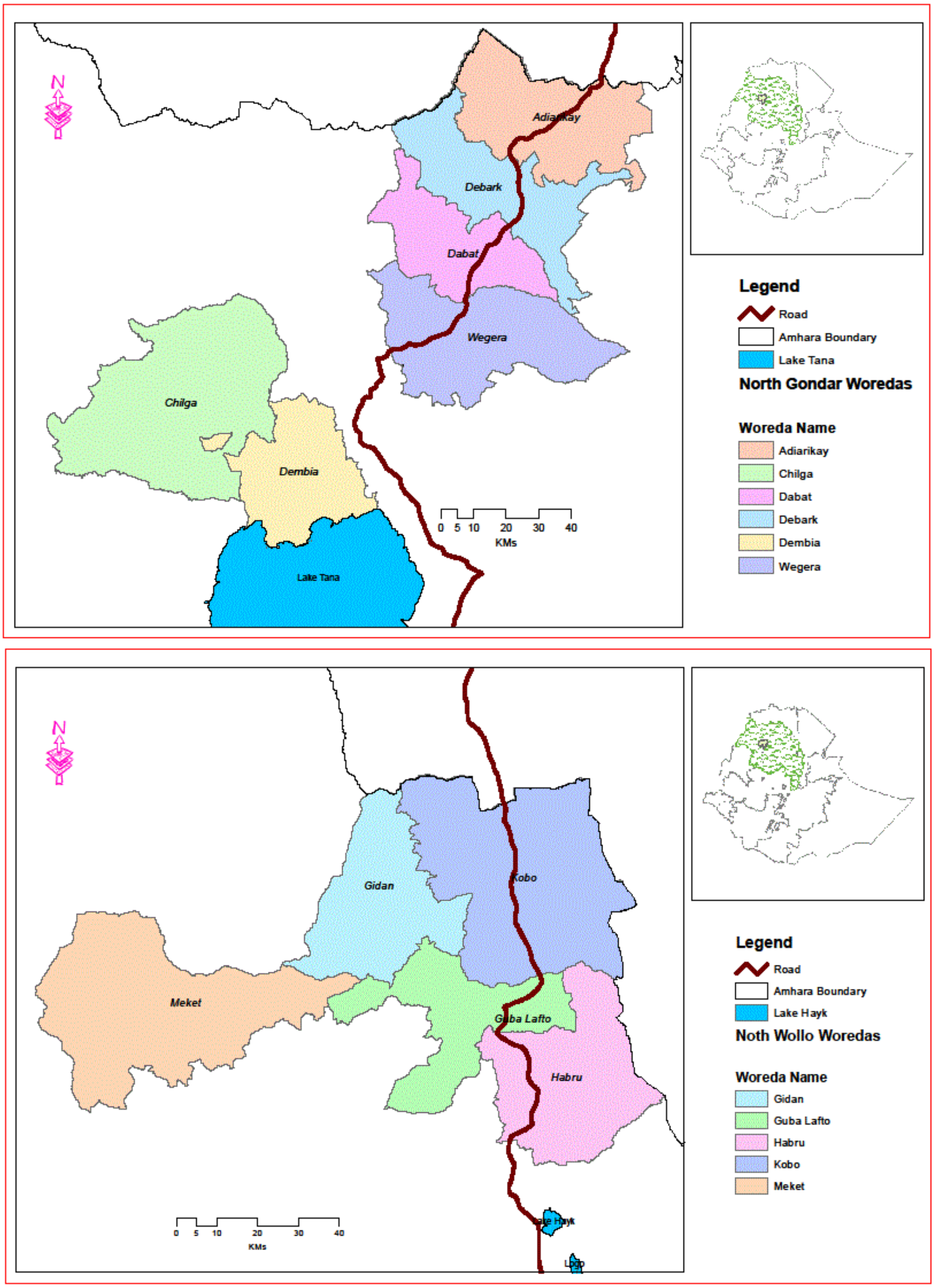

Figure 1: Map of the study sites.

