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Cultural and molecular detection of zoonotic tuberculosis and its public health impacts in selected districts of Tigray region, Ethiopia

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Abstract

Bovine tuberculosis (BTB) is a chronic infectious disease of animals characterized by the formation of granulomas in tissues and its detection is carried out most commonly on the basis of tuberculin skin testing, abattoir meat inspection and rarely on bacteriological techniques. A study was conducted to assess the epidemiology and zoonotic implication of bovine tuberculosis in three selected districts of Tigray region between September 2012 and June 2013 using Comparative Intradermal Tuberculin (CIDT) test, abattoir surveillance, bacteriology and molecular typing. Besides, livestock owners were interviewed for the evaluation of the zoonotic potential of BTB. On the basis of the CIDT test, animal and herd prevalence were 2.7% (14/524) and 9.3% (13/140), respectively, while in abattoir-based study the prevalence was 22% (117/531). Male animals were more likely (OR= 1.7; P=0.012; 95% CI: 1.12- 2.55) to exhibit tuberculous lesions as compared to female animals. The proportion of culture positivity was 32% (40/125) in tissue samples. Only nine isolates were typed and out of which seven isolates were positive for the genus *Mycobacterium* of which two were identified as *M. tuberculosis.* The two *M. tuberculosis* species had the same spoligotype pattern. Awareness of cattle owners about BTB was poor and detection of BTB both in live and slaughtered animals. The isolation of *M. tuberculosis* from animal tissues indicates the existence of transmission of this agent from humans to animals. Therefore, there should be practical and sound control methods such as strict meat inspection, boiling of milk and cooking of meat, and public education to raise the awareness on the transmission of the disease.

Keywords: Abattoir , Ethiopia, *Mycobacterium*, Tuberculosis, Zoonosis

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Introduction

Tuberculosis (TB) is an infectious disease that is caused by a bacterium called *Mycobacterium* tuberculosis complex and primarily affects the lungs, but it can also affect organs in the central nervous system, lymphatic system, and circulatory system among others (de la Rua-Domenech *et al*., 2006). It is an important disease among many zoonoses (Une & Mori, 2007) that affects humans and many vertebrate animals and is characterized by the formation of granulomas in tissues and organs (Amanfu, 2006). Tuberculosis is an ancient scourge that has plagued humankind throughout known history and human prehistory (Daniel, 2006). Tuberculosis remains a major global burden. The latest estimates included in the WHO (2012) report are that there were almost 9 million new cases in 2011 and 1.4 million TB deaths, 990 000 among HIV-negative people and 430 000 HIV-associated TB deaths.

In Ethiopia, tuberculosis and leprosy have been recognized as major public health problems since the 1950s. Tuberculosis is the most frequent cause of hospital admission (9.4% of all cases admitted to hospital) and the leading cause of hospital deaths. In the year 2005, the HIV prevalence among adult TB patients was determined to be 11%. Bovine tuberculosis (BTB) is an important disease caused by *Mycobacterium bovis*. It affects animal health, the

economic value of cattle and food products derived from cattle. Moreover, this disease is transmissible to other species of animals and humans (Jolley *et al*., 2007). It is classified as a list B disease by the Office International des Epizooties (OIE) and has a potential significant impact on the international trade of animals and animal products (OIE, 2009). Tuberculosis, caused by *M. bovis*, is emerging as the most important disease affecting cattle and continues to be a problem both in countries with and without active control policies (Thom *et al*., 2006). Bovine tuberculosis aggravates the 'triple trouble' of HIV/AIDS and TB infection and malnutrition (Awah-Ndukum *et al*., 2012). Tuberculosis cases due to *M. bovis* in HIV-positive persons also resemble disease caused by *M. tuberculosis*. Thus, they manifest as pulmonary disease, lymphadenopathy, or in a more profoundly immunosuppressed subjects as a disseminated disease. However, there is often little awareness about the potential for zoonotic *Mycobacterium* species, such as *M. bovis*, to cause human tuberculosis and our understanding of the contribution of the bovine tubercle bacilli to the human epidemic worldwide is very limited (Cleaveland *et al*., 2007).

In the Tigray region of Ethiopia, particularly in Wukro and Hintalo-Wajirat districts, little is known about the situation with animal tuberculosis. There is no information on the detection of bovine tuberculosis either on live animals or in abattoir. Hence, the degree of zoonotic transmission of tuberculosis from animals to humans is unknown. Various types of cattle husbandry methods and cultural practices that could facilitate transmission of BTB between cattle and humans exist. People in the area were observed with a great tendency to own cross-breed and exotic animals and implement intensive cattle production system via owning few productive animals. People residing in the area consume raw and locally soured milk, and raw meat. Moreover, cattle are kept in close proximity to farmers' homes and are often slaughtered in the backyard, where the slaughter men and butchers wear minimal protective clothing and process offal from diseased carcasses with their bare hands. These activities offer ample opportunity for zoonotic transmission of infection. Cases of TB patients who are admitted to the health centers of the study districts are increasing each year which depicts that tuberculosis is a major human health problem in the study areas (Gebretsadik, 2012). Therefore, the present study was conducted to determine the episode of zoonotic tuberculosis in cattle residing in the study areas; to identify the risk factors associated with zoonotic tuberculosis and to isolate and identify the agent from appropriate samples using cultural and molecular techniques.

