Mycobacterial Diseases
ISSN: 2161-1068

Worku et al., Mycobact Dis 2016, 6:3
DOI:10.4172/2161-1068.1000218

Research an open access journal

Cross-Sectional Study and Comparison of Different Diagnostic Methods of Bovine Tuberculosis in Gondar Elfora Abattoir, Ethiopia

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Rec date: May 11, 2016; **Acc date:** July 18, 2016; **Pub date:** July 25, 2016

Abstract

Background: The prevalence of bovine tuberculosis is high in developing countries due to lack of awareness, good diagnostic methods and prevention strategies. Therefore, this study aims to estimate the prevalence of bovine tuberculosis and compare the efficacy of different procedures of diagnosis.

Methods: A cross sectional study was conducted at Gondar Elfora abattoir from December, 2005 to June, 2006. To this effect, comparison has been made between the detailed postmortem examination and routine meat inspection procedures with gold standard culture result.

Result: Out of 402 animals examined at slaughter, 15.9% were diagnosed with gross tuberculous lesions by detailed laboratory examination. Routine abattoir inspection detected only 2.9% of the tuberculous cattle. From 64 cattle considered tuberculous, 10 show growth in Lowenstein-Jensen. The average number of lesions per infected cattle was 1.6% and 55.5% of cattle with tuberculous lesions possessed single lesion. All the traits (including sex, age and body condition score) measured in relation to tuberculous lesions did not show a statistically significant difference among the categories. The sensitivity of routine meat inspection was 18.8% with detailed postmortem examination and 30% with culture in comparison with 83.3% specificity. There was a poor agreement (k=0.18) between routine meat inspection and detailed postmortem examination procedures. Similarly, a poor agreement (k=0.12) was obtained between routine meat inspection procedure and culture result.

Conclusion: Relatively higher prevalence was recorded, and there is a need to improve the sensitivity of routine abattoir inspection procedures to diagnose tuberculous.

Keywords: Abattoir; Tuberculosis; Prevalence; Postmortem; Gondar

Introduction

Tuberculosis is an infectious with granulomatous characteristics. All species of vertebrates can be affected by tuberculosis and its distribution is worldwide. *Mycobacterium bovis*, which belongs to the *M. tuberculosis* complex, is acid fast bacilli that cause bovine tuberculosis. *M. tuberculosis* complex includes *M. africanum*, *M. microti* and *M. tuberculosis* [1].

Bovine tuberculosis in developed countries is rare because of the eradication of the disease by test and slaughter policy but in developing countries, especially in Africa where *M. bovis* infection is present in various animal species, there is a lack of knowledge on the epidemiological patterns, zoonotic implication and distribution of this disease [2].

In Ethiopia, BTB (Bovine Tuberculosis) is considered to be endemic based on tuberculin test surveys and abattoir inspection [3]. The prevalence of BTB in Ethiopia is high in intensive system which ranged from 3.4% in smallholder production systems to 50% in intensive production system [4].

During abattoir examination there is a tendency to lose small lesions because of time limitation for detailed examination of each tissue and very fine slicing of lymph nodes is very difficult. Highly acceptable (golden standard) for examination of *Mycobacteria* is culture, however long time for growth of colonies, intensity of labor required and possible presence of non-cultivate *Mycobacteria* in some clinical specimens require more appropriate methods. An agar based medium and egg based medium can be used for primary isolation [5].

To alleviate the problems during examination, different approaches have been investigated and developed. DNA technology recently advanced as a diagnostic method. Nucleic acid probes have been used to detect and identify of clinically important *Mycobacteria* which is now commercially available. The sensitivity of those nucleic acid probes found to be similar with Ziehl-Neelsen stain and it has limited importance for the detection of Mycobacteria in clinical samples. Tests based on Polymerase Chain Reaction (PCR) have shown more promise for mycobacterial detection in clinical samples [6].

Gondar is one of the towns with the largest number of population. There are several butchers that supply meat to the public and one abattoir that slaughters and distributes meat to the butchers. Estimation of the prevalence of bovine tuberculosis (BTB) in animals slaughtered in this abattoir on the basis of postmortem examination can give clue on the extent of the disease in the area where the animals are originated from. Therefore, the objectives of this study were:

To estimate the prevalence of bovine tuberculosis in slaughtered animals.

To assess the tuberculous lesion and its distribution.

