A Cross-sectional Study on Zoonotic and Reverse Zoonotic Transmission of Tuberculosis in Central Ethiopia

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Research article

Keywords: Ethiopia, Tuberculosis, Reverse Zoonosis, Zoonosis

Posted Date: June 1st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-26154/v1>

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Abstract

Background: The zoonotic transmission of tuberculosis (TB) from cattle to humans has long been recognized while its reverse zoonotic transmission from humans to animals has recently been reported. The socioeconomic situations of the rural communities of Ethiopia are conducive for the zoonotic and reverse zoonotic transmission of TB between cattle and human. The objective of this study was to investigate the zoonotic and reverse zoonotic transmission TB between humans and cattle central Ethiopia.

Methods: To achieve this objective a cross-sectional study was conducted on 1896 cattle slaughtered in two abattoirs and 392 TB suspicious human subjects visiting health institutions for treatment. Post mortem examination, mycobacteriological culture, and spoligotyping were used for the study. In addition, the Spoligotype International Types and VNTR (variable number of DNA tandem repeats) International Types (SITVIT2) database and the online “Run TB-Lineage” were used to identify SIT and lineages of the human and cattle isolates.

Results: Gross TB lesion was detected in 4.2% (80/1896) the slaughtered cattle and predominantly (52.5%) found in the thoracic cavity. But culture positivity was only 26.3% (21/80) in suspicious lesions. Of the 21 cattle isolates, 12 (57.2%) were *M. bovis* while the remaining nine (42.8%) were *M. tuberculosis*. SIT50, SIT118 and SIT1318 were isolated from both humans and cattle. Similar to the low culture yield in cattle tissues, only 22% (86/392) the sputa from TB suspicious humans were culture positive. These 86 human isolates were classified into 81 *M. tuberculosis*, three *M. africanum* and two *M. bovis* by spoligotyping. The two human *M. bovis* isolates had the pattern of SB1443, which was not isolated from cattle in this study.

Conclusion: The zoonotic and reverse zoonotic transmissions of TB were confirmed in Ethiopia by the isolation of two *M. bovis* from human and isolation of nine *M. tuberculosis* from cattle, which suggested higher role of *M. tuberculosis* in cattle compared to the role of *M. bovis* in humans.

Background

Bovine tuberculosis (bTB) is a chronic disease animals characterized by progressive development granulomatous lesions or tubercles in the lymph nodes, lung tissue and or other organs. BTB is caused by members of the *Mycobacterium tuberculosis* (*M. tuberculosis*) complex primarily by *M. bovis*, and to a lesser extent *M. tuberculosis*. The disease causes significant economic losses to the Agriculture Sector and public health problem in causing zoonotic TB in humans. According to the World Organization for Animal Health, 82 (44%) of the 188 countries and territories reported the presence of bTB during January 2017 and June 2018 [1]. Thus, the disease is found throughout the world although most of the developed countries have reduced or eliminated it from their cattle populations except in limited areas where wild animals are interfering with their control programs being reservoirs of the infection. On the other hand, the disease is prevalent in many African countries and in parts of Asian countries [1-4]. The widespread of bTB in animal populations in developing countries is due to the lack of or inadequate control measures for the socioeconomic problems associated with the presently available test and slaughter control measure [4].

The direct correlation between *M. bovis* infection in cattle and zoonotic TB in the human population has been well documented in industrialized countries [4]. Zoonotic TB is caused by *M. bovis* and transmitted from animals to humans through consumption of foods infected animals and through inhalation of cough droplets from infected animals during close physical contact with them. Zoonotic TB is also an occupational hazard for farmers, abattoir workers, butchers and veterinary professionals.

Epidemiological studies of TB in cattle and in humans in Zambia [5] and in Ethiopia [6] indicated associations between tuberculin-positive cattle and human TB. In Zambia, it was observed that households that reported a TB case within the last one year were seven times more likely to own herds containing tuberculin-positive cattle. Similarly, tuberculin positivity was three-fold higher in cattle owned by farmers with active TB than in those owned by farmers who did not have active TB in Ethiopia. These observations could suggest the transmission of mycobacteria between humans and cattle, which could be the transmission of *M. bovis* from cattle to humans or the transmission of *M. tuberculosis* from humans to cattle. A few studies in Ethiopia reported the isolation of *M. bovis* from sputum and fine needle aspirate of human TB cases [7-8]. According the literature review reported earlier [9], in countries where bTB is still common and pasteurization of milk is not practiced, an estimated 10–15% of human TB

is caused by *M. bovis* and the infections of *M. bovis* is thought to occur through drinking raw milk [10] and through inhalation in rural areas in people who live in close contact with cattle [11].

