An Abattoir Based Study of Bovine Tuberculosis in Adama and Bishoftu Abattoirs, Central Ethiopia

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Abstract

A cross-sectional study was conducted from November 2014 to August 2016 at Adama Municipal Abattoir and Bishoftu ELFORA Export Abattoir, central Ethiopia. The study aimed at estimating the prevalence and distribution of lesions of bovine tuberculosis (BTB) in organs on the basis of TB like gross lesion. Postmortem examinations were conducted on1896 cattle slaughtered at Adama (n=1266) and Bishoftu (n=630) abattoirs. The body condition scores, origins and ages of the animals were recorded during ante mortem examinations. The prevalence of BTB lesions was 4.2% (80/1896). Out of the 80 TB like lesions cultured, 26.3% of them were found to be culture positive *Mycobacterium* species. Higher proportion (52.5%) of TB like lesion was recorded in the respiratory pathway followed by lymph nodes of the head region (26.25%), mesenteric (7.5%), prescapular (7.5%) and hepatic lymph nodes (6.25%). Prevalence was higher in animals slaughtered at Bishoftu ELFORA export abattoir compared to Adama municipal abattoir. Animals coming to both abattoirs were from different origins and varied with their body condition score. ELFORA export abattoir slaughtered large proportion of lean animals not for export purposes but for local supermarkets while Adama municipal abattoir slaughtered fattened animals, which might account for difference in BTB prevalence. In conclusion, the lesion prevalence was low in cattle slaughtered at both abattoirs. Given the zoonotic importance BTB, this finding suggests the need for monitoring the prevalence and launching a feasible and practical control strategy of BTB.

Key words: Abattoir; Bovine Tuberculosis; Ethiopia, Prevalence; TB lesions.

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INTRODUCTION

Bovine Tuberculosis (BTB) is a chronic bacterial disease characterized by progressive development of tubercles in any tissue/organ of the body (Hlokwe et al., 2013; Pal et al., 2014). It has been reported from 176 countries as one of the important bovine diseases causing great economic loss (Awah-Ndukum et al., 2013). TB remains a major global health problem and causes ill health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide after the human immunodeficiency virus (HIV). TB can be difficult to diagnose based only on the clinical signs. Regular surveillance by skin test, bacteriology and molecular methods is not feasible due to lack of resources. Thus, conventional abattoir inspections continue to play a key role for national surveillance of BTB.

Ethiopia's increasing human population, coupled with expanding urbanization and higher average income is putting an increasing pressure on the meat supply. To meet this demand, millions of food animals slaughtered every year throughout the country. In 2007, for example, a total of 18.8 million cattle, sheep, goats and camels slaughtered at municipal abattoirs, primarily for domestic consumption (FAO, 2009). For this reason, close monitoring of meat hygiene, including proper implementation of meat inspection procedures during slaughter, should be a vital part of the national public health protection program.

BTB is characterized by the formation of nodules called tubercles whose location depends largely on the route of infection. In calves, BTB usually transmitted by ingestion and lesions involve the mesenteric lymph nodes with possible spread to other organs. In older cattle, infection usually transmitted by the respiratory tract with lesions in the lung and dependent lymph nodes (Carter and Wise, 2004). Recently, there has been increasing reports of human cases due to *M. bovis* especially in patients with HIV (Russell, 2003). Thus, a greater degree of transmission of infection with bacteria between

human and domestic animals could occur (Taracha et al., 2003).

In industrialized countries, animal TB is controlled and eliminated with milk test and slaughter, which has in turn drastically reduced the incidence of the disease caused by *M. bovis* in both cattle and human. In developing countries however, animals' TB is widely distributed, as control measures are not applied or applied sub optimally. In Ethiopia, where extensive production system predominates animals are kept in the same house with their owners; cow dung is used for painting of the wall and floor of houses as well as sources of energy for cooking. All these practices do exacerbate the chance of spread of TB to human (Asseged, 1999). The nationwide distribution of the disease and associated economic loss has not been fully determined due to lack of good diagnostic facilities (Asseged et al., 2004).