Materials and methods

Description of the study districts

Tigray region is the northern most of the nine ethnic regions of Ethiopia containing the homeland of the Tigray people. Wukro, Hiwane and Adigudem districts are three of the 36 districts in the Tigray Region. Wukro district is a town in the Eastern zone of the Tigray region; it is the administrative center of the district named after the town. The town has a total population of 30,210, of whom 14,056 are men and 15,154 are women. A total of 9,383 households were counted in this town, resulting in an average of 3.22 persons to a household, and 8,993 housing units. Hintalo-Wajirat district contains two towns, Adigudem and Hiwane, and it is located at 13°14′50"N and 39°-53°E with an elevation of 2100 meters above sea level. The districts have a cool tropical semiarid climate with mean annual temperature of around 18°C. The mean annual rainfall is about 650 mm and varies considerably between years (Sisay *et al*., 2013; Corbeels *et al*., 2000).

Study subjects

The study animals were cattle of household farmers and the study was made on live and slaughtered animals and the human population of the study areas. Comparative intradermal tuberculin test and abattoir-based surveillance was conducted on 524 and 531 cattle of 140 households, respectively. Among those animals tested, 331 (63.2%) were from Hintalo-Wajirat district and 193 (36.8%) were from Wukro district. The family members of the households were interviewed. Of those members 58 (41.4%) were females and 82 (58.6%) were males.

Study design and sampling

A cross-sectional study was used to study the cultural and molecular detection of Bovine tuberculosis (BTB) and its public health impacts between September 2012 and June 2013. First, urban and rural peasant associations (PAs) of the study districts were selected using simple random sampling method and then households were approached for questionnaire. Out of 13 rural and 9 urban PAs, a total of 22 PAs, which are known to keep indigenous and hybrid and/or exotic breeds of cattle and a total of 14 (7 rural and 7 urban PAs)

were selected randomly. Willingness of households was considered to include them in the study and 10 households were selected randomly. One hundred forty herds containing 524 cattle belonging to the selected 140 households were tested using the comparative intradermal tuberculin test and member of household that was found during the visit was interviewed. Abattoir-based BTB survey was also conducted on the basis of systematic random sampling method.

Sample size determination

The sample size required for the CIDT test was calculated according to the formula given by Thrusfield (2007) for cluster sampling. Using the CIDT test, the Hintalo-Wajirat Agriculture Office at Adigudem town reported that 14.5% (11/76) of traditionally managed cattle were reactors to bovine tuberculosis in the district. Therefore, the sample size, clusters or herds, required for the present study at Pexp = 14.5%, Vc = 0.01, d = 0.05 and n = 4 is 63 clusters and in order to cover larger area of the animals' population, this number was increased to 70 and it was then doubled to 140 herds to increase the precision as far as practically possible. For the determination of the magnitude of BTB in the slaughterhouse, the sample size required was 191 animals with the expected prevalence rate of 14.5%. To carry out bacteriological analysis, suspected tuberculous lesions from 531 animals were cultured.

Study methodology

Cattle were tested by house-to-house visits as early in the morning as possible. All cattle belonging to the selected households were individually identified by names which were previously given by their owners or assigned on the day of tuberculin inoculation, by their coat colour, age and sex. Their age, body condition score, sex, breed, lactation status, pregnancy status and skin test measurements were recorded. Body condition scoring was done according to Maurya *et al*. (2009) as poor, medium and good. In order to evaluate the effect of management on the occurrence of the disease, information was obtained regarding coughing in the herd and contacts made with other herds at watering points and during grazing. Households were asked for the presence of TB patient in their home or family. Comparative intradermal tuberculin test (CIDT) was carried out to differentiate between animals infected with tuberculosis or those sensitized to tuberculin due to exposure to other *Mycobacterium* or related genera. CIDT was performed by restraining the cattle and two sites on the skin of the mid neck were shaved and cleaned being separated by 12 cm on the same side of the neck; the areas were examined for the presence of any gross lesion. The skin fold thickness at the two sites was measured in millimeters with a skin-caliper and recorded before injection. Each animal was then injected with 0.1ml (28,000 IU/ml) of bovine purified protein derivative /PPD/ (Bovitubal, strain AN5, Bioveta Czech Republic) and 0.1ml (28,000 IU/ml) of avian PPD (Avitubal, strain D4ER, Bioveta, Czech Republic) intradermally using semi-automatic syringe at the anterior and posterior parts, respectively.

The injection sites were examined and the skin thicknesses were measured 72 hours post injection. When the difference in skin thickness is greater at the site of avian PPD inoculation than the bovine PPD inoculation site, an animal was considered as a non-specific reactor; whereas, the skin thickness increased at both sites and/or at the site of bovine PPD injection, the result was interpreted according to manufacturer's instruction. B2 – B1 = ⍰B; A2 – A1 = ⍰A. ⍰B - ⍰A ⍰ 2mm: Negative for *M. tuberculosis* complex; ⍰B - ⍰A= (2mm-4mm): Doubtful for *M. tuberculosis* complex and ⍰B - ⍰A⍰ 4mm: Positive for *M. tuberculosis* complex. Where: A1 = skin thickness before inoculation of avian PPD; B1 = skin thickness before inoculation of bovine PPD; A2 = skin thickness after inoculation of avian PPD; B2 = skin thickness after inoculation of bovine PPD; ⍰A=Change in skin thickness at the site of avian PPD inoculation, and ⍰B= Change in skin thickness at the site of bovine PPD inoculation.

For the abattoir survey of BTB, animals and their carcasses and offal were identified using the butcher number and information on breed, sex and type of organs affected were recorded during carcass inspection. The lungs and lymph nodes were carefully and skillfully inspected for tuberculous lesions.