The few studies conducted in Ethiopia are limited in their geographic coverage and representations. Geographic regions such the East Shewa Zone of the central Ethiopia where dairy cattle and beef farming are widely practiced have not been considered. East Shewa Zone of central Ethiopia is prone to risk of transmission of mycobacteria between cattle and humans. There are many butchers in the area and cattle from the beef farms and nearby rural areas are slaughtered by butchers for selling raw meat and the area is known by the business of raw meat. In addition to beef cattle, dairy cows are kept by the farmers living in the towns and in the rural districts surrounding the towns. A significant percentage of raw milk are supplied to the consumers in living in the area. These common customs of raw milk and raw meat consumptions could facilitate the transmission of *M. bovis* from cattle to humans. On the other hand, Ethiopia stands 7th in the 20 human TB burden countries with the incidence rate of 165 individuals per 100,000 population [12]. East Shewa Zone is one of the densely populated areas of Ethiopia with high rate of human mobility since the main road to the Port of Djibouti passes through the Zone. Therefore, there are conducive environment for the transmission of human TB, and there could be high chance of transmission of *M. tuberculosis* from humans to cattle because of the close physical contact between humans and their cattle. It is paramount important to investigate if there is transmission TB between cattle and humans since there are conducive epidemiological situations. The present study was conducted to investigate the zoonotic and reverse zoonotic transmission TB between humans and cattle East Shewa, central Ethiopia.

Results

Prevalence of BTB in feedlots and dairy farms East Shewa

The average lesion prevalence was 4.2% (80/1896) in the two abattoirs. Lesion prevalence was 7.6% (48/630) in cattle slaughtered at Bishoftu abattoir while it was 2.5% (32/1266) in Adama abattoir (Table 1). However, only 26.3% of the 80 suspicious lesions were turned to be culture positive. The e distribution and frequency of lesions in different tissues of cattle was presented in Table 2. The highest proportion (52.5%) of the lesion was recorded in the lungs and thoracic lymph nodes followed by the lymph nodes of the head and neck.

Sputum culture positivity and demographic characteristics of the study subjects

Table 3 shows selected demographic characteristics of the human TB suspicious cases, who were used a sources sputum at the two towns. Sputum culture positivity was only 21.9% (86/392). Fifty culture positive samples were from the samples collected from Adama while 36 were from Bishoftu. Culture positivity was 18.2% (50/274) in samples collected from Adama Town while it was 30.5% (36/118) in samples collected from Bishoftu Town.

Molecular identification of species and strains of mycobacteria

Animal isolates

Of the 21 isolates, 12 (57.2%) isolates were confirmed to be *M. bovis* while the remaining nine (42.8%) were *M. tuberculosis*. The 12 *M. bovis* isolates were grouped into nine spoligotype patterns; three of which were clustered each consisting two isolates while the remaining six were singleton each consisting of one isolate (Table 4). Two of the nine *M. bovis* spoligotype patterns were shared types (SB1265 and SB1176) while the remaining seven types were orphans. Cattle isolates of *M. tuberculosis* were grouped into nine spoligotype patterns in which case one isolate constituted one spoligotype pattern. Four of the nine spoligotype patterns (SIT50, SIT117, SIT118 and SIT1318) were shared types while the remaining five were orphans (Table 4). Almost all (eight) of the nine spoligotypes of cattle isolates of *M. tuberculosis* were members of the Euro-American Lineage while the remaining one was a member of Indio-Oceanic Lineage.

Human isolates

A total of 86 human isolates were identified that were classified into 81 *M. tuberculosis*, three *M. africanum* and two *M. bovis* (Table 5). The two isolates of *M. bovis* had SB1443 pattern, that has already been registered in *M. bovis* database. The spoligotype pattern of the human *M. bovis* isolates did not match with any of the patterns of the cattle *M. bovis* isolates. As indicated in Table 5, 75% (61/81) of the *M. tuberculosis* isolates were grouped under the shared spoligotypes while the remaining 25% (20/81) were grouped under orphan spoligotypes. The dominantly identified spoligotypes were SIT149, SIT53 and SIT118 each consisting of 18, 11 and six isolates, respectively. SIT50, SIT118 and SIT1318 which were isolated from cattle were also isolated from humans. Totally, the 81 *M. tuberculosis* isolates were grouped into 32 different spoligotype patterns and the majority (84.4%) these patterns were members of the Euro-American Lineage followed by the East African-Indian Lineage (12.5%) and Indio-oceanic Lineage (3.1%).