Study subjects/populations

TB patients who owned cattle were identified in their respective health institutes (purposefully selected public health centers as well as hospitals) and traced back to their home guided by health workers or veterinarians in the respective sites. A human TB case was defined as a smear positive adult pulmonary TB patient (aged 18 and above years) diagnosed at the respective health institutes in the study Zones while pulmonary TB negatives were apparently healthy individuals (aged 18 and above years, as a household head) who did not have any clinical signs suggestive of pulmonary TB or had no history of TB in the family in the past as well as no chronic cough, which lasted for more than two weeks prior to data collection. All cattle older than six months of age in herds owned by Households with and without TB cases were tested using a comparative cervical intradermal tuberculin test. Cattle included in the study were local zebu breeds and some crosses kept under extensive management system. For each tested cattle, sex, breed, age, source (home-bred or purchased) and body condition score (BCS) were collected and recorded at the time of the test. The body condition of each of the study cattle was scored using guidelines established by Nicholson and Butterworth (1986) [13]. Cattle which were under 6 months of age, new additions to the herd (less than 6 months duration), pregnant cows near to term, sick cattle during the time of the visit, cattle under treatment, highly aggressive cattle and oxen used for ploughing during the time of study days were excluded from tuberculin skin testing.

Sample size determination

The sample size was calculated taking into consideration a comparative cross-sectional design with the assumption of 15% bovine TB among the non-pulmonary TB households and 41.38% among the pulmonary TB households, confidence interval of 95% with a power of 80%, a ratio of cases to comparison group of 1:3; (findings of 15.4% *Mycobacterium tuberculosis* and 44.1% *Mycobacterium bovis* from collected milk samples by Fetene et al. [11] were used to calculate the sample size). Adding 10% non-response the required sample sizes were 35 households with pulmonary TB and 105 households without pulmonary TB cases. The distribution of the samples was based on the identified, confirmed pulmonary TB cases during the follow up periods in both study Zones. Those eligible cattle owned by both household types were tested.

Bovine TB diagnosis procedure: Tuberculin intradermal skin test

Comparative cervical intradermal skin test was performed to know the status of bovine tuberculosis in the study areas. For this test AVITUBAL-25000 IU/ml-inj. ads us.vet, *Mycobacterium avium* (strain D4 ER, 25000 IU/ml) and BOVITUBAL (*Mycobacterium bovis*, strain AN-5 25.000 IU/ml) PPD antigens were used at a dose rate of 0.1 ml each intradermally [14]. First the injection sites were prepared; for injection of avian tuberculin about 10 cm from the crest of the neck and bovine tuberculin about 12.5 cm lower on a line roughly parallel with the line of the shoulder or on different sides of the neck in young cattle, if there is no room to separate the two, were shaved and cleansed. Then a fold of skin within each clipped area taken between the forefinger and the thumb was measured using digital caliper and the test antigen injected using a BD1/2 tuberculin syringe (graduated) based on manufacturer recommendations [14]. A correct administration was confirmed by palpating a small pea-like swelling at each site of injection. Evaluation of results was done 72 ± 4 hrs after injection. The result was considered as positive, if the reaction number to bovine exceeds the reaction to avian tuberculin by more than 2 mm and 4 mm; or clinical symptoms such as diffuse or extensive edema, exudation, necrosis soreness or inflammatory reaction of relevant lymphatic vessels or lymphatic nodes were discovered in the application place of bovine tuberculin. Results considered inclusive [15] if the reaction measurement for bovine TB is from 1–2 mm and 1–4 mm greater than the reaction to avian tuberculin or no clinical symptoms were discovered. If the reaction number is the same or lower than for avian tuberculin with no clinical symptoms were observed in either site; the result considered as negative.

Ethical considerations

Ethical clearance was obtained from the Institutional Review Board (IRB) of the College of Health Science, Addis Ababa University and AHRI/ALERT (Armauer Hansen Research Institute) ethics committee. Permission was obtained from the Amhara Regional Health Bureau and from North Gondar and North Wollo Zones as well as respective health departments. Permission was also obtained from Zonal department of agriculture and agricultural offices in each Zone. Written consent or assent was taken from each participant TB cases and comparative groups. Confidentiality was maintained by using codes instead of participants' names. During the study period advice was given to those households identified as bovine TB positive or doubtful. Individuals still had cough and other TB related signs after treatment were consulted to go to health centers for possible check-ups. All participants were given health education on safe food practices. All tested cattle received antiparasitic drugs as per the recommended dosage. All participants were enrolled only upon giving written consent, which was previously approved by the ethics committee.