Bacteriological culture: For the isolation and identification of mycobacteria, a total of 125 tissues containing tuberculous lesions were collected. They were carefully removed from the carcass and placed in a 50 ml capacity tubes with screw caps containing 5 ml of sterile 0.85% saline water in a cooler with ice, and stored at -20^{0}C and transported at +4^{0}C to the TB laboratory of the Armauer Hansen Research Institute (AHRI) for bacteriological examination.

Löwenstein-Jensen media was used for culturing and prepared according to the procedures of WHO (2012). In processing samples of lesions from cattle in the laboratory, first the fat was removed from

tissues containing the tuberculous lesion and then sectioned into pieces using sterile scissors and forceps. Thereafter, 0.85% saline water and sterile sand were added and grinding with pestle and mortar. Using a sterile 15 ml centrifuge tube with a screw cap, equal amounts of specimen and 4% NaOH of about 5 ml each were added. The centrifuge tube was capped and mixed on a vortex mixer until the specimen was liquefied. Thereafter, they were centrifuged at 3000 rpm for 15 minutes. The supernatant was decanted, and 2 ml of 0.85% saline was added to re-suspend the sediment. The samples were neutralized by adding 2NHCl drop by drop until the color of the indicator (0.1% phenol red) turned yellow. The suspension was inoculated onto two Löwenstein-Jensen slopes, one with pyruvate and the other with glycerol and incubated at 37°C for between 8 and 12 weeks (Lambert *et al*., 2006). Cultures were checked once a week for the growth of mycobacteria.

Slow growth and colony characteristics were considered as an evidence of growth of mycobacteria and further subculturing to obtain sufficient number of colonies for further molecular analysis was done from the positive cultures. The observation of isolates with euogenic growth on Löwenstein–Jensen media supplemented with glycerol were tentatively identified as *M. tuberculosis* while those with euogenic growth on the pyruvate-containing media were regarded as suggestive of *M. bovis*. Finally, isolates were harvested for molecular typing analysis by scrapping the growth from a slope into 200 µl of sterile distilled water and heating at 80°C for 1 hour (Cadmus *et al*., 2006). Then, the harvest was kept at -20^{0}C until the day of transportation to the Veterinary Laboratory Agency (VLA) in UK for molecular analysis. In the Veterinary Laboratories Agency (VLA), the current methods of choice for the molecular typing of *M. bovis* isolates are spacer-oligonucleotide typing (spoligotyping) and variable number of tandem repeat (VNTR) typing (Hewinson *et al*., 2006).

Multiplex polymerase chain reaction (m-PCR): For m-PCR, the procedure described by Savita *et al*. (2012) was followed. This analysis differentiates *M. tuberculosis* complex from *M. avium*, *M. intracellularae* and other mycobacterial species. The PCR targets the sequence of the genus *Mycobacterium* within the 16S rRNA gene (G1, G2), sequences within the hyper-variable region of 16S rRNA that is known to be specific to *M. intracellularae* (MYCINT-F) and *M. avium* (MYCAV-R), and the MTB70 gene specific for *M. tuberculosis* complex (TB-1A, TB-1B). The primers used were MYCGEN-F, 5’AGA GTT TGA TCC TGG CTC GA 3’, (35 ng/µl); MYCGEN-R, 5’-TGC ACA CAG GCC ACA AGG GA 3’, (35 ng/µl); MYCAV-R, 5’-ACC AGA AGA CAT GCG TCT TG 3’, (35 ng/µl); MYCINT-F, 5’-CCT TTA GGC GCA TGT CTT TA 3’, (75 ng/µl); TB1-F, 5’-GAA CAA TCC GGA GTT GAC AA 3’, (20 ng/µl); TB1-R, 5’-AGC ACG CTG TCA ATC ATG TA 3’, (20 ng/µl). The reaction was carried out using Thermal Cycler (Applied Biosystems, GeneAMP 9700) for 10 minutes at 95^{0}C; 35 cycles of 1 minute at 95^{0}C, 1 minute at 61^{0}C, and 1.5 minutes at 72^{0}C; and 10 minutes at 72^{0}C. Each PCR tube consisted of 5.2 µl H2O Qiagen, 8 µl HotStarTaqMasterMix, 0.3 µl of each of the six primers, 5 µl of DNA templates of samples or controls making the total volume 20µl. *M. avium*, *M. intracellularae*, H37Rv and *M. bovis* strain 2122/97 were used as positive controls while H2O Qiagen, was used as a negative control. The product was electrophoresed in 2% agarose gel in TAE running buffer 10X SYBR safe at a ratio of 1:10 in 2% agarose gel, 100bp DNA ladder, and orange 6x loading dye were used in gel electrophoresis.

All members of the genus *Mycobacterium* produce a band of 1030bp. On the other hand, *M. avium* or subspecies such as *M. avium* subsp. Paratuberculosis, *M. intracellularae* and members of *M. tuberculosis* complex produce a band, 180bp, 850bp and 372bp, respectively.