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Discussion

In the present study, zoonotic transmission of *M. bovis* from cattle to humans and the reverse zoonotic transmission of *M. tuberculosis* from humans to cattle were investigated on 1896 cattle and 392 TB suspicious humans using mycobacterial culture and spoligotyping. TB suspicious lesions were sampled from cattle while sputum samples were the isolation mycobacteria and their identification using spoligotyping.

The result of the study indicated that the prevalence of bTB was low in cattle slaughtered on the basis of lesion. This observation was similar with the observations made by other studies in different abattoirs in the country prior to the present study [21-23]. This observation can be related to the fact that almost all (about 98%) the cattle that are slaughtered in the country are indigenous zebu breed which are relatively resistant to bTB [17,24-26]. Moreover, the majority of the zebu cattle that are slaughtered in Ethiopia are raised by the traditional farmers who raise their cattle under extensive cattle farming which does not favor the transmission of bTB [17]. Furthermore, the percentage of isolation of *M. tuberculosis* complex from lesions was low in this study similar to the results of other studies conducted earlier [18,21,27]. Such low percentage of isolation of *M. tuberculosis* complex in zebu cattle in Ethiopia could be associated with different factors. One of the factors could be the containment of the *M. tuberculosis* complex in the granulomas, which are usually calcified and could lead to the death of *M. tuberculosis* complex leading to the low yield of culturing of such lesions. As observed during the last two decades, most of the lesions detected in

zebu cattle were localized to one anatomical site and calcified [17,25-26]. The other factor could be human errors that may be introduced during the transportation, storage and processing of the lesioned tissue for culturing.

The culture of positivity the sputum samples collected from TB suspicious human cases was also low. Similar to this observation, earlier study in Ethiopia reported low percentage of isolation of *M. tuberculosis* from the sputum of TB patients [28]. The same study indicated that 83.2% (247/297) pulmonary TB suspected patients had a negative smear results for acid fast bacilli. The chance of isolation of *M. tuberculosis* complex from smear negative TB cases low. Culture positivity of sputum is directly related to the availability of the bacilli in the sputum, which can also be affected on the nature of the granuloma. If the granuloma is dense and solid, the chance of excretion of the bacilli in the sputum is minimal, which in turn leads the low yield of sputum culture. In addition, if sputum proper is not collected, and if the sputum storage and processing for culture are not performed using the optimum procedures, the yield of sputum culture is low.

In the present study, *M. bovis* was isolated from human TB patients while on the other hand, *M. tuberculosis* was isolated from TB lesions of cattle. Thus, this study indicated that TB is a good example of zoonosis and reverse zoonosis. Similarly, earlier studies reported the isolation of *M. bovis* from human TB patients in Ethiopia although the percentage of isolation was low [29-33]. Besides, *M. tuberculosis* was isolated from TB lesions of cattle in Ethiopia and in other countries [18,29,34-37]. Moreover, *M. tuberculosis* was also isolated from other species domestic animals and zoo animals [38-40]. Although such studies are in generally scarce in Africa, the isolation of *M. tuberculosis* was reported from animals in few African countries [41-42]. The possible sources of infection of animals with *M. tuberculosis* could be sputum, urine and feces of humans, which arise from active cases of human TB in the respiratory tract, urinary tract or gastrointestinal tract, respectively [43].

The present studies and earlier Ethiopian studies reported greater percentage of isolation of *M. tuberculosis* (reverse zoonosis) in cattle tissues as compared to the percentage of the isolation of *M. bovis* from humans. This observation could suggest that regarding B transmission in Ethiopia reverse zoonosis is more important than zoonosis, which is directly influenced by the prevalence TB in the primary host. The prevalence of bTB in the indigenous cattle, which constitute about 98% of the Ethiopian cattle population is low, and subsequently, there is a low chance of transmission of bTB from cattle to humans. On the other hand, Ethiopia is one of the 10 countries of the world with high burden of human TB [12], which gives a high chance of transmission of *M. tuberculosis* from humans to cattle because of the sociocultural link of Ethiopian farmers with their cattle. But, contrary to the Ethiopian reports, studies from other African countries reported the isolation of higher percentage of *M. bovis* from humans as compared to the percentage of isolation of *M. tuberculosis* from animals [8, 44-46].

Conclusion

Zoonotic transmission TB was observed by the isolation of two *M. bovis* from human subjects while the reverse zoonotic transmission was confirmed by isolation of nine *M. tuberculosis* isolates from cattle tissues. This observation could suggest that the prevalence of *M. tuberculosis* infection in cattle is higher than the prevalence of *M. bovis* infection in humans.