Data management and analysis

During the study period, good data management practices (including survey monitoring as well as on site supervision, timely data archiving and transfer, sorting and filing) were implemented. The collected data were coded and entered into EpiInfo version 3.2.1 [16] and exported to SPSS [17] for analysis. The manufacturer [14] >4 mms and Ameni et al. [18] >2 mms cutoff values of the skin test measurements were used to determine the skin test result. Descriptive statistics was done using frequency distributions, percentages, mean and standard deviations. Multiple logistic regressions were used to assess factors associated with positive Bovine tuberculosis. During the analysis a household/herd was considered positive if one head of cattle among others was positive for the skin test.

Results

Of the total 481 cattle tested for bovine TB, 76% and 1:1.2 were from North Gondar Zone and with male to female ratio, respectively. The mean age of cattle was 5.6 ± 3.0 and 5.2 ± 3.7 years for those cattle owned by households with pulmonary TB and without pulmonary TB, respectively. Among them, 76% (n=481) cattle were homebred (reared at home) and 87.1% were local; 61.3% had medium size, 24.1% were lean and 14.6% fat (Table 1).

Using >2 mms as a cutoff value, 4.6% (22/481) and 4.2% (20/481) had skin test positive cattle in households with PTB and without PTB, respectively. With the same cutoff value, the individual cattle prevalence of bovine TB was 15.3% (95% CI: 9.4–1.2) and 5.9% (95% CI: 3.4–8.5) among PTB and non-PTB households, respectively. In this study an overall individual apparent prevalence of 8.7% (95% CI: 6.2–11.3) was recorded.

On the other hand, using >4 mms cutoff value, the individual cattle

Category	Households (n=481)					
	With PTB		Without PTB		Total	
	No	%	No	%	No	%
Study area						
North Gondar	117	81.2	247	73.3	364	75.7
North Wollo	27	18.8	90	26.7	117	24.3
Sex						
Male	75	52.1	145	43	220	45.7
Female	69	47.9	192	57	261	54.3
Cattle age (Mean) in years	5.6 ± 3.0			5.2 ± 3.0		
Breed						
Local	126	87.5	293	87	419	87.1
Cross	18	12.5	44	13	62	12.9
Source						
Homebred	103	71.5	262	77.7	365	75.9
Purchase	41	28.5	75	22.3	116	24.1
Body condition						
Lean	28	19.5	88	26.1	116	24.1
Medium	85	59	210	62.3	295	61.3
Fat	31	21.5	39	11.6	70	14.6
Bovine tuberculosis						
>4 mm						
Inclusive	20	13.9	45	13.4	65	13.5
Negative	116	80.5	290	86	406	84.4
Positive	8	5.6	2	0.6	10	2.1
>2 mm						
Inclusive	6	4.2	27	8	33	6.9
Negative	116	80.6	290	86	406	84.4
Positive	22	15.2	20	6	42	8.7

Table 1: Characteristics of tested cattle in North Gondar and North Wollo Zones, Ethiopia, 2014.

Measurements	Test Result	Household with TB (n=35)		Household without TB (n=105)	
		No	%	No	%
BTB>4mms	Negative	28	80	103	98.1
	Positive	7	20	2	1.9
BTB>2 mm	Negative	18	51.4	89	84.8
	Positive	17	48.6	16	15.2

Table 2: Skin test result at household/herd level in North Gondar and North Wollo Zones, Ethiopia, 2014.

prevalence of bovine TB was 5.6% (95% CI: 1.8–9.3) and 0.6 % (95% CI: 0.2–1.4) among PTB and non-PTB households, respectively, making the overall individual cattle prevalence of 2.1%, 95% CI: 0.8–3.4). About 30% (42/140) of the households/herds and 13.5% (65/481) of the individual cattle had inclusive bovine TB results based on >4 mms cutoff value, of which 31.4% (11/35) were from PTB households and 29.5% (31/105) without PTB households.

As it is shown in Table 2, households with PTB showed 20% (7/35) (95% CI: 6.7–33.7) and 48.6% (17/35) (95% CI: 32.0–65.1) while those without PTB revealed 2% (2/105) (95% CI: 0.7–4.5) and 15.2 (16/105) (95% CI: 8.4–22.1) bovine tuberculosis using >4 mms and >2 mms cutoff values, respectively. An overall household/herd apparent prevalence of 6.4% (9/140) (95% CI: 2.4–10.5) and 23.6% (33/140) (95% CI: 16.5–30.6) were registered using >4 mms and >2 mms cutoff values, respectively.