To compare the efficiency of the diagnostic methods used in this study.

Materials and Methods

Study Area

The study is conducted in Amhara region, originated from Dembia, Wogera, Maksegnit, Gondar and Aymba and collected in Gondar town, which is located at latitude of 12.40 North, longitude of 27.250 East and stands at an altitude range of 1800-2200 meters above sea level. The ranges of maximum and minimum temperature of the area vary between 22-30.70˚C and 12.3-17.10˚C, respectively. The region receives a bimodal rainfall, the average annual precipitation rate being 1000 mm. the short rains occur during the months of March, April and May while the long rains extend from June through September [7].

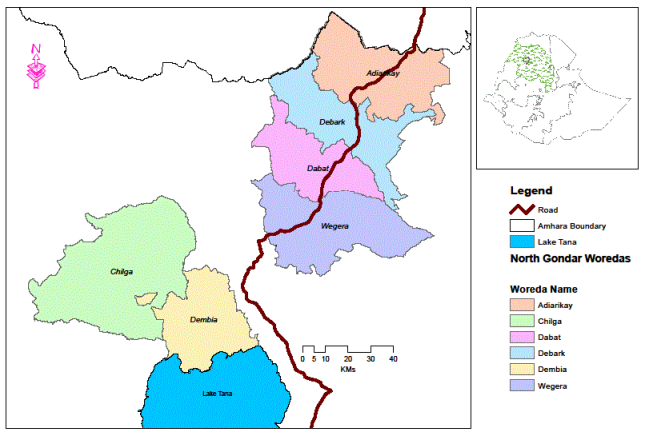

Figure 1: Map of the study areas.

Study Design, Sampling and Sample Transportation

A cross sectional study was done on 402 animals slaughtered at the abattoir for tuberculous lesions. The study was conducted at Gondar Elfora abattoir from December, 2005 to June, 2006. Figure 1 comprises 394 males and 8 females.

Dembia, Wogera, Maksegnit, Gondar and Aymba were the towns form which cattle brought for slaughter. Sterile universal bottles were used to collect lymph nodes/tissues samples and kept at -200˚C before transported to Armauer Hansen Research Institute (AHRI), Addis Ababa for culturing.

Ante-mortem, Postmortem and Microscopic Examination

Routine physical examination (Ante-mortem examination) of animals and routine inspection (postmortem examination) to detect tuberculous lesions at the abattoir is conducted and it was done according to the method developed by the meat inspection and quarantine division of the Ministry of Agriculture [8]. The methods used were palpation and incision of the mediastinal, prescapular and bronchial lymph nodes, as well as incision and visual inspection of the lungs, kidney, udder and liver. Lymph nodes were incised if there was lesion in the tissues. These lymph nodes were parotid, mandibular, retropharyngeal, bronchial, mediastinal, mesenteric, prescapular and prefemoral lymph nodes. In addition, tissue specimens such as lungs, liver, mammary gland and kidneys were collected.

Lymph nodes sliced in to 2 mm thin sections and other tissues were cut in to slices of 2 cm using new surgical blades for each. The cut surfaces of tissues were examined in a good light source for the presence of tubercles and abscesses [3,9]. In the presence of suspected tuberculous lesions, tissue samples were collected in sterile universal bottles and sent for culturing. Direct smear of each tissue with tuberculous lesions (Table 1) was prepared and stained using the Ziehl-Neelsen acid-fast staining technique [10].

Anatomical site	No of tuberculosis lesions	Percent (%)	M. bovis positive	Percent (%)
Lung	4	5.12	1	25
Bronchial lymph node	23	29.49	3	13.04
Mediastinal lymph node	24	30.77	4	16.67
Retropharyngeal lymph node	18	23.08	1	5.56
Mesenteric lymph node	9	11.54	1	11.11
Total	78	100	10	12.82

Table 1: Distribution of tuberculous lesions in tissues.

Isolation and Identification of *Mycobacteria*

Isolation of the bacteria done according to standard stated [10,11]. Samples of tissues and lymph nodes were collected and using sterile blades, individual tissues in sterile petridishes were macerated to get fine pieces.

