

## Evaluation of Routine Meat Inspection Procedure to Detect Bovine Tuberculosis Suggestive Lesions in Jimma Municipal Abattoir, South West Ethiopia

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**Abstract:** A survey was conducted in Jimma municipal abattoir from October 2010-January 2011 to evaluate the effectiveness of the routine meat inspection procedure as compared to the detailed inspection method in detecting BTB (bovine tuberculosis) suggestive lesions and concomitantly to determine the prevalence of BTB on the basis of post mortem inspection and Zihel-Neelsen microscopy. Carcasses of seven hundred eighty randomly selected cattle were inspected through both methods. Analysis of the data revealed that 21 (2.7 and 95%; CI, 1.5-3.8%) had BTB suggestive lesions using detailed inspection; while it was detected only in 2 carcasses (0.3 and 95%; CI, 0.1-0.6%) by the routine method. Thus, the routine inspection method missed most of the carcasses with BTB suggestive lesions (90.5%) and the agreement between the detailed and routine meat inspection methods was slight ( $\text{Kappa}=0.17$ ). The mean time spent to inspect a carcass ( $1.2\text{min}\pm 0.4$ ) by the routine method was virtually five times shorter than the detailed method ( $5.8\text{min}\pm 1.9$ ). There was a statistically significant difference ( $P < 0.05$ ) in the mean time spent to inspect each carcass between the two methods. Zihel-Neelsen stained smears that were directly made from twenty one suspected tuberculosis lesions showed eight acid fast bacteria positive. There was a moderate agreement between the detailed meat inspection and Zihel-Neelsen staining methods ( $\text{Kappa}=0.54$ ). In conclusion, the routine meat inspection has limitations in detecting BTB suggestive lesions. Consequently, people who used to consume raw meat might be at risk of acquiring BTB infection. Hence, due attention should be given to redress the concern.

**Key words:** Bovine tuberculosis % Evaluation % Jimma % Lesions % Meat inspection

### INTRODUCTION

Bovine tuberculosis (BTB) is an endemic disease of cattle in Ethiopia and has been reported from different regions of the country based on tuberculin tests ranging from 3.4% in small holder production system to 50% in peri-urban (intensive) dairy production system [1-3] and 3.5 to 5.2% in slaughter houses in various places [4, 5]. It has been reported that approximately 85% of cattle and 82% of human populations in Africa have been estimated to live in areas whereas BTB is either partly controlled or uncontrolled at all [4, 6]. Moreover, zoonotic tuberculosis in eastern and southern Africa is increasing [7].

Bovine tuberculosis is prevalent in animals of many developing countries whereas surveillance and control activities are often inadequate or unavailable; Ethiopia is one of these countries where many epidemiologic and public health aspects of the infection remain largely

unknown [8]. Leite *et al.* [9] estimated that the proportion of human cases in developing countries due to *Mycobacterium bovis* accounted is 3.1% for all forms of tuberculosis. The disease has a negative impact on livestock production in developing countries through reduced production efficiency, carcass or organ condemnation and restriction of international trade [10]. The Office International des Epizooties classifies BTB as a list B transmissible disease of public health importance and is of high significance to the international trade of animals and animal products [11].

Although milk is considered as the main route for the transmission of BTB from cattle to human beings [12], contaminated meat can also play its own role for the transmission. It has been indicated that the behaviors and traditions of consuming raw animal products could be one of the routes of transmission of tuberculosis of animal origin and raw meat consumption is a welcomed tradition

in Ethiopia [13-15]. Increasing human population coupled with expanding urbanization and higher average income raised the demand for meat [16-18]. To meet this demand, millions of food animals are slaughtered every year throughout Ethiopia [19]. Thus, implementation of appropriate meat inspection procedures during slaughter is supposed to be an integral part of the national public health safeguard plan.