As presented in Table 3, based on >2 mms as a cutoff value adjusted for age, body condition, source, sex and breed of the individual animal

Category	BTB (n=481)		Tot.	P-value	AOR	95% CI	
	Positive	Negative				Lower	Upper
Sex							
Male	18	202	220	1	-	-	-
Female	24	237	261	0.4	1.35	0.7	2.7
Mean cattle age§	5.6+3.0	5.2+3.0	-	0.81	0.99	0.9	1.1
Breed							
Local	34	385	419	1	-	-	-
Cross	8	54	62	0.43	1.42	0.6	3.41
Source							
Homebred	28	337	365	1	-	-	-
Purchase	14	102	116	0.21	1.6	0.8	3.4
Body condition							
Lean	6	110	116	1	-	-	-
Medium	29	266	295	0.17	1.9	0.8	4.8
Fat	7	63	70	0.45	1.6	0.5	5.1
Household TB status							
Negative	20	317	337	1	-	-	-
Positive	22	122	144	0.002	2.9	1.5	5.54

§age was not categorized.

Table 3: Bovine tuberculosis in North Gondar and North Wollo Zones based on >2 mm as a cutoff value, Ethiopia, 2014.

the odds of bovine TB that cattle owned by PTB cases was about 3 times (2.90, 95% CI: 1.50–5.54) higher compared to those cattle owned by without PTB households and the difference was highly significant ($p<0.05$). In this study, sex, age, breed, body condition and source of the cattle were not associated with the occurrence of bovine TB.

Discussion

The current study, which mainly conducted on local zebu cattle and some cross breeds, indicated the importance of Bovine tuberculosis (BTB), particularly in cattle owned by human PTB diagnosed households. Based on >4 mm measurement difference 6.4% an overall household/herd and 2.1% of an individual cattle apparent prevalence of BTB was documented. Herd (household) and individual cattle apparent prevalence of 20% and 5.6% registered, respectively in PTB households (cases). In the comparative groups 2% and 0.6% of herd and individual cattle apparent prevalence, respectively were recorded. Using >2 mms as a cutoff value 23.6% and 8.7% of an overall household/herd and individual cattle apparent prevalence of bovine TB was observed. Using the same cutoff value the apparent prevalence in households with PTB and households without PTB were 48.6% and 15.2%, respectively.

Bovine TB is recorded in different animal species in Ethiopia. In a far area (Ethiopia) an individual animal BTB prevalence of 0.4% and 0.2% were recorded in Camels and Goats, respectively [19]. Sera of wild-lives tested with rapid test in five regions of Ethiopia revealed 23% (20/87) of BTB [20]. A herd prevalence of 19% [21], 44% [22] and 51.4% [23] of BTB were reported in Boji district, Afar and Jima dairy herds, respectively. A study conducted in and around Mekelle area, Tigray regional state, Ethiopia, revealed 54% of herd BTB prevalence [10]. A cross sectional study of BTB conducted in Ambo and Toko Kutaye districts, Ethiopia, showed an overall individual and herd prevalence of 1% and 7.02%, respectively [22]. In all cases, the difference might be attributed to the epidemiological factors that favor the transmission of BTB, which include herd sizes, farm types, communal grazing and watering of diverse species of animals [24].

The authors [22] also showed that BTB was more prevalent in cattle owned by tuberculosis households (1.36% at an individual and 12% at herd levels) compared to non-tuberculosis households (nearly 0.6% at an individual and 3.13% at herd levels), which in general supports the current finding. However, contrary to the above mentioned study from cattle owned by the TB patient's higher prevalence of BTB at herd level was registered in this comparative cross-sectional study.