Methods

Study design

This study was a cross-sectional study in which the zoonotic transmission of *M. bovis* from cattle to humans and the reverse zoonotic transmission of *M. tuberculosis* from humans to cattle were investigated in the East Shewa Zone of central Ethiopia.

Study setting

The field sample collection part of the study was conducted from August 2015 to August 2016 at Adama and Bishoftu towns East Shewa Zone of Oromia Regional State, central Ethiopia (Figure 1). Bishoftu and Adama towns are located at 47km and 95km southeast of Addis Ababa, respectively. These two towns are known by beef production and selling of beef meat. Cattle from the beef farms and rural areas surrounding the two towns are slaughtered in the abattoirs located at these towns and raw meat consumption is very common in the area. In addition to beef cattle, dairy cows are kept by the farmers living in these towns

and in the rural districts surrounding the two towns. Thus, a significant percentage of raw milk are supplied to the consumers in living in these towns and the surrounding districts. Unfortunately, as BTB is prevalent in dairy farms in the country and these dairy farms are not expected to be free from the disease.

Sample size

The determination was based on the availability of fund and logistics to support the research activities of the project. Since the main objective of this research was to evaluate the transmission of *M. tuberculosis* between cattle and humans, it was tried to collect the maximum number of samples by examining large number of cattle and also collecting large number sputum samples. No statistical calculation was used to estimate the sample size in both human and cattle studies. Thus, the available fund and logistics could cover the examination of 1,896 cattle at the two (1266 at Adama and 630 at Bishoftu) abattoirs. In addition, 392 (274 from Adama and 118 from Bishoftu) sputum samples were collected from the two towns.

Participants of the study

The study subjects were comprised of cattle slaughtered at these two abattoirs and human TB patients who visited the health care centers at the two towns during the study period. Post mortem examination for BTB was conducted on cattle, which were originated from the beef farms in located either at the two towns or in the surrounding districts. Similarly, sputum samples of individuals who were living in these towns and or the surrounding districts plus who had connections with either dairy or beef cattle were used for bacteriological and molecular analysis.

Variables

The primary outcome was the isolation the same species of *M. tuberculosis* complex from both cattle and humans, which could suggest the transmission of TB between cattle and humans. Other secondary outcomes were percentage of cattle with TB lesion in their tissues and the percentage of suspicious TB lesion that were positive by culture and spoligotyping. With regard to the human study, the secondary outcome was the percentage of TB suspicious patients who were positive by culture and spoligotyping. The diagnostic criteria for cattle study were gross BTB lesion, and confirmation by culture and spoligotyping. While the diagnostic criteria for human study was clinical examination by the responsible health professionals in the health care centers, and then further confirmation of culturing of sputum and spoligotyping the isolates. The final confirmation of the transmission of *M. tuberculosis* complex species between cattle and humans was made by spoligotyping.

Sample collection

As the primary objective the study was to confirm the existence of the transmission of *M. tuberculosis* species between humans and animals, focus was made on the search of BTB lesions in tissues of cattle and then collection of suspicious lesions. Data on the body condition and origin of each study animal were collected. However, data on the age of the animal not collected. In addition, the tissues from which suspicious lesions detected were recorded. The BTB suspicious tissue samples were collected into universal bottles with normal saline and transported to the TB laboratory Aklilu Lemma Institute of Pathobiology of the Addis Ababa University in cold chain. In total, 1896 cattle were examined at the two abattoirs and 80 BTB suspicious lesions were collected for culturing. The number of the animal recruited was dependent on the availability of logistics and convenience of the slaughterhouses.

Sample collection in humans was conducted as part of the routine diagnostic procedures of TB and sputum samples submitted to the hospitals and health centers by 392 TB suspicious cases for diagnosis were shared. Thus, 274 sputum samples were collected from Adama Town while 118 sputum samples were collected from Bishoftu Town. The sputa samples were transported in cold chain to the TB laboratory of the Aklilu Lemma Institute of Pathobiology of the Addis Ababa University for culturing.

Post-mortem examination and tissue sampling

Body condition scoring was made using the method developed earlier based on the different prominent anatomical structures such as the visibility of the ribs, the gluteal muscles, tuber coxae, the head of the tail, transverse processes and others [13-14]

Postmortem examination was performed following the protocol set previously by another researcher [15]. Each of the seven lobes of the lungs were thoroughly inspected and palpated for any suspicious gross TB lesions. Similarly, mandibular, retropharyngeal, cranial and caudal mediastinal, left and right bronchial, hepatic, and mesenteric lymph nodes were sliced into 2mm size sections and then inspected for the presence of visible lesions according to the protocol described earlier [16-17]. The tissues showing macroscopic lesions compatible with bTB were collected into universal bottle in 0.9% saline solution and then transported to the laboratory in cold chain. A total of 80 suspicious lesions were collected from the total 1896 cattle investigated, and processed for culturing as described below.