Though detection of BTB in Ethiopia is most commonly carried out on the basis of tuberculin skin testing and abattoir meat inspection [14], regular surveillance through skin testing of millions of individual cattle, bacteriology and molecular methods are not realistic methods for logistic reasons. In connection, the 'test-and-slaughter' approach and pasteurization of milk, which have been used successfully in industrialized countries, might not be the optimal tools in Africa [20]. To this end, abattoir inspection at the moment remains economically affordable and valuable technique to detect BTB in carcasses of slaughtered animals [21, 22]. On one hand, the main purpose of post mortem examination of carcasses at slaughterhouse is protection of the public health [23]; on the other hand, however, failure to detect a lesion during abattoir inspection in cattle with a single lesion will have a huge zoonotic implication. To this effect, it is imperative to evaluate the efficiency of the routine abattoir inspection in identifying bovine tuberculosis suggestive lesions.

Therefore, the objectives of this study were to evaluate the effectiveness of the routine meat inspection procedure as compared to the detailed inspection in detecting BTB suggestive lesions and concomitantly to determine the prevalence of bovine tuberculosis on the basis of post mortem inspection and Zihel-Nelseen staining methods.

## MATERIALS AND METHODS

**Study Area:** Jimma town is located in Jimma administrative zone, Oromia Regional State of Ethiopia, 355 kilometers South West of Addis Ababa at the latitude of about 7° 13' -8° 56' N and longitude of about 35°52' -37° 37' E and an

elevation ranging from 880 to 3360 meters above sea level. The climate varies from wet and humid during heavy rains between May and September to hot and semi-arid condition between November and April. The area receives annual rain fall of 1200 to 2400 mm with mean annual minimum and maximum temperature range of about 11°C to 28°C. The administrative zone has a total cattle population of 2,016,823 [24].

**Jimma Municipal Abattoir:** The abattoir which is administered under Jimma town municipality is the only source of inspected beef for all inhabitants of the town. The average number of animals slaughtered per day during the study period was about 45 with more than 85% of the slaughtered animals being cattle (Table 1). The overall abattoir sanitary conditions and internal facilities were very poor.

**Study Design:** A survey was conducted in Jimma municipal abattoir from October 2010 to January 2011 to evaluate the effectiveness of the routine meat inspection procedure as compared to the detailed inspection in detecting BTB suggestive lesions and concomitantly to determine the prevalence of BTB on the basis of post mortem inspection and Zihel-Nelseen staining methods.

**Study Animals and Sampling Method:** Animals were supplied to the abattoir by traders who used to purchase and bring them from different directions of Jimma zone. A total of 780 cattle was randomly selected according to the formula for simple random sampling [25]. Virtually all cattle slaughtered during the study period were male indigenous zebu breed that have been managed under traditional husbandry system with very little or no veterinary services.

**Ante-mortem Inspection:** Physical examination of animals was carried out before they are slaughtered. Body temperature, pulse rate, respiratory rate and type of nasal discharge if present, condition of superficial lymph nodes and visible mucous membrane were examined and age was recorded for individual animal.

Table 1: Number of animals slaughtered in Jimma municipal abattoir from October 2010 to January 2011

Months	No of animals slaughtered		
	Cattle	Sheep	Goats
October	1039	344	24
November	1196	165	15
December	1237	102	18
January	1143	78	10
Total	4615	689	67

**Body Condition Scoring:** Body condition of the animals was determined using the guidelines established by Nicholson and Butterworth [26]. Accordingly, nine scores were used which are abbreviated as F+, F, F-; M+, M, M-; L+, L and L-. Each scoring was given a number from 1 (L-) to 9 (F+). The animals slaughtered in the abattoir were from 5 to 8 scores.

**The Routine Meat Inspection:** This procedure was carried out as usual by the assistant meat inspector of the abattoir following meat inspection protocols issued by the former Meat Inspection and Quarantine Division of the Ministry of Agriculture, Ethiopia [27]. It involved visual examination and palpation of intact organs like the liver and kidney as well as palpation and incision of the head, lung and pleural lymph nodes. Other lymph nodes are incised if lesions were detected in one of these tissues.