Deletion typing: In this analysis the procedure described by Cadmus *et al*. (2006) was followed. Primers directed against the RD4, RD9 and RD10 loci were used to generate a deletion profile that would allow species identification of the isolate. Primers that were used include RD4intF ACA CGC TGG CGA AGT ATA GC, RD4flankF CTC GTC GAA GGC CAC TAA AG and RD4flankR AAG GCG AAC AGA TTC AGC AT to check for the presence of RD4 locus; RD9intR CTG GAC CTC GAT GAC CAC TC, RD9flankF GTG TAG GTC AGC CCC ATC C and RD9flankR GCC CAA CAG CTC GAC ATC to check for the presence of RD9 locus; RD10intR GAA GTC GTA ACT CAC CGG GA, RD10flankF CTG CAA CCA TCC GGT ACA C and RD10flankR AAG CGC TAC ATC GCC AAG to for the presence of RD10 locus. The HotStarTaq Master Mix system from Qiagen was used for PCR, with primers described previously. The reaction mixture was 10 µl of HotStarTaq Master Mix, 0.3 µl ×3 of each primer (flank R, F and int), 2 µl DNA template and distilled water to a final volume of 50 µl. The PCR cycle was performed on a Perkin-Elmer GeneAmp machine using an initial hot start of 95°C

for 15 minutes, followed by 35 cycles of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute; a final extension step of 72°C for 10 minutes completed the cycle. Products were visualized by electrophoresis through 1% agarose gel.

The presence of RD4 (i.e. *M. tuberculosis*, *M. africanum*), RD9 (i.e. *M. tuberculosis*) and RD10 (i.e. M. tuberculosis), is shown by the amplification of a product size of 335bp (RD4intF + RD4flankR), 1.421kb (RD9flankF + RD9intR) and 308bp (RD10flankF + RD10flankR), respectively, whereas the occurrence of an amplification product size of 446 bp (RD4flankF + RD4flankR), 472bp (RD9flankF + RD9flankR) and 202bp (RD10falnkF + RD10flankR) indicates deletion of RD4 (*M. bovis*), RD9 (*M. africanum* and *M. bovis*) and RD10 (*M. africanum* and *M. bovis*), respectively.

Spoligotyping: Spoligotyping was performed as described by Cadmus *et al*. (2006) and the direct repeat (DR) region was amplified by PCR with oligonucleotide primers derived from the DR sequence. Twenty-five microliters of the following reaction mixture was used for the PCR: 12.5 µl of HotStarTaq Master Mix (Qiagen; this solution provides a final concentration of 1.5 mM $MgCl_2$ and 200 µM each deoxynucleoside triphosphate.), 2 µl of each primer (20 p mol each), 5 µl of the suspension of heat-killed cells (approximately 10 to 50 ng), and 3.5 µl of distilled water. The mixture was heated for 15 minutes at 96°C and subjected to 30 cycles of 1 minute at 96°C, 1 minute at 55°C, and 30 seconds at 72°C. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequences within the DR-locus. After hybridization, the membrane was washed twice for 10 minutes in 2× SSPE (1×SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7]) – 0.5% sodium dodecyl sulfate at 60°C and then incubated in 1:4,000-diluted streptavidin-peroxidase conjugate (Boehringer) for 45 to 60 minutes at 42°C. The membrane was washed twice for 10 minutes in 2× SSPE–0.5% sodium dodecyl sulfate at 42°C and rinsed with 2× SSPE for 5 minutes at room temperature. Hybridizing DNA was detected by the enhanced chemiluminescence method (Amersham Plc. Lt, UK) and by exposure to X-ray film (Hyper-film ECL; Amersham Plc. Lt, UK) as specified by the manufacturer.

The questionnaire survey: A total of 140 respondents whose animals were tested or their family members were interviewed with a questionnaire. Their recognition of bovine TB, awareness on BTB transmission onto human beings and dairy product consumption and meat eating habits were investigated.

Data analysis

The data were analyzed using statistical package for the social sciences (SPSS®) version 20. Descriptive and analytic statistics were computed and logistic regression and Chi-square test (χ^2) were employed to see the association of risk factors with that of the disease; the degree of association was computed using Odds ratio (OR) and 95% confidence interval (CI). Odd ratio (OR) was used to indicate the degree of risk factor association with the disease occurrence signified by 95% confidence intervals.

Results

Animal characteristics

Out of 524 animals tested with CIDT, 14 (2.7%) were found positive for tuberculosis and 14 (2.7%) were determined as doubtful reactors. Therefore, the individual animal prevalence of bovine TB obtained was 2.7% (95% CI: 1.5, 4.4) if doubtful animals were considered negative. On the other hand, if doubtful reactors were considered as positive the prevalence of tuberculosis would be 5.3% (95% CI: 3.6, 7.6). Besides, 3.5% (18/524) of the tested animals reacted to both bovine and avian type tuberculin and classified as non-specific reactors, which resulted from infection with other mycobacteria. The prevalence of BTB was found to be 2.6% in Wukro district and 2.7% in Hintalo-Wajirat district (Table 1). Many animal characteristics were considered (Table 8) to determine the risk factors predisposing animals to BTB infection. However, all the considered animal level characteristics were found to be not associated with tuberculosis ($P > 0.05$).

Herd level characteristics

Out of 140 herds tested, 27 (19.3%) and 13 (9.3%) were found to be TB infected with and without considering doubtful reactors as positives, respectively. Therefore, the herd level prevalence was 19.3% (95% CI: 13.1-26.8%) with and 9.3% (95% CI: 5-15.4%) without considering doubtful reactors as bovine TB infected herds. However, only positive reactors were considered in investigating the possible risk factors. The type of management, herd size and status of infection of owners with TB were not risk factors associated with the episode of tuberculosis infection of herd ($P > 0.05$). But the prevalence rate was slightly

higher in cattle under extensive-management system (11.1%) than those under semi-intensive management (6.8%). Likewise, the prevalence of BTB was higher in those herds containing more than 4 animals (11.4%) than in those herds with lower number of animals (7.6%) (Table 2).