Culturing of sputum and tissues samples

Regular visits were made to the hospital and health centers in the study area for collection of sputum samples that were submitted to the routine diagnosis of TB. In total, 392 sputum samples were collected and transported to the TB laboratory in cold chain for culturing. The samples were stored at -20°C freezer until being thawed and processed for culturing. Culturing of sputum samples was done on Lowenstein-Jensen (LJ) media following the procedure used earlier [6]. Similarly, suspicious tissue samples were processed for culturing on LJ media following the procedure used by other researchers [1]. The colonies were harvested and re-suspended in sterile distilled water and heat-killed at 80°C for 50 minutes so that mycobacterial DNA is released and used for subsequent spoligotyping.

Spoligotyping of mycobacterial isolates

Spoligotyping was performed on 107 *M. tuberculosis* complex isolates at the Aklilu Lemma Institute of Pathobiology, Addis Ababa University, following the standard operating procedure that was used by Berg *et al.* [18] and primarily developed by Kamerbeek *et al.* [19]. The DNA released by heat-killing of the colonies was used as a template to amplify the direct repeat (DR) region of *M. tuberculosis* complex by polymerase chain reaction (PCR) using oligonucleotide primers derived from the DR sequence, RDa (5'GGTTTTGGTTTGAACGAC3') and RD_b (5'CCGAGAGGGGACG GAAAC3') primers [19]. The total volume of the reaction the PCR reaction mixture was 25 µl and constituted of 12.5 µl of HotStarTaq Master Mix (Qiagen; this solution provides a final concentration of 1.5 mM MgCl₂ and 200 mM of each deoxoribonucleotide triphosphate), 2 µl of each primer (20 pmol each), 5 µl suspension of DNA template (approximately 10–50 ng), and 3.5 µl Qiagen water. The mixture was heated for 15 minutes at 96°C and then subjected to 30 cycles of 1 minute at 96°C, 1 minute at 55°C, and 30 seconds at 72°C, and the final extension at 72°C for 10 min. Immediately before running spoligotyping, the PCR product was denatured using thermocycler at 96°C for 10 minutes and then removed from the thermocycler and kept on ice so as to prevent renaturing of the PCR products. Thereafter, the denatured PCR product was loaded onto a membrane covalently bounded with a set of 43 oligonucleotides, each corresponding to one of the unique spacer DNA sequence within the DR locus of *M. tuberculosis* complex and then hybridized at 60°C for 1h. After hybridization, the membrane was washed twice for 10 minutes in 2x SSPE (1x SSPE is 0.18 M NaCl, 10 mM NaH₂PO₄, and 1 mM EDTA [pH 7.7])-0.5% sodium dodecyl sulfate at 60°C and then incubated in 1:4000 diluted streptavidinperoxidase (Boehringer) for 1 h at 42°C. The membrane was washed twice for 10 minutes in 2 x SSPE-0.5% sodium dodecyl sulfate at 42°C and rinsed with 2 x SSPE for 5 minutes at room temperature. Hybridizing DNA was detected by the enhanced chemiluminescence method (Amersham, Biosciences, Amersham, UK) and by exposure to X-ray film (Hyperfilm ECL, Amersham). A mixture of 10 ml of ECL reagent 1 and 10 ml of ECL reagent 2 was prepared, and then added onto the membrane, and the membrane was rinsed in the solution for 5min at room temperature. Then, the membrane was attached onto a film in the dark room and placed in the cassette and incubated for 15 minutes a room temperature. Thereafter the film was removed and placed in a developer solution for 2 minutes. The film was removed from the developer and rinsed with tap water for 15 seconds and then placed in a fixer solution for 1 minute. Thereafter, the film was dried and used for interpretation of the result. The presence of the spacer was identified as a black square while absence of the spacer was identified as a white square on the film. Thereafter, the black squares were converted to 1 while the white squares were converted to 0 and then transferred to the spoligotype international types-VNTR international types (SITVIT) database for the identification of the spoligotype international types (SIT) and the lineages of the isolates.