**Detailed Meat Inspection:** Inspection of each of the carcass was undertaken in detail according to Ameni *et al.* [28] and Demelash *et al.* [22]. Particular emphasis was given during examination to certain organs and lymph nodes that were carefully inspected for presence of suspected BTB lesions. The seven lobes of the two lungs, including the left apical, left cardiac, left diaphragmatic, right apical, right cardiac, right diaphragmatic and right accessory lobes, were inspected externally and palpated. Then, each lobe was sectioned into 2-cm-thick slices to facilitate the detection of lesions. Similarly, lymph nodes, namely, the parotid, submaxillary, mandibular, medial retropharyngeal, tracheobronchial, cranial and caudal mediastinal, hepatic, mesenteric iliac, precrucial, prescapular, supramammary, inguinal, apical and ischiatic lymph nodes, were sliced into thin sections (circa 2 mm thick) and inspected for the presence of visible lesions. Moreover, organs such as liver, kidneys, mammary gland and intestines were also thoroughly examined. The cut surfaces were examined under bright light for the presence of abscess, cheesy mass and tubercles [29]. When gross lesions suggestive of BTB were found in any of the tissues, the animal was classified as having lesions.

**Recording the Time Spent for Inspection:** The time used to inspect each carcass by the routine and detailed inspection methods was recorded. The mean time spent to inspect a carcass (Minutes $\pm$ SD) was calculated for both methods.

**Microscopic Examination:** Zihel-Neelsen staining was directly done from tuberculosis suspected lesions. The stained slide were observed under a microscope for

the presence of acid fast bacteria, which appear as red bacillary cells occurring singly or in clumps [10].

**Data Analysis:** The data were entered in to a computer on a Microsoft Excel spreadsheet and analyzed using SPSS version 16.0 software program. Kappa was calculated to see the agreement between the diagnostic methods; accordingly, 0: poor agreement; 0-0.20: Slight agreement; 0.21-0.40: Fair agreement, 0.41-0.60: Moderate agreement; 0.61-0.80: Substantial agreement; >0.81: Almost perfect agreement [25]. Independent samples t-test was used to compare the mean time spent to inspect a carcass using the routine and detailed methods. The difference between various factors was also analyzed using chi-square ( $P^2$ ). A p-value < 0.05 was considered statistically significant.

## RESULTS

**Detection of BTB Suggestive Lesions:** Analyses of the data revealed that 21 carcasses (2.7 and 95%; CI, 1.5-3.8%) had BTB suggestive lesions using detailed postmortem examination; while it was detected only in 2 carcasses (0.3% and 95%; CI,-0.1-0.6%) by the routine method. Thus, the routine inspection method missed most of the carcasses with BTB suggestive lesions (90.5%). Hence, the sensitivity of the routine meat inspection procedure was very low (9.5%) and the agreement between the detailed and routine meat inspection methods was slight (Kappa=0.17). Zihel-Neelsen stained smears that were directly made from twenty one suspected tuberculosis lesions showed eight acid fast bacteria positive. There was a moderate agreement between the detailed meat inspection and Zihel-Neelsen staining methods (Kappa=0.54).

**Time Spent to Inspect Carcasses:** The mean time spent to inspect a carcass (1.2min $\pm$  0.4) by the routine method was virtually five times shorter than the detailed method (5.8min $\pm$ 1.9). There was a statistically significant difference ( $P < 0.05$ ) in the mean time spent to inspect each carcass between the two methods.

**Distribution of BTB Suggestive Lesions in Different Organs/ Lymph Nodes:** The lesions identified in different organs/ lymph nodes according to the methods of inspection and anatomical site involved is presented in table 2. Hence, large proportions of gross lesions were observed in the lungs and mediastinal lymphnodes whereas the least in the intestines and sub-mandibular lymph nodes.

Table 2: Comparison of the routine and detailed inspection in terms of distribution of BTB suggestive lesions in different organs/ lymph nodes

Meat inspection methods							
Anatomical site	Routine meat inspection			Detailed meat inspection			
	No of organs/ lymph nodes with lesions	Percentage (%) out of BTB suggestive lesions	[95% Confidence Interval]	No of organs/ lymph nodes with lesions	Percentage (%) out of BTB suggestive lesions	[95% Confidence Interval]	P <sup>2</sup> (p-value)
Organs Lung	2	9.5	-3.03-22.03	9	42.9	21.69-64.03	4.405E2* (0.000)
Liver	-	-	-	2	9.5	-3.03-22.03	
Intestine	-	-	-	1	4.8	-4.35-13.87	
Lymph nodes Mediastinal	-	-	-	5	23.8	5.59-42.03	1.820E2*(0.000)
Retro-pharyngeal	-	-	-	3	14.3	-0.68-29.26	
Sub-mandibular	-	-	-	1	4.8	-4.35-13.87	
Total	2	9.5		21	100		