Using mortar and pestle, samples were homogenized and decontaminating homogenate was done using 2 ml of 4% NaOH for 15 min, centrifuged at 300 rpm for another 15 min and neutralized by 1% (0.1N) hydrochloric acid using phenol red as the indicator. The sediment was inoculated on to a set of Lowenstein-Jensen slants supplemented with 0.4% sodium pyruvate and glycerol (standard L-J). Cultures were incubated at 370˚C for up to 12 weeks under aerobic environment.

The growth of white, moist, flat and non-pigmented friable colonies were considered as the primary cultures of *M. bovis*. Observation for growth done weekly and a suspected Mycobacterial growth was examined using the Ziehl-Neelsen technique for confirmation of acid fast bacilli.

For initial identification, the rate of growth and colony morphology of Mycobacterial species was used. Species in the *M. tuberculosis* complex are known to show a slow growth rate. For further Mycobacterial species identification (especially *M. bovis* from *M. tuberculosis*), positive cultures were sub-cultured on Lowenstein-Jensen media supplemented with glycerol or pyruvate and incubated for another three to four weeks.

Biochemical test done based on growth of the bacteria on Lowenstein-Jensen media supplemented with glycerol or pyruvate and the result was determined as negative if there is growth on glycerol and positive when there is growth on pyruvate. In addition niacin test for niacin and nitrate reduction was used and the result considered when there is no niacin production and nitrate reduction.

Data Management and Analysis

During the study, individual animal identification number, sex, breed and ante-mortem examination findings were recorded; age was categorized using tooth eruption and wear.

Body condition scoring was made using the method developed for zebu cattle based on anatomical structures such as tail head, brisket, and transverse processes of lumbar vertebrae, ribs and hips (Nihcolson and Butterworth, 1986).

The range and frequency of affection of anatomical sites were also recorded for individual tuberculous suspected cattle. Sensitivity and specificity were calculated and expressed in percentage.

Statistical analysis was included and comparison of proportions using a chi-square (x2) test and a test of agreement (kappa) for all the analysis were performed and confidence level was 95% and $p<0.05$ was set for significance.

Results

Abattoir Survey

During the study period, the prevalence of BTB in slaughtered cattle was 15.9% (64/402) on the basis of detailed postmortem examination. The distribution of the tuberculosis lesions in different tissues of positive animals is represented in Table 1.

About 54.1% of the lesions were found in lungs and thoracic lymph nodes. The average number of lesions in tissues of infected animal was 1.6 and about 55.5% of the infected animal found with only a single lesion.

Routine abattoir inspection	Detailed Laboratory Examination		Total
	Positive	negative	
Positive	12	0	12
Negative	52	338	390
Total	64	338	402
Sensitivity = 18.8% (95% CI9.19-28.31); Specificity = 100%; Kappa (k) = 0.18			

Table 2: Comparison of routine abattoir inspection and detailed laboratory examination.

Routine abattoir inspection	Culture		Total
	Positive	negative	
Positive	3	9	12
Negative	7	45	52
Total	10	54	64
Sensitivity = 30% (95% CI 1.59-58.4); Specificity = 83.3% (95% CI 73.39-93.27); Kappa (k) = 0.13			

Table 3: Evaluation of routine abattoir inspection to detect *M. bovis.*

In contrast to detailed abattoir inspection, routine abattoir inspection classified only 12 out of 402 carcasses examined found with tuberculous lesion (Table 2). As indicated in Table 3, from 64 samples cultured, routine abattoir inspection indicated only 12 samples as tuberculous and the other 52 samples as non tuberculous. From the 12 samples found tuberculous during routine abattoir inspection (Table 3) only three specimens confirmed as *M. bovis* using culture, and of 52 cases identified as non tuberculous in routine abattoir inspection, 7 specimens tested positive for *M. bovis* using culture. Thus poor agreement (k=0.18) was recorded between detailed laboratory inspection and routine abattoir (Table 2). Similarly poor test agreement (k=0.13) was observed between routine inspection and culture method.

Direct Microscopy and Isolation of *Mycobacterium*

When direct smear was used to detect acid fast bacilli on 12 tuberculous specimens, only four bacilli with positive smears found to be negative upon bacteriological culture but 2 species were positive for both direct smear and bacteriological culturing. The kappa value (k=0.25) shows a moderate agreement between direct microscopy and bacteriological culturing (Table 4).