Abattoir findings

Abattoir-based surveillance revealed that the distribution of suspected tuberculous lesions in tissues of positive animals and is presented in (Table 3). Among the lesions observed, 89.6% were localized lesions involving frequently a single organ and the majorities (73.8%) of the lesions were located on the mesenteric lymph nodes meanwhile 19.1% and 7.1% of the lesions were found in the thoracic cavity and cranial regions, respectively (Table 3).

Of the two risk factors considered, only sex was found to be significantly associated with BTB infection (χ^2 = 6.85, P = 0.009). Male animals were found harboring tuberculous lesions 1.7 times more than that of females (OR= 1.7; P=0.012; 95% CI: 1.1-2.5%). However, there was no statistically significant association between the presence of BTB lesions among animal breeds (X^2 = 2.23, P = 0.327) (Table 4).

Isolation of Mycobateria

Out of 125 tuberculous lesions that were cultured, 32% (40/125) showed growth on the primary culture media. The result of primary culture is presented in Table 5. The cultures which showed visible growth on primary cultures were 82.5% (33/40) and the isolates were subcultured. However, due to the scarcity of resources only nine isolates were subjected to molecular analysis.

Molecular analysis

The PCR analysis of the nine isolates, which were subjected to molecular analysis, indicated the presence of *Mycobacteria* in 77.8% (7/9) of the samples. However, only 28.6% (2/7) of them showed the presence of *Mycobacterium tuberculosis* complex and 14.3% (1/7) was positive for the presence of *M. avium* complex. The remaining 22.2% (2/9) isolates didn't show the amplification products characteristic to mycobacteria. Figure 1 presents the electrophoesis separation of PCR products. Upon deletion typing of those two samples (that showed amplification product for *M. tuberculosis* complex in m-PCR), neither of them showed amplification products for RD4, RD9 and RD10 primers.

Spoligotyping of those two isolates that showed amplification products characteristic to both the genus *Mycobacterium* and to *Mycobacterium tuberculosis* complex revealed in identical strains of *M. tuberculosis.*

The questionnaire survey

A total of 140 respondents or members of these households were interviewed. Of these, 36 (25.7%; 95% CI: 18.7-33.3%) respondents reported that they knew or have heard about TB of cattle and 72.2% of them didn't recognize the symptoms of the disease and almost all of them cannot identify the post-mortem tissue lesions. Besides, only 27.8% (10/36) of them were aware of BTB transmission between the cattle population, whereas 36.6% (13/36) described possible transmission of the disease from sick cattle to humans through inhalation and ingestion of contaminated milk and meat. Several demographic characteristics and other factors were considered (Table 7) to investigate their possible association with BTB recognition of the respondents. Residential area, sex, age, occupation and level of education were not associated with the knowledge of residents on BTB (P > 0.05).

Concerning dairy product consumption habits, 1.4% (2/140) and 82.9% (116/140) of the respondents drink raw milk and locally soured milk (yoghurt) respectively. Moreover, 45% of the respondents have the habit of eating mixed (raw and cooked) meat. Table 8 summarizes the milk and meat consumption habits of the households.

Table 1: Summary of the CIDT test result in cattle of the three study districts

Study district	Number of Tested animals	Positive reactors	Doubtful reactors	Prevalence
Wukro district	193	5	3	2.6%
Hintalo-Wajirat district	331	9	11	2.7%
Total	524	14	14	2.7%

Table 2: The level of association of herd level characteristics with bovine tuberculosis

Herd characteristics	Number of herds			χ^2 Value	P-value
	Positive	Negative	Total		
Management					
-Semi-intensive	4(6.8%)	55(93.2%)	59(100.0%)	0.7603	0.383
- Extensive	9(11.1%)	72(88.9%)	81(100.0%)		
Herd size					
$\leq$ 4 cattle	6 (7.6%)	73(92.4%)	79(100.0%)	0.6177	0.734
$\geq$ 5 and $\leq$ 8 cattle	5 (11.4%)	39(88.6%)	44(100.0%)		
$\geq$ 9 cattle	2(11.8%)	15(88.2%)	17(100.0%)		
Owners' health					
- Sick with TB	-	10 (100%)	10(100.0%)	1.1024	0.296
- Not sick	13(10.0%)	117(90%)	130(100.0%)		

Table 3: The distribution of tuberculous lesions in tissues of infected animals

Anatomic site	Number of infected tissue	Percent (%)
Medial retropharyngeal LN	7	5.6
Mandibular LN	2	1.6
Mediastinal LN	4	3.2
Tracheobronchial LN	5	4.0
Lung	15	12.0
Mesentric LN	92	73.6
Total	125	100.0

LN: Lymph node

Table 4: The level of association of sex and breeds of animals with tuberculous lesions

Variable		Number of animals			χ^2 Value	P-Value
		Positive	Negative	Total		
Sex	Male	66 (27.2%)	177 (72.8%)	243 (100.0%)	6.8544	0.009*
	Female	51(17.7%)	237 (82.3%)	288 (100.0%)		
Breed	Local	100 (21.4%)	367 (78.6%)	467 (100.0%)	2.2331	0.327
	Cross	15 (25.0%)	45 (75.0%)	60 (100.0%)		
	Exotic	2 (50.0%)	2 (50.0%)	4 (100.0%)		

* Statistically significant

Table 5: The result of primary culture of tuberculous tissues from slaughtered cattle