The primary reason for post mortem examination of carcasses in slaughterhouses is for the protection of public health and containment of disease spread among livestock. The knowledge of TB in cattle slaughtered provides useful information and is a proxy indicator for the epidemiology of the disease in the cattle population from which the slaughtered cattle are originated. Furthermore, it could serve as a good indicator of risk to humans through consumption of infected meat. Apart from providing data for regulatory programs, carcass examination also provides clues as to whether the infection is in its early stage or has reached the transmissible stage. This provides better programmatic awareness with subsequent development of targeted guidance on how to reduce the risk of TB spread within the specific geographic area, as well as opportunities to trace back the source of infection to the herds. Hence, having the knowledge of distribution, prevalence and risk factors of the disease are fundamental to look for effective control strategy. Therefore, the objectives of this study were to estimate the prevalence of bovine tuberculosis at Adama municipal abattoir and Bishoftu ELFORA export abattoir, central Ethiopia and to assess the distribution of tuberculous lesions in organs of slaughtered animals.

MATERIALS AND METHODS

Study Area

A cross sectional study was conducted from November 2014 up to August 2016 in Adama and Bishoftu towns of East Shewa Zone of Oromia Regional State, Central Ethiopia (Fig. 1). East Shewa, human population is 1,919,994 and cattle 1,031,652 (CSA, 2013). Adama and Bishoftu towns are the major towns in East Shewa zone. Adama town is located at 8.54^0N 39.27^0E, 99 km southeast of Addis Ababa along the road that connects Addis Ababa to Dire Dawa - Djibouti. Adama is among the largest metropolitan city in Ethiopia with estimated 450,000 human population and many resorts that make it suitable for conference and tourism. Adama abattoir slaughtering has the capacity of about 150 cattle and 500 sheep and goats per day. However, to fulfill the standard abattoir level it lacks many facilities including drainage system, sterilizers of the equipment, adequate light, workers' clothes. The inspection system is not detailed and only performs routine inspections.

Bishoftu is one of the major towns in east Shewa zone Oromia regional state with human population of 104 215 (World City Population, 2020). This town is located about 45 km southeast of Addis Ababa. Bishoftu is an important town where most governmental institutions including national air force, national and international research centers and others Universities and colleges are located. It has three export abattoirs and one municipal abattoir. In addition, it has many private intensive and extensive dairy, poultry and swine farms. This highly populated town is main supplier of animals, poultry and swine meat and its products such as milk, egg and others to Addis Ababa supermarkets. Bishoftu export abattoir has slaughtering capacity of 200 cattle 1500 sheep and goats. Cattle slaughters in Bishoftu abattoir were mostly emaciated cattle (mainly for local consumption) compared to Adama municipal abattoir that slaughter fattens animals. It is export abattoir and entails all conditions that ideal export abattoir requires. Bishoftu has a relative humidity varying between 70% and 80% during the rainy season and 40% to 50% during the dry season.

