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# Bovine Tuberculosis Prevalence in Slaughtered Cattle at Gondar Elfora Abattoir, Based on Meat Inspection and Microscopic Methods

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**Abstract:** Tuberculosis is a well-known infectious disease in human beings and domestic animals caused by Mycobacterium. It affects millions of people worldwide. Bovine tuberculosis (BTB) is often neglected in developing regions of the world in which it normally infects animals. We sought to describe the prevalence of bovine tuberculosis at Gondar Elfora abattoir, North Ethiopia to provide background data for appropriate control measures to reduce the spread of BTB in the region. A cross-sectional study on bovine tuberculosis (BTB) was conducted at Gondar Elfora abattoir from April to July 2013 on the basis of post mortem inspection and Ziehl-Neelsen stained samples for microbial identification. A total of 369 cattle were inspected following slaughtering. Of the total 369 animals, 43 (11.7%) had symptomatic lesions. Acid fast staining was performed for all 43 tissue specimen and only 2.7 % (10/369) of the specimens were microscopically confirmed smear positive for tuberculosis. There were high, gross tuberculosis lesions in the lung and related lymph nodes of the head. During abattoir meat inspection more attention needs to be given to the lungs and associated lymph nodes. Detailed meat inspection has limitations in exactly confirming BTB-suggestive lesions. Consequently, integrated methods for detecting tuberculosis lesions for economic and public health concerns are necessary. To safe guard people who are used to consume raw meat, appropriate control measures should be taken for the control of the spread of BTB in the Gondar area.

**Key words:** Bovine Tuberculosis・Meat Inspection・Ziehl-Neelsen Microscopy・Lesions・Gondar

## INTRODUCTION

Tuberculosis is a well-known infectious disease in human beings and domestic animals caused by Mycobacterium species [1]. It is the cause of significant morbidity and mortality affecting millions of people worldwide [2, 3]. Despite highly successful eradication efforts in several countries, *Mycobacterium bovis* infection of cattle remains a significant health concern worldwide [4]. It is characterized by the formation of nodules called tubercles. In calves, lesions involve the mesenteric lymph nodes with possible spread to other organs. In older cattle, infection usually spreads in the respiratory tract with lesions in the lung and nearby lymph nodes [5].

BTB in cattle remains to be a great concern due to the susceptibility of humans to the disease. Large numbers of livestock, for which Ethiopia is known, are kept in human dwellings and maintained under very poor management [6] and hygienic conditions [7]. Consequently, Zoonotic TB (Caused by *Mycobacterium bovis*) is common in animals in Ethiopia. The endemic nature of tuberculosis in humans and cattle has long been documented in Ethiopia [7]. Yet, surveillance and control activities are often inadequate or unavailable and many epidemiologic and public health aspects of infection remain largely unknown. The disease is still widely distributed and often termed a neglected disease; its public health consequences have scarcely been investigated and are still largely ignored in Ethiopia [8]. Until recent years there was little concern

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with cross infection of Mycobacterium bovis between cattle and man. Currently, however, difficulties in controlling the disease adequately in domestic animals have increased the probability of infection of humans with bovine tuberculosis [9]. The Ethiopian habits of eating raw meat, drinking raw milk and high incidence of HIV infection have significantly escalated the chance of contracting bovine TB.

Recent studies indicate the prevalence rate of Bovine TB of 3.5% to 5.2% in slaughterhouses in various places of Ethiopia [7]. Because of the animal and public health consequences of *M. bovis,* disease surveillance programs should be considered a priority, especially in areas where risk factors are elevated [8]. Research is needed to determine when *M. bovis* is of zoonotic importance and what the underlying mechanisms of transmission are. Locally operative risk factors for zoonotic TB should be identified to determine people at risk and to develop appropriate control measures [8]. The objectives of this study were to determine the prevalence of bovine tuberculosis at Gondar Elfora abattoir, north Ethiopia and to evaluate the efficiency of abattoir inspection for the diagnosis of *M. bosvis* infection as well as to assess the distribution of tuberculosis lesion in organs of slaughtered animals.