Table 3: Proportion of carcasses with BTB suggestive lesions based on body condition score

Examination results							
Body condition (Score) of the animals	No Examined	Detailed meat inspection results			Zihel-Neelsen positive		
		No of carcasses with BTB suggestive lesions	Percentage (%) out of BTB suggestive lesions	P <sup>2</sup> (p-value)	No of positives	Percentage (%) out of BTB suggestive lesions	P <sup>2</sup> (p-value)
M (5)	329	8	42.2	5.46 (0.14)	4	50.0	2.3 (0.51)
M+ (6)	318	6	40.8		3	37.5	
F-(7)	97	5	12.4		1	12.5	
F (8)	36	2	4.6		-	-	
Total	780	21	100.0		8	100	

Where:

M = Ribs usually visible, little fat cover, dorsal spines barely visible.

M+ = Animal smooth and well covered; dorsal spines cannot be seen, but are easily felt.

F=Animal smooth and well covered, but fat deposits are not marked. Dorsal spines can be felt with firm pressure, but feel rounded rather than sharp.

F = Fat cover in critical areas can be easily seen and felt; transverse processes cannot be seen or felt.

Table 4: Comparison of prevalence of BTB suspected carcasses based age group

Examination results							
Age, years	No Examined	Detailed meat inspection results			Zihel-Neelsen positive		
		No of carcasses with BTB suggestive lesions	Percentage (%) out of BTB suggestive lesions	P <sup>2</sup> (p-value)	No of positives	Percentage (%) out of BTB suggestive lesions	P <sup>2</sup> (p-value)
# 5	284	3	14.3	7.4 (0.025)	1	12.5	2.1(0.35)
>5 # 7	375	11	52.4		4	50.0	
>7 #10	121	7	33.3		3	37.5	
Total	780	21	100		8	100	

**Association of Body Condition Score and the Proportions of Carcasses with BTB Suggestive Lesions:** The association between the proportions of carcasses with BTB suggestive lesions among the various body condition scores was assessed. Accordingly, the results of the detailed inspection and Ziehl-Neelsen staining methods among the different body condition scores were not statistically significant ( $P > 0.05$ ) (Table 3).

**Association of Age and Proportion of Carcasses with BTB Suggestive Lesions:** The relationship between the proportions of carcasses with BTB suggestive lesions among the different age group was

assessed. Accordingly, the detailed inspection result showed statistically significant association with age ( $P < 0.05$ ) (Table 4).

## DISCUSSION

It has been suggested that abattoir meat inspection is the utmost obligatory and fundamental step in detecting bovine tuberculosis in Ethiopia, whereas other diagnostic options are limited [4]. To this effect, detection of tuberculosis lesions in cattle through abattoir inspection is so far the common procedure in this country [14]. Our work showed that the routine abattoir inspection

following the method developed by the Meat Inspection and Quarantine Division of the Ministry of Agriculture, Ethiopia [27] missed most of the carcasses with BTB suggestive lesions (90.5%) as compared to the detailed inspection method. This indicated that the likelihood of such carcasses to be unwittingly passed safe for human consumption is high. In support of our observation, Shitaye *et al.* [14] and Demelash *et al.* [22] reported the limitation of the routine abattoir inspection procedure; moreover, Etter *et al.* [30] undertook a risk assessment on the poor standard of meat inspection in slaughterhouses with regard to bovine tuberculosis in Ethiopia. Accordingly, the risk varied between from 23.8% (95th percentile assuming that all infected animals have lesions) to 33.2% (95th percentile assuming infected animals could have no lesions). Thus, it was concluded that the quality of meat inspection is low with a high risk to release BTB infected carcass in to the food chain.

According to our observation, the time used to inspect a carcass by the routine meat inspection procedures was by far shorter than that of the detailed inspection method. Hence, most of the tuberculosis suggestive lesions can easily be missed due to insufficient time spent for inspection. This is in accord with Shitaye *et al.* [4] and Corner *et al.* [29] who reported that postmortem surveillances for detection of BTB lesions in particular depend on the work load, time and diligence of the inspector conducting the examination.