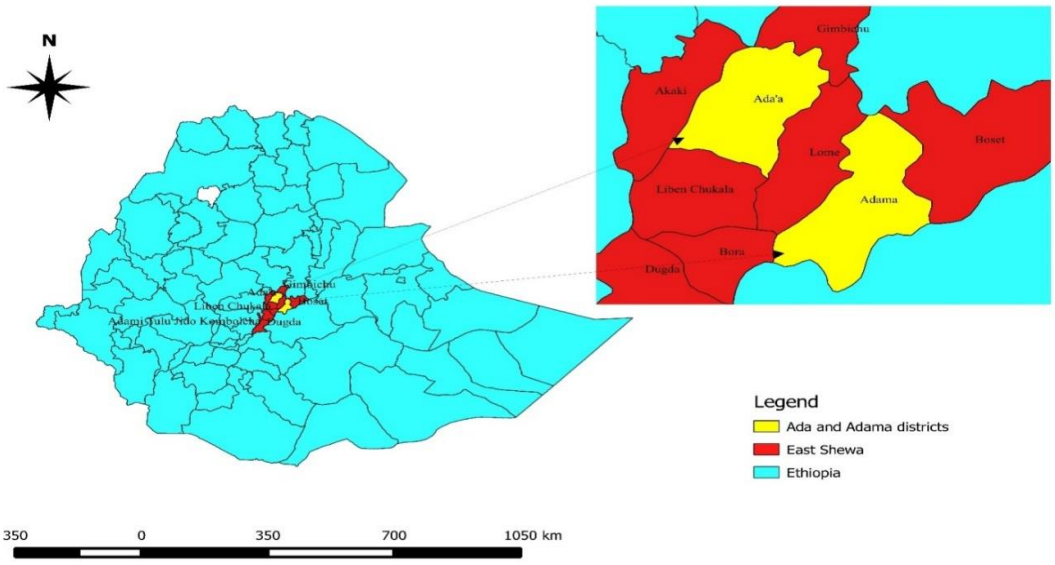

Figure 1: Map of Ethiopia showing Location of study area

Study Subjects

The study subjects comprised of abattoir slaughtered cattle at Adama and Bishoftu abattoirs East Shewa Zone, Central Ethiopia. Animals presented to these Abattoirs were from the surrounding Adama and other different sites. These animals were transported to abattoir by vehicles.

Sampling and sample size determination method

The sample size was calculated according to Thrusfield (2005) by taking 6.79% of expected prevalence reported by Terefe (2014), and 5% accepted error at 95% confidence interval. The general formula is:

$$\text{Required sample Size} = \frac{Z_{1-\propto/2}{}^2 \text{p}(1-\text{p})}{\text{d}^2}$$

Where

$Z_{1-\alpha/2}$ is standard normal variant (1.96 for 5% accepted error).
P = expected prevalence
d = desired absolute precision

Accordingly, the estimated sample size was 80 cattle for each month which is 960 cattle each year for two years. A total of 1,896 cattle were sampled during the study period (from November 2014 up to August 2016).

Post-mortem examination

Cattle and cattle related variables such as age, breed, sex and body condition were recorded during ante-mortem inspection. Postmortem examination was carried out as described previously by Vordermeier et al. (2002). Each of the seven lobes of the lungs were thoroughly inspected and palpated for suspicious gross TB-like lesions. Similarly, mandible, retropharyngeal, cranial and caudal mediastinal, left and right bronchial, hepatic, and mesenteric lymph nodes were sliced into 2mm size sections and then be inspected for the presence of visible lesions according to the protocol described earlier by Vordermeier et al. (2002).

Body condition scoring (BCS) and age determination

The body condition scores, origins and ages of the animals were recorded before slaughtering during ante mortem examinations. Body condition scoring was made using a method developed for Zebu cattle (Nicholson and Butterworth, 1986), accordingly, based on observation of anatomical parts such as vertebral column, ribs, and spines. The study animals were classified as lean (1), medium (2 and 3) or good (4 and greater). Animals coming to the abattoir were apparently healthy and leanness considered as thinness. Age of the study animals was determined by using the dental eruption and wear as described by De-Lahunta and Habel (1986) and for the present

study, animals were categorized as young age ≤2 and adult age > 2.

Collection of suspected tuberculous lesions in slaughtered cattle

The tissues showing macroscopic lesions suggestive of BTB were collected from slaughtered cattle carcasses during the postmortem inspection at Adama Municipal and Bishoftu ELFORA Export abattoirs. From 1896 inspected carcasses, from which BTB suspected lesions were collected in sterile plastic bags and transported in cold chain to Akililu Lemma Institute of Pathobiology (ALIPB) for culturing. At the TB lab of the Institute, the samples were kept at –20°C until processed for culturing.

Mycobacterium culture of suspected tuberculous lesions

The suspected TB lesions were incubated using pyruvate and glycerol enriched LJ slants following standard operation procedures (RNTCP, 2009). All the bovine specimens were processed in biosafety level 2 cabinet. Ziehl-Neelsen staining and microscopic demonstrations of acid-fast bacilli were used to confirm successful inoculation and growth (WHO, 1998).