Sample type	Outcome of primary culture			
	L-J media containing glycerol		L-J media containing pyruvate	
	Total	Positive	Total	Positive
Mesenteric LN	92	20 (21.7%)	92	18(19.6%)
Bronchial LN	5	-	5	3(60.0%)
Mandibular LN	2	1 (50.0%)	2	-
Mediastinal LN	4	2 (50.0%)	4	2(50.0%)
Retropharyngeal LN	7	2 (28.6%)	7	1(14.3%)
Lung	15	2 (13.3%)	15	1(6.7%)
Total	125	27 (21.6%)	125	25 (20.0%)

LN: Lymph node

Table 6: Summary of the result of m-PCR analysis of isolates from cattle tissues

Sample number	Sample type	Result of m-PCR		
		Genus *Mycobaterium*	*M. tuberculosis* Complex	*M. avium* complex
SS65Med	Mediastinal LN	Positive	Negative	
SS26BR	Bronchial LN	Positive	Positive	
SS10Mes	Mesenteric LN	Positive	Negative	
SS16Mes	Mesenteric LN	Negative	Negative	
SS14Med	Mediastinal LN	Positive	Negative	
SS34Br	Bronchial LN	Positive	Negative	Positive
SS12Br	Bronchial LN	Positive	Positive	
SS13Mes	Mesenteric LN	Positive	Negative	
SS15Mes	Mesenteric LN	Negative	Negative	

LN: Lymph node

Table 7: Awareness of respondent to the level of association of risk factors with BTB

Variables	Knowledge of BTB Yes	No	Total	χ2 Value	P-Value
Residence (district)					
Wukro	14 (20.0%)	56(80.0%)	70 (100.0%)	2.393	0.122
Hintalo-Wajirat	22 (31.4%)	48(68.6%)	70 (100.0%)		
Sex					
Female	11(20.0%)	47(80.0%)	58(100.0%)	2.361	0.124
Male	25(30.5%)	57(69.5%)	82(100.0%)		
Age					
≥11 and ≤ 30 years	15(30.0%)	35(70.0%)	50(100.0%)	0.920	0.820
≥31 and ≤ 50 years	12(21.8%)	43(78.9%)	55(100.0%)		
≥51 and ≤ 70 years	7(25.9%)	20(74.1%)	27(100.0 %)		
≥71 years	2(25.0%)	6(75.0%)	8(100.0%)		
Occupation					
Farmer	17(27.4%)	45(72.6%)	62(100.0%)	5.238	0.264
House made	5(13.9%)	31(86.1%)	36(100.0%)		
Student	5(31.3%)	11(68.7%)	16(100.0%)		
Civil servant	5(45.5%)	6(54.5%)	11(100.0%)		
Others	4(26.7%)	11(73.3%)	15(100.0%)		
Education					
Illiterate	2 (11.1%)	16 (88.9%)	18 (100.0%)	2.306	0.129
Literate	34 (27.9%)	88 (72.1%)	122 (100.0%)		

Table 8: Questionnaire result of milk and meat consumption habit of the respondents

Habit of respondents	Number of respondents	Percent (%)
Milk consumption	140	100.0
Raw milk	2	1.4
Boiled milk	95	67.9
Both raw and boiled milk	32	22.9
Do not drink	11	7.8
Soured milk (yoghurt) consumption	140	100.0
Consume	116	82.9
Do not consume	24	17.1
Meat consumption habit	140	100.0
Cooked meat	77	55.0
Both raw and cooked meat	63	45.0

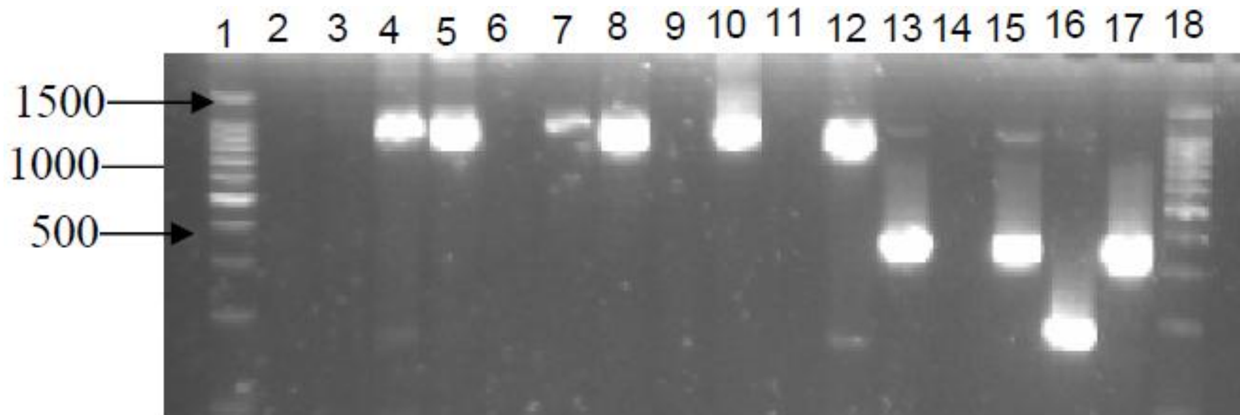

Figure 1: Electrophoretic separation of PCR products of multiplex PCR typing of the genomic DNA of mycobacteria isolated from tuberculous lesions of cattle. Lanes 1 and 18, 100bp DNA ladder; Lane 17, *Mycobacterium tuberculosis* complex positive control; Lane 16, *M. avium* positive control; Lane 14, water negative control. Lane 4, 5, 7, 8, 10 and 12, are positive only for *genus Mycobacterium* and lane 13 and 15 for genus *Mycobacterium* and *Mycobacterium tuberculosis* complex. Lane 2, 3, 6, 9, 11 and 14 are negative.