Adjusted for age, sex, body condition, breed and source, at an individual cattle level the risk of a cattle to be positive for bovine TB, when owned by PTB affected individuals were about three times (2.9) using >2 mms as a cutoff, higher compared with those cattle owned by non-PTB households. Studies done in different parts of Ethiopia indicated the presence of high prevalence of BTB in cattle owned by PTB diagnosed cattle owners than PTB negative ones at herd and individual cattle levels. For instance, of 11 TB households, 4 (4/11, 36.4%) [25] and 5 (5/8, 62.5%) [23] in northwest Ethiopia and Jimma dairy herd, respectively, had BTB reactor herds. Fetene et al. [11] reported a significantly higher prevalence of BTB in cattle owned by TB patients than in cattle owned by non-TB owners and at the same time ascertained by isolating *M. tuberculosis* and *M. bovis* from sputum and fine needle aspiration specimens of TB patient cattle owners. Besides, *M. tuberculosis* was isolated in grazing cattle in Central Ethiopia. In Nigeria 11.8% prevalence was reported in cattle owned by TB positive owners [26] and Aishatu [27] denoted that the high TB prevalence trend among human patients in Nigeria has similar trend among cattle populations.

The presence of higher BTB reactor cattle in cattle owned by PTB positive households than PTB negative ones could suggest that either of them could be a source of infection for the other creating a sort of vicious cycle [6]. From the current study and other literature human TB may be transmitted to cattle or that cattle TB may be acquired from humans [9,24,28] and, therefore, *M. tuberculosis* might be a possible cause of the skin test positivity in cattle. A study carried out, in and around Mekele reported that those who owned cattle had 5% TB patients in their house compared to 3% TB patients in the house for those who did not own cattle [10]. A report in Spain indicated *M. tuberculosis* infection of cattle and the source of infection was human TB patient [29]. Apart from the possible contribution of *M. tuberculosis* for skin test positivity, the difference in skin test result might be related to the differences in the cattle husbandry system, nutritional status of the animal, breed differences [6] number of PTB case households, number of cattle (herd size) [30] owned by the PTB cases, extent of study area coverage as well as cattle duration of cattle stay in the households, the use of proper cold chain systems and technical disparity as well as cattle immunity/circulating antibodies [31].

Based on the current comparative intradermal skin test referring to >4 mms 30% (42/140) of the herds as well as 13.5% (65/481) of individual cattle were tested inclusive and this seems important because of the fact that such cattle could serve as a source of infection to other cattle or humans, as this was evidenced by culturing of *Mycobacterium* species from suspected BTB lesions [32] and milk samples [27]. Besides, a study conducted in India showed 26.7% doubtful/inclusive skin test result, and among the inclusive cattle 66.7% revealed positive results with interferon assay [33]. Besides, test positive cattle, particularly the young ones could be seen a source of human TB for the negative comparative households. Purposive selection of the study sites, smaller tuberculosis cases, inability to recheck inclusive cattle due to logistical and financial problems as well as a lack of determination/identification of the causal agent to establish possible cross infections were some of the limitations of the this study.

Conclusion and Recommendations

The current study indicates the presence and importance of BTB in North Gondar and Wollo Zones of Amhara National Regional State and denotes the possible transmission of the disease in the studied rural areas. Using >2 mms as a cutoff value 23.6% an overall herd/household and 8.7% an individual apparent prevalence of BTB was recorded. The odds of cattle owned by households with TB were about three times compared to their counterparts. Existing cattle management practices, the free and mixed grazing as well as using common water sources and free livestock movement in the study area is likely to facilitate the expansion of the disease in the areas. BTB eradication from livestock is expensive, but it is possible to minimize the possible spread of the disease by regular testing/surveillance, segregation and creation of awareness among livestock owners. In addition the investigation of the BTB situation in humans in the study areas should be given emphasis to see the possible cross infections or reverse zoonoses verification. The possible contribution of *M. tuberculosis* to cattle skin test positivity in those cattle owned by households with a TB case needs to be verified by tubercle lesion or milk sample collection and culturing with subsequent molecular typing. BTB finding in Deberak (where Ras Dashen Mountain harboring endemic wild lives is located) adjacent Kebeles could be seen as a risk to wildlife, thereby hampering the tourism industry and this might require additional studies to highlight the possible spread of BTB at the wildlife-domestic animal interface. The current finding obtained by comparing one case with three comparative groups is strong and this will help in developing policies related to animal husbandry systems in the country. At this moment test and slaughtering strategy might be difficult for Ethiopia and therefore, frequent education and creation of awareness among the farming communities should be strengthened to prevent the public from possible BTB infections. The contribution of BTB inclusive cattle was high and this should be given attention in future studies.

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