Briefly, suspected tuberculous cattle specimens were cut into tiny pieces and then homogenized separately in 0.85% saline in sterile blenders to obtain fine pieces. Frozen samples were allowed to thaw to room temperature before processing. The cattle tissue homogenates were decontaminated with equal volumes of sterile 4% NaOH; mixed well by shaking for a few seconds and allowed to stand for 10 minutes at room temperature before neutralization with 1 mol/L HCl using phenol red as the indicator. Neutralization was achieved when the suspension changed to a yellowish colour; which was centrifuged at 3,000 rpm for 15 min. The supernatant was discarded leaving about 2ml and spread generously (~ 0.3 ml) on the LJ slants as follows: 2 LJ medium enriched with glycerol and 2 LJ medium enriched with Pyruvate. Incubation at 37°C for up to 12 weeks with weekly observation for growth of colonies was done. On observation of visible growth, a few colonies were gently mixed into one drop of sterile saline and smeared on a clean, grease-free microscopic slide, heat-fixed using the Bunsen burner flame without burning and stained by the ZN method to confirm the presence of acid-fast bacilli. The smeared slide was flooded with ZN carbon fusion, gently steamed without boiling with the Bunsen burner flame from the underside for 5 min. It was then rinsed gently until all free stain was washed away. The slide was flooded with 3% acid-alcohol decolorizing solution for 2 – 3 minutes until the red color disappeared, then rinsed again with water and the excess water drained. The slide was then flooded with Methylene blue counter stained for 1 minute, rinsed thoroughly with water, and excess water drained from the slide and the smear allowed to air dry without blotting. The smear was examined under a microscope (100 x oil immersion objective) for the presence of acid-fast bacilli. The presence of bacilli in 100 immersion fields was recorded as positive. Smears in which no acid-fast bacilli were seen in 100 fields were considered negative.

Growth of colonies on LJ media

Cultures with growth/colonies on LJ medium were checked for being AFB using ZN staining. Isolates which are AFB positive were harvested and kept into two separate nunc tubes (one contained 1 ml freezing media and the other 0.3 ml dH_2O). Isolates in freezing media were kept at -20^oC. Isolates in dH_2O were heated in a water bath at 80^oC for 50 minutes to release the mycobacterium genomic DNA material. Released mycobacterium genomic DNA material were kept at -20^oC until used for molecular characterization (Lowenstein, E. 1933).

RESULTS

The prevalence of BTB was 4.22% (80/1896) on the basis of gross TB lesions. There was statistically significant difference ($p<0.001$) between origin of animals (Table 1), as well as between abattoirs and body condition score ($p<0.001$) of the animals. Bishoftu abattoir slaughtered significantly more number of cattle with TB lesions because the animals that they slaughter were emaciated due to TB & they slaughter these animals for supermarkets or retailers and Adama municipal abattoir has lower TB cases because the animals they slaughter were fatten animals for butchers' house (Table 2).

Table 1. Association of BTB lesion prevalence with origins of animals in abattoir slaughtered cattle carcasses

Origin of animal	No of cattle inspected	No of carcasses positive (%)	χ^2	P-value
Southeast	1018	16(1.6)	45.9	0.000
Northeast	878	64(5.6)		
Total	1896	80(4.2)		

Table 2. Association of BTB lesion prevalence with BCS of animals andthe two abattoir

Variable	No of cattle inspected	No of lesion positive carcasses (%)	χ^2	P-value
Abattoirs				
Adama municipal	1266	32(2.5)	26.9	0.000
Bishoftu export	630	48(7.6)		
BCS				
Lean	460	33(7.2)	19.4	0.000
Medium	440	23(5.2)		
Good	996	24(2.4)		
Total	1896	80 (4.2)		

Distribution of tuberculosis lesions

The distribution of TB lesions in different tissues of cattle was presented in Tables 3. Identifying each study animal organs is marked with identifying number, so during collection of the organs there was no problem to identify which organ from which animal. About 78.75% of the lesions were observed in the lung and associated lymph nodes. The lung region contributes a higher percentage of tubercle lesions than the head and the gastrointestinal area.

Table 3. Percent of distribution of tuberculosis lesion in organs and lymph nodes

	Postmortem	
Organs	**Number**	**(%)**
Lung tissue	14	17.5
Bronchial LN	17	21.25
Mediastinal LN	11	13.75
Retropharyngeal LN	17	21.25
Mandibular LN	4	5.0
Mesenteric LN	6	7.5
Prescapular LN	6	7.5
Liver tissue	5	6.25
Overall	80	100

Tuberculosis lesions were present in different tissues of cattle (Table 3). About 52.5% of the lesions were observed in the lung and associated lymph nodes. The lung region contributes a higher percentage of tubercle lesions than the head and the gastrointestinal area (Table 4). Figure 2 and 3 also show BTB lesions in thoracic cavity and the lung tissue.