Discussion

Infection of cattle with *M. bovis* constitutes a human health hazard as well as an animal welfare problem. Furthermore, the economic implications in terms of trade restrictions and productivity losses have direct and indirect implications for human health and the food supply (Awah-Ndukum *et al*., 2012). The present study using the comparative intradermal tuberculin test (CIDT) showed that the prevalence of BTB in the study districts is 2.7%. The disease is assumed to be more prevalent in dairy cattle kept under intensive management system than in extensive due to closer confinement, longer life spans and greater productivity stress (Shitaye *et al*., 2007). Comparable results with the present study were reported by Ashenafi *et al*. (2013) who described prevalence rates of 3.5% (18/514) in Assela and 3.8% (12/320) in Bodji district. This similarity may be related to the resemblance in the type of management system (extensive) and environmental conditions. The lower prevalence rate obtained with the CIDT test in the present study may be due to extensive management system, small herd size of cattle (Cleaveland *et al.*, 2007), or increased resistance due to non-specific response to environmental mycobacteria (Oloya *et al*., 2006). Abattoir inspection of carcasses revealed a high infection rate of BTB and the present survey showed 22% (117/531) in animals slaughtered at Wukro and Adigudem municipal abattoir. However,

comparable results with the present study were obtained by Cleaveland *et al.* (2007) who determined 19.8% (1502/7589) prevalence in cattle carcasses examined by meat inspectors in Tanzania. Majority of the animals slaughtered in the study districts' municipal abattoir were obtained from the surrounding areas. Out of 125 tissue samples cultured, only 32% (40/125) showed growth on primary culture media. The low isolation rate of mycobacteria may have resulted from reduced sensitivity of culture arising from prolonged storage at field sites and the freeze–thaw cycles that occurred during transportation and contamination of tissue samples and overgrowth of *M. bovis* with environmental *Mycobacteria* (Cleaveland *et al.*, 2007). Besides, *M. bovis* grow poorly on standard Löwenstein–Jensen medium (Amanfu, 2006).

Molecular typing of the isolates using m-PCR revealed that seven isolates belong to the genus *Mycobacterium* and only two of them were found to be members of the *Mycobacterium tuberculosis* complex. Strain typing of these isolates indicated that both belong to a single species of *M. tuberculosis* and found to be identical strains. These *M. tuberculosis* strains identified in this study were previously reported by Ameni *et al*. (2013). Isolation of *M. tuberculosis* from animal samples has been reported by many authors. Cadmus *et al*. (2006) reported the finding of two *M. tuberculosis* strains on spoligotyping among 17 isolates from cattle in Nigeria. Moreover, in India Prasad *et al*. (2005) discovered *M. tuberculosis* infection using nested-PCR (N-PCR). Therefore, the finding of *M. tuberculosis* in animals might indicate the potential of transmission of the agent between cattle and man in the study area. In the present study it was found to be only 25.7% of cattle owning family who knew or have heard about bovine tuberculosis. This finding is consistent with the previous reports (Ameni *et al.*, 2008) that the knowledge of cattle owning family was not found to vary among the various demographic characteristics.

In conclusion, this study demonstrates a widespread presence of BTB infection in cattle residing in and around Wukro and Hintalo-Wajirat districts. The majority of cattle owning family in the study area were not aware of the existence BTB and its public health consequence. Moreover, large portion of the human community of the study districts has the custom of consumption of raw food of animal origin, milk and meat which predispose them to zoonotic tuberculosis. The attempt of molecular typing of *M. bovis* was not successful because of its poor adaptation in subcultures. On the other hand, the finding of *M. tuberculosis* isolates from animal samples indicates that cattle could serve as a source of the disease agent to humans. The extent of bovine tubercle bacilli as a contributor to the tuberculosis epidemic in humans remains undetermined. Thus, the presence of the BTB in cattle determined by the CIDT test and abattoir-based study together with the lack of awareness of the public on the presence of TB in cattle, consumption habits and the finding of *M. tuberculosis* from animal tissues highlights the risks of infection. In addition, the cattle with the pulmonary form of BTB, inevitably contribute to the risk of aerosol transmission to humans. This is of particular importance in the study areas since animals are kept in very close proximity to human dwellings and have contact with animals while feeding, herding, milking and ploughing. There should be public awareness creation regarding the risk associated with the consumption of raw and/or unpasteurized dairy products and undercooked meat of food animals. Meat inspection procedures must be strictly implemented with skilled meat inspectors. Locally operative risk factors for zoonotic TB should be identified to determine persons at risk and develop appropriate control measures.

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References

Amanfu W (2006). The situation of tuberculosis and tuberculosis control in animals of economic interest. *Tuberculosis*. **86**(3): 330-335.

Ameni G, Hewinsen G, Abraham A, Young D & Vorderdermeir M (2008). Appraisal of interpretation criteria for the comparative intradermal tuberculin test for the diagnosis of tuberculosis in cattle in central Ethiopia. *Clinical Vaccine Immunology.* **15** (8): 1272-1276.

Ameni G, Tadesse K, Hailu E, Deresse Y, Medhin G, Aseffa A, Glyn Hewinson Vordermeier M & Berg S (2013). Transmission of *Mycobacterium tuberculosis* between Farmers and Cattle in Central Ethiopia. PLoS ONE 8(**10**): e76891.