Direct microscopy	Culture		Total
	Positive	Negative	
Positive	2	2	4
Negative	2	6	8
Total	4	8	12
Sensitivity = 50% (95% CI 5.1.0-99.0); Specificity = 75% (95% CI 44.9-100.0); Kappa (k) = 0.25			

Table 4: Results of direct microscopic examination and culture to confirm mycobacterial infection.

During assessment of host factors like body condition, age and sex on status of tuberculous lesion, the infection rate between different categories showed no significant difference (Table 5).

Samples from 64 suspected carcasses that contain tuberculosis lesion were sent for mycobacterial culture.

Out of these 64 samples cultured, routine abattoir inspection classified only 12 as tuberculosis and 52 as non-tuberculosis but in detailed laboratory examination, from 64 samples cultured mycobacterial growth was observed in 10 of the samples.

Variable	Categories	No of animals examined	Number (Percentage positive)	95% CI	X2 (P)
Sex	Male	394	10(2.5)	1.4-4.7	0.221 (0.65)
	Female	8	0(0)	-	
Age	1-4 years	15	1(6.7)	1.0-44.3	1.37 (0.503)
	5-8 years	205	4(2)	0.7-5.1	
	>8 years	182	5(1.8)	0.7-4.2	
Body	Poor	144	3(2.1)	0.7-6.4	1.12 (0.57)
condition	Medium	139	5(3.6)	1.5-8.5	
Score	Good	119	2(1.7)	0.4-6.6	

Table 5: Proportion of TB infection by the type of animal, age and body condition.

Discussion

The report of this study on the prevalence of BTB based on detailed laboratory examination (culturing and biochemical tests) is in line with a study conducted in Gondar area using comparative intradermal tuberculin test at the same year which showed an overall prevalence of 14.7% [12]. Hence, both research results may indicate that there is a high rate of tuberculosis infection in the general population.

This finding is lower than the reports in the dairy farms of Ethiopia by other researchers [13,14] which was 15.9% of the cattle with gross lesion was detected by routine inspection in abattoir, therefore, this result indicates that the routine inspection procedures should be improved to increase the sensitivity of postmortem examination which is affected by the anatomical sites examined and diagnostic method used [5].

During the study period, postmortem examination was found with lower kappa value and lower sensitivity that may indicate mistaken classification of tuberculous lesions or it might also be due to the absence of gross (visible) lesions in the examined lymph nodes and organs. The sensitivity of laboratory diagnosis is significantly higher than the sensitivity of routine inspection methods. Previous studies have reported that the detailed necropsy alone detects above 84% of all lesions [15]. To diagnose tuberculous lesions, detailed laboratory examination considered to be a satisfactory procedure than the routine examination.

The moderate agreement between bacteriology and direct microscopy showed that good results in using direct microscopy and this result found to be different from previous study done by Woldesenbet [16] in Ethiopia. The difference in results found by direct microscopy may be due to killing of the Mycobacteria when there is contamination or environmental bacteria may contaminate the smear.

During the study period, frequent tuberculosis lesions (gross) were found in thoracic lymph node which was followed by retropharyngeal and mesenteric lymph nodes which is in line with the previous report by Woldesenbet [16]. The result found may indicate higher aerosol infection route than oral (ingestion) route. However, the above result is different from other studies done previously [8,17] that they found lymph nodes in the head to be most frequently infected.

In this study the mean number of infected tissue per carcass was different from a previous report [15], which may show the probability of missing a tuberculous lesion in carcass during routine abattoir procedures.

The host factors like sex, body condition and age was found to have no significant difference in infection rates which was similar with the previous studies done in Ethiopia [3,16] which may indicate factors other than host factors have played a major role in the spread of the disease in the area of the present study.

In conclusion, the prevalence of BTB recorded in this study was relatively higher than previous report in the slaughter houses of Ethiopia. Differences in the prevalence of BTB among cattle origination from different places were recorded. The routine meat inspection procedure was observed to be less sensitive than the detailed postmortem examination in detecting TB lesion. The prevalence of the disease in this abattoir is high even though there is no organized postmortem examination especially with regard to tuberculosis.

Conflict of Interest

No conflict of interest declared.

Acknowledgements

The authors would like to acknowledge Armauer Hansen Research Institute (AHARI) for their support in funding this study and providing laboratory for our work. Addis Ababa University, College of Veterinary Medicine and Agriculture and Gondar Elfora Abattoir staffs should also be acknowledged for their support.

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