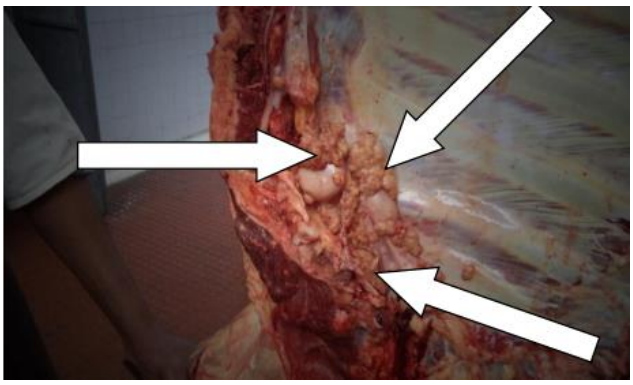

Figure 2. Tuberculosis lesions in the thoracic cavity of adult cattle at ELFORA abattoir, in Central Ethiopia.

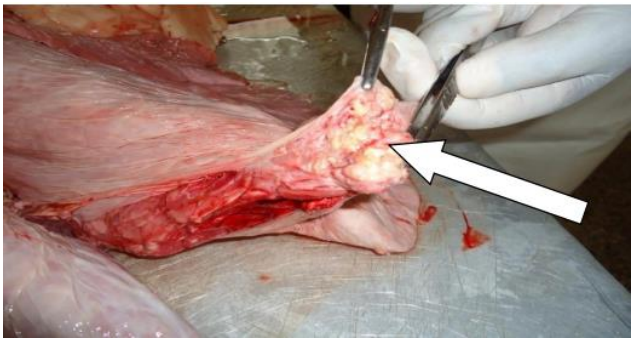

Figure 3. Tuberculosis lesions in lung tissues of adult cattle at ELFORA abattoir, in Central Ethiopia.

Table 4. Pooled TB lesions distributions among lymph nodes by regions.

Anatomic sites	**Lesions**	**Relative proportion***
Lymph nodes around head	21	26.25
Lung and associated lymph nodes	42	52.5
Mesenteric lymph nodes	6	7.5
Prescapular LN	6	7.5
Liver and hepatic lymph nodes	5	6.25
Total	80	100

Isolation of mycobacterium from tissue

The suspected TB lesions were collected from lung tissues and lymph nodes of different regions and a total of 80 samples were cultured using pyruvate and glycerol enriched LJ slants following standard procedures under biosafety level 2 cabinet. Then Ziehl-Neelsen staining and microscopic demonstrations of acid-fast bacilli were used to confirm successful inoculation and growth. The presence of at least three acid-fast bacilli in 100 immersion fields was recorded as positive but fields without bacilli were considered negative.

Accordingly, the growth of MTC species were observed in 26.3% (21/80) of tissue samples of BTB-like lesions on primary culture.

DISCUSSION

The abattoir survey revealed that 4.22% of inspected cattle were harboring TB suspected lesion with 26.25% (21/80) AFB positive culture yield. Abattoir prevalence in present study is comparable to a previous report of 4.2% in Yabello municipal abattoir (Demelash et al., 2009), 4.5% in Hosanna in cattle (Teklu et al., 2004). Culture positivity in present study is comparable with the results reported (Biffa et al. 2010) with 25.9% culture positivity from Addis Ababa, Adama, Hawassa. Statistically a significant difference was observed in the prevalence of the lesion ($p<0.05$) in terms of body condition scores (BCS), where the prevalence was found to be higher in animals with lean or poor BCS (7.2%) compared to medium (5.2%) and good (2.4%) body conditioned animals. The present result is consistent with the previous reports, which indicated that animals with good BCS have relatively strong immunological response to the infectious agent than animals with poor or medium BCS. It also indicates the wasting nature of the disease and as a result affected animals are chronically in poor body condition (Radostits et al., 2007).

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CONCLUSIONS

In conclusion, BTB the abattoir survey result of the present study has shown that bovine tuberculosis was prevalent in cattle slaughtered at Adama municipal abattoir and Bishoftu ELFORA export abattoirs with moderately low prevalence (4.2%). However, higher proportion of BTB lesions recorded in the lung and associated lymph nodes implies that the respiratory route might be the principal route of TB infection and attention should be given on detail inspection of the lungs and associated lymph nodes during post-mortem examination.

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