## MATERIALS AND METHODS

**Description of the Study Area:** The study site (Gondar) is located at 727 Km North of the capital Addis Ababa, Ethiopia. The farmers in the vicinity of the town practice mixed farming activity where cattle, sheep and goat are reared in combination with traditional crop cultivation farming system. Usually, animals are kept within the dwellings of humans or at nearby outdoor dwellings.

**Study Design:** A cross-sectional study was conducted to determine the prevalence of bovine tuberculosis (BTB). The efficiency of detailed abattoir meat inspection to diagnosed TB lesions was evaluated using samples stained with the Ziehl-Neelsen approach and then examined by microscopy. All animals slaughtered during the period (March 2012 to July 2012) were inspected for bovine tuberculosis lesions to determine the disease prevalence in slaughtered cattle using detailed meat inspection as described in [10, 11] and Ziehl-Neelsen microscopy.

**Study Animals:** Animals used for the study were those which were ready to be slaughtered at Gondar ELFORA abattoir, came from surrounding areas, were male and all of them were local breeds (*Bos indicus)*. Most of the cattle were kept under fattening conditions for a short period of time, so their body condition was medium or good. The cattle were examined physically before slaughtering. Body condition score (BCS) were recorded during ante-mortem examination and the age of the study animals were also determined [12]. The age was categorized as young less than 2 years old, young adult between two to six years old and adult greater than six years old [13]. All animals slaughtered during the study period were greater than two years old so they were categorized as young adult and adult.

**Sample Size Determination:** The sample size calculation [14] was used to determine sample size.

$$n = \frac{Z^2 P(1-P)}{d^2}$$

where,
$n$ = sample size,
$Z$ = Z statistic for a level of confidence, for 95 % Z= 1.96,
$P$ = expected prevalence or proportion (4%, $P$ = 0.04, Shitaye *et al*. [7]. $d$ = precision ($d$ =0.02). Bovine Tb in slaughter house Prevalence in Ethiopia was 3.5% to 5.2% on average 4% which lies below 10% hence, (d=0.02) as recommended by Naing *et al*. [15]. Therefore, sample size = $\frac{(1.96)^2\ 0.04\ (1\text{-}0.04)}{(0.02)^2} = 369$

**Sample Collection and Preparation:** Specimens were taken from symptomatic meat lesion on liver, lymphadenitis and lung after inspection by one doctor of veterinary medicine (DVM) supervisor and one animal health expert. The collection was performed in sterile Ependorff tubes using a surgical knife. Then, Ziehl-Neelsen staining was directly performed from tuberculosis suspected lesions. The stained slides were observed by microscopy for the presence of acid fast bacteria, which appears as red bacillary cells occurring singly or in clumps [16].

**Data Analysis Procedure:** Data analysis was accomplished using SPSS version 16.0 software packages. á< 0.05 was considered significant during the analysis.

## RESULTS

**Distribution of Tuberculous Lesions:** The distribution of tuberculous lesions in different tissues of cattle was examined. Eight organs and/or lymph nodes were found

Table 1: Distribution of TB suggestive lesions in organs of cattle slaughtered at Gondar Elfora abattoir (Postmortem)

| | Observed Meat inspection | | Ziehl-Neelsen staining microscopy | |
|---|---|---|---|---|
| Organ | N | % | Positive | Negative |
| Mandibular ( right and left) MD | 2 | 4.7 | 0 | 2 |
| Medial retropharyngeal MR | 7 | 16.3 | 2 | 5 |
| Mediastinal cranial MDF | 2 | 4.7 | 0 | 2 |
| Mediastinal lymph node MDR | 3 | 7 | 0 | 3 |
| Bronchial lymph node right BR | 5 | 11.6 | 2 | 3 |
| Bronchial lymph node left BL | 6 | 14 | 1 | 5 |
| Liver and hepatic lymph node HP | 3 | 7 | 0 | 3 |
| Mesenteric lymph node MES | 6 | 14 | 1 | 5 |
| Lung | 9 | 20.9 | 4 | 5 |
| Total | 43 | 100 | 10 | 33 |