Data analysis

Proportion was used to show the number of cattle with TB lesions. Tissue lesion and sputum culture positivity were presented using proportions. Comparisons of proportions was made by using chi-square (χ^2) test. Statistical significance was considered at $P < 0.05$. In molecular aspect of the study, the identification of the SIT numbers and lineages of isolates was done using SITVIT1Database and Run TB-Lineage. The results of spoligotyping were converted into octal and binary formats, and then entered into query box so that the names of the strains are retrieved from the database if the spoligotype pattern of the strain in question fits the pattern that has already been registered in the SPolDB4 database and at <http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/> (SITVIT1Database) [20]. If the pattern of the strain in question has not been registered in the database prior to this study, the strain was considered as an orphan. The lineages were also generated by entering binary and octal formats into the query box of SITVIT1Database and Run TB-Lineage.

Abbreviations

BTB: Bovine tuberculosis; CAS: Central Asian Strain; EA: Euro-American; EAI: East-African-Indian; IO: Indio-Oceanic; LJ: Lowenstein-Jensen; SITVIT: Spoligotype International Types-VNTR International Types; APHA: Plant and Animal Health Agency; UAE: United Arab Emirates; VNTR: Variable Number of DNA Tandem Repeats

Declarations

Acknowledgements

The authors acknowledge the study participants, owners of the animals and the administrations of the abattoirs and health care provider centers (hospitals and health centers) for their willingness to facilitate the study.

Authors' contributions

TW contributed in the conception and designing of the research, performed field data collection, did the analysis and drafted the manuscript. GM contributed in the conception, designing and in reviewing the manuscript. AZ contributed in the field and laboratory work of the manuscript. MC contributed in the laboratory work of the manuscript. TM contributed in field and laboratory activities of the manuscript and the writing of the manuscript. GA contributed in the conception, designing of the research, supporting in analysis and its interpretation of the data and in reviewing the manuscript. All authors read and approved the final manuscript.

Funding

The study was financially supported by the Office of the Vice President for Research and Graduate Studies of the Addis Ababa University (AAU) under the Thematic research Project entitled as "Mycobacterial Infections in Animals and Their Livestock" with Account Number of 15000018800006223. The AAU was monitoring the execution of this research project through scientific and financial reports on annual basis. In addition, the final scientific and financial reports were submitted to the AAU.

Competing Interests

The authors declare that there are no competing interests

Availability of data and materials

All data generated and /or analyzed during the current study are not publicly available but they are kept at the Data Management Unit of the Aklilu Lemma Institute of Pathobiology, Addis Ababa University. They can be available by request through the corresponding author.

Ethical approval and consent to participate

The study was approved by the Institutional Review Board (IRB) of the Oromia Health Bureau (Reference No. IRB/BEFO/HBTFH/1-8/3647). Verbal consent was included in the proposal and was approved by the IRB. The IRB approved

verbal consent since the project was PhD student's research project and did not involve international collaboration and material transfer. Thus, informed consent was obtained verbally from the patients for using sputum samples for this research. Furthermore, permission was obtained from the owners of animals for post mortem examination and sampling

Consent for publication

Not applicable

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Tables

Table 1. Association of selected risk factors with the percentage of lesion BTB at Adama and Bishoftu abattoirs

Variable	Number of cattle examined	Number (%) positive	Pair-wise comparison	Chi-square (χ^2) test	P-value
Origin of animal					
Southeast to the study site (Arsi, Bale and Borena zones)	1018	16(1.6)	Southeast versus North east	45.9	<0.001
Northeast to the study site (Afar, Harar, Wollo zones)	593	51(8.6)	Southeast versus West	4.56	<0.05
West to the study site (Wellega zones)	285	13(4.6)	Northeast versus West	9.08	<0.01
Abattoir					
Adama abattoir	1266	32(2.5)		27.21	<0.001
Bishoftu export abattoir	630	48(7.6)			
Body condition					
Lean	460	33(7.2)	Lean versus Medium	1.54	>0.05
Medium	440	23(5.2)	Lean versus Good	19.25	<0.001
Good	996	24(2.4)	Medium versus Good	7.59	<0.01
Total	1896	80			

Table 2. Distribution and frequency of TB lesions in the lymph nodes and organs of cattle slaughtered at Adama and Bishoftu abattoirs, central Ethiopia