Thorough examination of all predilection tissues and organs using detailed inspection in our case improved the very low sensitivity (9.5%) of the routine meat inspection procedure by 90.5%. This might imply that the sensitivity of post mortem examination could depend on the methods of meat inspection employed and the organs examined. Baltzare [31] reported that examination of as few as six pairs of lymph nodes, the lung and the mesenteric lymphnodes can increase to 95% the probability of cattle with macroscopic lesions being identified.

In this study, BTB suggestive lesions were detected predominantly in the lungs and associated lymph nodes, which was consistent with the previous findings [11, 29, 32-34]. According to Corner [35], between 70 and 90% of the lesions are found in either the lymphnodes of the thoracic cavity or in the head, hence the wide range of lymphnodes are required to be examined. Besides, Opara, [36] reported that tuberculosis was the major cause of bovine lung condemnation in abattoirs. Thus, our finding supports the theory that most cases of BTB infections in cattle are acquired by inhalation. This indicates that meat inspectors in the slaughterhouses need to give special

attention to the lungs and associated lymph nodes particularly when inspecting large number of carcasses so that BTB suggestive lesions will not be missed.

The proportion of carcasses with BTB suggestive lesions based on the detailed inspection procedure was found to be 2.7%. Even though our finding is low, it is relatively not as such lower than Shitaye *et al.* [4] and Teklue *et al.* [37] and who reported 3.5 and 3% in Addis Ababa municipal abattoir, respectively. Nonetheless, the present finding is lower than Hussen [38] who reported 12.5% in Butajira abattoir. It has been reported that, the spread of bovine tuberculosis among cattle is linked to the type of production system [4, 39]. Hence, the low prevalence in our study might be due to the fact that all animals were kept under extensive production system.

The reduced number of positives using Zihel-Nelseen staining in our work might be associated with the fact that *M. bovis* is often low in bovine specimens and they can be visualized by Zihel-Nelseen staining only if a limited quantity (at least  $5 \times 10^4$  Mycobacteria /ml) of material is present [40]. The result can also justify that viable Mycobacteria may not be present in calcified lesions [4]. Moreover, the result of Zihel-Nelseen staining may also be affected by the sample taking technique during smear preparation as Mycobacteria are not evenly distributed in the tissue sample [41]. Besides, the presence of acid fast organisms in histological sections may not be detected although *M. bovis* can be isolated in culture [11].

This study revealed that the prevalence of tuberculosis increases as the age increases and post mortem examination result showed statistically significant difference among the various age group ( $P < 0.05$ ). This is consistent with the previous reports of Hussein [38] in Butajira. Since bovine tuberculosis is a debilitating disease, as the age of the animal increases, the likelihood of being infected with BTB also increases. It has also been reported that the duration of exposure to different stressors, malnutrition and various immunosuppressants increases with age; older animals are more likely to have been exposed than younger ones [42]. Similarly, studies in Canada and Northern Ireland indicated an increased incidence of BTB with increased age [43]. It has been suggested that increased incidence of BTB in older animals can be explained by a waning of protective capability in aging animals, as experimentally confirmed in the murine system [44].

The difference in the prevalence among the various body condition scores was assessed in table 4. Accordingly, the results of the post mortem suspected

lesion and Ziehl-Neelsen stained smears among the different body condition scores were statistically not significant. This might be due to the fact that the majority of cattle slaughtered were in good body conditions where there is a less likelihood of obtaining BTB infection in such animals. In support of this finding, Collins [45] reported that animals under good body condition are with good immune status that can respond to any foreign protein better than those with poor body condition.

Even though, the detection of BTB in carcasses in Ethiopia mainly depends on postmortem examination, there are still infected carcasses which do not show visible lesion and might be a risk to public health. Few studies previously done in this country have indicated that not all cattle infected with *M. bovis* have visible tuberculosis lesions at slaughter [34, 37]. On the other hand, the absence of visible lesions may not often lead to credible conclusion as *M. bovis* does not exist in the inspected carcasses. The presence of caseous and/or calcified lesions may not necessarily always found to be of mycobacterial origin [35, 46].

In conclusion, the routine meat inspection has limitations in detecting BTB suggestive lesions. Consequently, people who used to consume raw meat might be at risk of acquiring BTB infection. Hence, due attention should be given to redress the concern.

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