Ashenafi D, Mamo G, Ameni G, & Simenew K (2013). Epidemiology and Molecular characterization of causative agents of bovine tuberculosis in ruminants. *Journal of Bacteriology & Parasitology*, **4** (1): 161-167.

Awah-Ndukum J, Kudi AC, Bradley G, Ane-Anyangwe I, Titanji VPK, Fon-Tebug S & Tchoumboue J (2012). Prevalence of bovine tuberculosis in cattle in the highlands of Cameroon based on the detection of lesions in slaughtered cattle and tuberculin skin tests of live cattle. *Veterinarni Medicina*, **57**(2): 59–76.

Cadmus S, Palmer S, Okker M, Dale J, Gover K, Smith N, Jahans K, Hewinson RG & Gordon SV (2006): Molecular a nalysis of human and bovine tubercle bacilli from a local setting in Nigeria. *Journal of Clinical Microbiology.* **44**(1): 29-34.

Cleaveland S, Shaw DJ, Mfinanga SG, Shirima G, Kazwala RR, Eblate E & Sharp M (2007). *Mycobacterium bovis* in rural Tanzania: Risk factors for infection in human and cattle populations. *Tuberculosis*. **87**(1): 30–43.

Corbeels M, Abebe S, & Mitiku H. (2000). Farmers' knowledge of soil fertility and local management strategies in Tigray, Ethiopia. Managing Africa's Soil; No. 10. Pp 1-37.

Daniel TM (2006).The history of tuberculosis. *Respiratory Medicine*. **100**(11): 1862–1870.

De la Rua-Domenech R, Goodchild AT, Vordermeier HM, Hewinson RG, Christiansen KH & Clifton-Hadley RS (2006). Antet-mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, γ-interferon assay and other ancillary diagnostic techniques. *Research in Veterinary Science*. **81**(2): 190-210.

Gebretsadik B, Fikre E & Abraham A (2012). Treatment outcome of smear-positive pulmonary tuberculosis patients in Tigray Region, Northern Ethiopia. *BMC Public Health*, 12:537.

Hewinson RG, Vordermeier HM, Smith N & Gordon SV (2006). Recent advances in our knowledge of *Mycobacterium bovis*: a feeling for the organism. *Veterinary Microbiology*. **112**(2-4):127-139.

Jolley ME, Nasir MS, Surujballi OP, Romanowska A, Renteria TB, De la Mora A, Lim A, Bolin SR, Michel AL, Kostovic M & Corrigan EC (2007). Fluorescence polarization assay for the detection of antibodies to *Mycobacterium bovis* in bovine sera. *Veterinary Microbiology*. **120**(1-2): 113–121.

Lambert W, Ameni G, Manaye K & Mekonnen Y (2006). Study on Bovine Tuberculosis in the Holeta Dairy Farm, Central Ethiopia. *Journal of Animal and Veternary Advances*. **5**(12): 1150-1154.

Maurya VP, Sejian V, & Naqvi SMK (2009). Body condition scoring system-a strategic tool for optimising productivity in animal farm. *Indian Farming.* 58(**12**):28-33.

OIE (2009). Bovine Tuberculosis. Version adopted by the World Assembly of Delegates of the World Organization for Animal Health (OIE) in May 2009. OIE Terrestrial Manual, Pp 1-16.

Oloya J, Opuda-Asibo J, Djønne B, .Muma JB, Matope G, Kazwala R & Skjerve E (2006). Responses to tuberculin among Zebu cattle in the transhumance regions of Karamoja and Nakasongola district of Uganda. *Tropical Animal Health and Production*. **38**(4): 275-283.

Prasad HK, Singhal A, Mishra A, Shah NP, Katoch VM, Thakral SS, Singh DV, Chumber S, Bal S, Aggarwal S, Padma MV, Kumar S, Singh MK & Acharya SK (2005). Bovine tuberculosis in India: Potential basis for zoonosis. *Tuberculosis*. **85**(5-6):421–428.

Savita K, Singh P, Aafreen M, Gita N, Swapna K, Rohini K & Rajan MGR (2012). An in-house multiplex PCR test for the detection of *Mycobacterium tuberculosis*, its validation & comparison with a single target TB-PCR kit. *Indian Journal of Medical Research.* **135** (5): 788-794.

Shitaye JE, Tsegaye W & Pavlik I (2007). Bovine tuberculosis infection in animal and human populations in Ethiopia: a review. Shola Veterinary Clinic and Laboratory, Ethiopia. *Veterinarni Medicina*. 52 (**8**): 317–332.

Sisay WZ, Reta DH, Abay AG & Zelelew YB (2013) Detection of human and bovine tuberculosis using an existing diagnostic practice in residential districts of Tigray region, Northern Ethiopia. *Journal of Environmental and Occupational Sciences.* **2** (2): 77-88.

Thom ML, Hope JC, McAulay M, Villarreal-Ramos B, Coffey TJ, Stephens S, Vordermeier HM & Howard CJ (2006). The effect of tuberculin testing on the development of cell-mediated immune responses during *Mycobacterium bovis* infection. *Veterinary Immunology and Immunopathology.* **114**(1-2): 25–36.

Thrusfield M (2007). Veterinary Epidemiology, (*3rd edition*). Blackwell Science Ltd., London, Pp 227-247.

Une Y & Mori T (2007). Tuberculosis as a zoonosis from a veterinary perspective. *Comparative Immunology, Microbiology and Infectious Diseases*. **30**(5-6): 415-425.

WHO (2012). Global Tuberculosis Report. World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland. Basic facts about tuberculosis (TB). Pp 3-5.

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