Organs	Postmortem findings						
	Number of organs with lesion	Percent of organs with lesion	Summary on the basis of anatomical sites	Percent of lesion	Pairwise comparison	χ^2 (95% confidence interval)	P value
Lungs	14	17.5%	Head/neck region	26.3%			
Bronchial lymph nodes (LN)	17	21.3%	Thoracic cavity	52.5%	Head/neck region and thoracic cavity	11.43 (11.08, 39.71)	<0.001
Mediastinal LN	11	13.7%	Abdominal cavity	13.7%	Head/neck region and abdominal cavity	3.88 (0.02, 24.61)	P=0.05
Retropharyngeal LN	17	21.3%	Pre-scapular LN	7.5%	Head/neck region and pre-scapular LN	10.00 (7.26, 30.11)	<0.01
Mandibular LN	4	5.0%			Thoracic cavity and abdominal cavity	26.86 (24.49, 50.81)	<0.001
Mesenteric LN	6	7.5%			Thoracic cavity and pre-scapular LN	38.33 (31.61, 56.31)	<0.001
Pre-scapular LN	6	7.5%			Abdominal cavity and pre-scapular LN	1.66 (-3.52, 16.26)	>0.05
Liver	5	6.2%					
Total	80	100%		100%			

Table 3. Selected demographic characteristics of the study participants and their association with culture positivity

	Number (%) positive	Total	Pairwise comparison	Chi-square test (95% confidence interval, CI)	P value
Adama Town					
Age					
Group 1: 10-29	12 (10.3%)	116	Group 1 and Group 2	6.48 (2.8, 20.8)	P<0.05
Group 2: 30-59	31 (22.3%)	139	Group 1 and Group 3	9.40 (7.5, 49.00)	p<0.01
Group 3: ≥60	7 (36.8%)	19	Group 2 and Group 3	1.9 (-4.8, 37.5)	p>0.05
Total	50	274			
Sex					
Female	25 (18.4%)	136		0.0 (-8.9, 9.5)	p>0.05
Male	25 (18.1%)	138			
Total	50	274			
Residence					
Rural	34 (18.5%)	184		0.32 (-6.9, 14.8)	P>0.05
Urban	16 (21.6%)	90			
Total	50	274			
Bishoftu Town					
Age					
Group 1: 10-29	15 (31.9%)	48	Group 1 and Group 2	0.68 (-9.7, 24.3)	p>0.05
Group 2: 30-59	14 (24.6%)	58	Group 1 and Group 3	1.5 (-9.1, 44.0)	p>0.05
Group 3: ≥60	7 (50%)	14	Group 2 and Group 3	3.38 (-1.2, 50.5)	p>0.05
Total	36	120			
Sex					
Female	20	63		0.17 (-13.0, 19.5)	p>0.05
Male	16	57			
Total	36	120			
Residence					
Rural	10	46		2.34 (-3.8, 28.4)	p>0.05
Urban	26	74			
Total	36	120			

Table 4. Spoligotype patterns of *M. bacterium tuberculosis* complex species isolated from cattle in East Shewa, central Ethiopia

SIT/SB	Isolates with similar pattern	Lineage	SIT/VIT2 Lineage/	Octal number	Binary
SB1176	1	M. bovis	BOV_1	602773761000200	ggccccgcggggggcggggggcccgcccccccccgcccc
SB1256	2	M. bovis	BOV_1	616763777777600	ggcccgggcgggggccggggggggggggggggggggggcccc
50	1	Euro-American	H3	77777777720771	ggggggggggggggggggggggggggggggcgccccggggggg
118	1	Euro-American	T2	77776777760771	ggggggggggggggcgggggggggggggggggccccggggggg
117	1	Euro-American	T1	77776777760731	ggggggggggggggcgggggggggggggggggccccgggcggg
1318	1	Euro-American	T1	57776777760771	gcggggggggggggcgggggggggggggggggccccggggggg
Orphan	1	M. bovis	BOV	416743777756600	gccccgggcggggcccggggggggggggggggcgggcgggcccc
Orphan	1	M. bovis	BOV_1	612763761000200	ggcccgcgcgggggcggggggcccgcccccccccgcccc
Orphan	1	M. bovis	BOV_1	612773761000200	ggcccgcgcgggggcggggggcccgcccccccccgcccc
Orphan	1	M. bovis	BOV_1	400201434200000	gccccccccgcccccgcccggcccgcccccccccccccc
Orphan	1	M. bovis	BOV_1	402773761000200	gcccccgcgggggcggggggcccgcccccccccgcccc
Orphan	2	M. bovis	BOV_1	416763577756600	gccccgggcgggggccgggcggggggggggcgggcgggcccc
Orphan	2	M. bovis	BOV_1	612773761000200	ggcccgcgcgggggcggggggcccgcccccccccgcccc
Orphan	1	Euro-American	H3	57776777700771	gcggggggggggggcgggggggggggggggggcccccgggggg

Orphan	1	Indo-Oceanic	MANU1	77776777777771	ggggggggggggggcggggggggggggggggggggggggggg
Orphan	1	Euro-American	T	775767577760771	gggggggcgggggcggggcggggggggggggcccccggggggg
Orphan	1	Euro-American	T	777767777760661	ggggggggggggggcgggggggggggggggggcccccggcggcg
Orphan	1	Euro-American	X2	417763575600200	gccccggggggggggccgggcgggggcgggccccccccgcccc

Table 5. Spoligotype patterns of *M. tuberculosis* complex species isolated from humans in East Shewa, central Ethiopia.