Table 2: Pooled TB lesions distribution

| | | | | Ziehl-Neelsen | |
|---|---|---|---|---|---|
| Anatomic site | Lesion | Percent(N=369) | Organ proportion (N=43) | -ve | +ve |
| Lymph nodes around Head | 9 | 2.44 | 20.93 | 7 | 2 |
| Lung and lymph nodes around it | 25 | 6.78 | 58.14 | 25 | 7 |
| Mesenteric lymph nodes | 6 | 1.63 | 13.95 | 5 | 1 |
| Liver and hepatic lymph nodes | 3 | 0.81 | 6.98 | 3 | 0 |
| Total | 43 | 11.66 | 100 | 33 | 10 |

Table 3: Comparison of the detailed meat inspection and Ziehl-Neelsen staining microscopy

| | Detailed Meat Inspection | | Ziehl-Neelsen staining microscopy | |
|---|---|---|---|---|
| Result | N | % | N | % |
| Positive | 43 | 11.66 | 10 | 2.7 |
| Negative | 326 | 88.34 | 359 | 97.3 |
| Total | 369 | 100 | 369 | 100 |

Table 4: Prevalence of bovine tuberculosis with respect to age and body condition score (Ani-mortem)

| Variables | Number examined | Positive | % | $X^2$ cal | P-Value |
|---|---|---|---|---|---|
| Age | | | | | |
| Young adult | 157 | 19 | 5.2 | 0.00 | 0.99 |
| Adult | 212 | 24 | 6.5 | | |
| BCS | | | | | |
| Good | 287 | 8 | 2. 2 | 79.01 | 0.01 |
| Medium | 82 | 35 | 9.5 | | |

BCS= body condition score; % = percent, X = Chi square

to contain tuberculous lesions. About 20.9% of the lesions were observed in the lung alone. The lung contributes to a higher percentage of tubercle gross lesions than the other areas followed by the retropharyngeal lymph node. The least infected tissues examined were the intestine and sub-mandibular lymph nodes (Table 1). About 58.14% of the lesions were observed in the lung and associated lymph nodes. The lung region contributed a higher percentage of tubercle lesions than the head and the gastrointestinal area (Table 2).

**Prevalence of the Disease:** The overall prevalence of BTB in ~~the~~ cattle slaughtered at Gondar Elfora abattoir during the study period was 11.7% (43/369) based on detailed post-mortem examination. Visually, the presence of changes seen in the affected organs and/or lymph nodes were the presence of circumscribed lesion suspected for the presence of tuberculosis disease. However, only 2.7% (10/369) cattle were found to have observable mycobacterium by the Ziehl-Neelsen staining microscopy. Thus, the proportion of lesion detected by detailed examination to that of Ziehl-Neelsen staining microscopy was in the ratio of 4.3:1. There was high deviation between the diagnoses in detecting tuberculosis between the two methods (Table 3).

Based on age and body condition score, the prevalence of tuberculosis in slaughtered cattle at Gondar Elfora abattoir is presented in table 3. There was no statistically significant ($P > 0.05$) difference between age groups. However, there was a variation in occurrence of tuberculous lesions across body condition scores (Medium and good), with considerable higher prevalence recorded in medium scored cattle (Table 4).