STT	Isolates with similar pattern	Lineage	SITVIT2 sublineage	Octal number	Binary
149	18	EA	T3 ETH	777000377760771	ggggggggc-ccccccggggggggggggggc-ccgggggg
777	3	EA	H4	777777777420771	ggggggggggggggggggggggggggggc-ccggccgggggg
336	2	EA	X1	777776777760731	ggggggggggggggggc-gggggggggggggc-ccgggc-ggg
	11	EA	T1		ggggggggggggggggggggggggggggggc-ccgggggg
53	2	EA		777777777760771	
50			H3	777777777720771	ggggggggggggggggggggggggggggc-gcccgggggg
134	3	EA	H3	777777777720631	ggggggggggggggggggggggggggggc-gcccggc-ggg
1745	1	EA	T3	773737777760771	gggggc-ggggc-gggggggggggggggggc-ccgggggg
245	1	EA	T1	777777777760671	ggggggggggggggggggggggggggggc-ccggc-ggg
37	4	EA	T3	777737777760771	ggggggggggc-gggggggggggggggggc-ccgggggg
221	1	EA	X1	777766777760771	ggggggggggggc-ggc-gggggggggggggc-ccgggggg
1221	1	EA	T1	757767777760771	gggc-gggggggc-gggggggggggggggc-ccgggggg
1547	4	EA	T3	777727777760771	ggggggggggc-gc-gggggggggggggggc-ccgggggg
51	1	EA	T	777777777760700	ggggggggggggggggggggggggggggggc-ccggccccc
75	1	EA	H3	777767777720771	ggggggggggggc-ggggggggggggggc-gcccgggggg
7	1	EA	T1	377777777760771	cggggggggggggggggggggggggggggc-ccgggggg
118	6	EA	T2	777767777760771	ggggggggggggc-gggggggggggggggggc-ccgggggg
1318	3	EA	T1	577767777760771	gcggggggggggc-gggggggggggggggc-ccgggggg
117	3	EA	T1	777767777760731	ggggggggggc-gggggggggggggggc-ccgggc-ggg
1687	1	EA	T1	777767775760771	ggggggggggc-gggggggggc-gggggc-ccgggggg
1802	1	EA	H3	777767777720631	ggggggggggc-ggggggggggggggc-gcccggc-ggg
611	1	EA	T1	77776777560771	ggggggggggc-ggggggggggggc-gggc-ccgggggg
834	1	EA	U	777767777740771	ggggggggggc-gggggggggggggc-cccggggggg
	1				ggggggggggc-gggggggggc-gggc-gcccgggggg
116		EA	H3	777767775720771	
Orphan	1	EA	H3	477767777720571	gc-gggggggggc-ggggggggggggggc-gcccgc-ggg
Orphan	1	EA	X2	777737777760000	ggggggggggc-gggggggggggggggggc-cccccccc
Orphan	1	EA	T	755777777760671	gggc-ggc-gggggggggggggggggggc-ccggc-ggg
Orphan	1	EA	X2	777727777760000	ggggggggggc-gc-gggggggggggggggc-cccccccc
309	1	EAI	CAS1	703767740003171	gggcccggggggc-gggggggc-ccccccccggc-gggg
25	1	EAI	CAS1	703777740003171	gggcccggggggggggggc-ccccccccggc-gggg
	2	EAI	MTB CAS		gggcccggggggc-gggggggc-ccccccccgggggg
141				703767740003771	gggcccggggggc-gggggggc-ccccccccgggggg
Orphan	1	EAI	CAS	703777400002061	gggcccggggggggggc-ccccccccggc-ccggc
Orphan	2	<i>M-africanum</i>	Manu2	575347777763661	gcggggc-gc-gggc-gggggggggggggggc-gggc-ggc
Orphan	1	<i>M-africanum</i>	X2	554377777760660	gcggc-ggc-ccggggggggggggggggc-ccggc-gggc
Orphan	1	IO	CAS	713600000001171	gggcgcgggcccccccccccccccccccgcg-gggg
1443	2	<i>M. bovis</i>	BOV	616777777777600	ggcccggc-gggggggggggggggggggggggc-cccc

Figures

Figure 1

Location of the study area on the map of Ethiopia.