## DISCUSSIONS

In the present study, gross tuberculous lesions were found most frequently in the lymph nodes of the lung (20.9%). In total, seven confirmed cases are from lung and related lymph nodes of the head. Hepatic lymph nodes contributed the least to such lesions. This finding is consistent with previous studies (3) [17-20] which reported higher TB suggestive lesions in lungs and associated lymph nodes. This is an indication that inhalation might be the principal route of TB infection in cattle. Therefore, during post-mortem examination, focus should be given to lungs and associated lymph nodes. Mesenteric lymph nodes should also be given due attention as infection can occurs through ingestion [16].

As revealed by detailed meat inspection and Ziehl-Neelsen staining microscopy, the prevalence of tuberculous lesions in slaughtered cattle at Gondar Elfora abattoir were 11.7% and 2.7% respectively. The findings of overall prevalence of tuberculosis reported in Ziehl-Neelsen staining microscopy confirmed cases are in agreement with different study results in Ethiopia [21-24] and findings of detailed post-mortem examination coincides with higher results from Butajira municipality abattoir (11.50%) [19] and Adama (24.70%) [25]. This indicates the endemicity of the disease and high infection rate prevailing in the general population of slaughter cattle in Ethiopia. Bringing animals from different farms together without any pre-examination might have also played a role in increasing its prevalence due to possible transmission between animals kept under very poor hygienic condition. A better management of the breeding would reduce this incidence [26]. This study provides vital information for the stakeholders in the Public Health Sector for informed steps [27] to be taken to better position the livestock workers especially butchers in the drive towards eradication of BTB [28].

Ziehl-Neelsen stained smears that were directly made from 43 suspected tuberculosis lesions showed ten positive acid fast bacteria. There was high deviation between the detailed meat inspection and Ziehl-Neelsen staining methods. The detailed meat inspection has limitations in exactly detecting BTB suggestive lesions. Hence, due attention should be given to confirm cases before making decisions on the carcass use as it may be wastage for the butchers but still used for economic reasons[29,30]. Some combination with other methods for confirming BTB, such as PCR is necessary, in addition to the existing inspection methods [31]. The most probable explanation for the failure of standard meat inspection to correctly detect tuberculosis infection could be the manner of examination [21]. Test and slaughter policy should be designed and started as a major control measure to avert the spreading of the BTB infection. Similarly, routine abattoir meat inspection procedures have to be made for the detection of tuberculous lesions and special attention has to be given when a large number of animals are needed to be examined. The result can be upgraded when other methods are simultaneously used for economic and public health concerns [7, 21, 32].

The prevalence of the disease was statistically insignificant when comparing different age categories. This result agrees with previous reports [28]. This might be explained in part due to the type of animals slaughtered in the abattoir; i.e. in which most were of adult age. In contrast, there was a statistically significant difference ($P < 0.05$) in the prevalence of the disease between body condition scores (BCS), the prevalence being higher in medium (9.5%) than good (2.2%) body conditioned animals. The animals were kept in temporarily fatting farms before slaughter in the vicinity of the municipality of Gondar. Consequently, the majority of cattle slaughtered were in good body conditions where there is a less likelihood of obtaining BTB infection in such animals [28] Animals with good BCS also have relatively strong immunological response to the infectious agent than animals with medium BCS and the result could also indicate the wasting nature of the disease [16]. Those animals with previously manifested disease progression and brought to the temporary fatting farm had a low immunologic status and might have led to significant variation in the population.

In conclusion, this study suggests presence of high, gross tuberculous lesions in the lung and related lymph nodes of the bovine head. Hence, during abattoir meat inspection more attention has to be given to the lungs and associated lymph nodes. Detailed visual meat inspection has limitations in exactly confirming BTB suggestive lesions. Hence, due attention should be given to redress the concern. Integrated methods for detecting tuberculosis lesion for economic and public health concerns are necessary for efficient bovine tuberculosis control. This study indicates high prevalence of BTB in Gondar Elfora abattoir. Consequently, people who consume raw meat might be at increased risk of acquiring BTB infection. Therefore, appropriate control measures should be taken for the control of BTB spread in the region.

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