

Tuberculous lesions not detected by routine abattoir inspection: the experience of the Hossana municipal abattoir, southern Ethiopia

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Summary

The efficacy of the meat inspection procedures implemented for the detection of tuberculous cattle was evaluated by testing for bovine tuberculosis in 751 animals. The study involved routine inspection at slaughter, collection of tissues for detailed examination in the laboratory, and bacteriological investigation to identify *Mycobacterium bovis*.

Of the 751 carcasses examined, 34 (4.5%) were found to have tuberculous lesions. Routine abattoir inspection detected only 29.4% of the carcasses with visible lesions. Eighty-four percent of the tuberculous lesions were found in the lungs and thoracic lymph nodes, 11.5% in the lymph nodes of the head, and the remaining 4.5% in the mesenteric and other lymph nodes of the carcasses. In addition, *M. bovis* was isolated from a carcass that presented no gross tuberculosis lesions. The low sensitivity of routine abattoir inspection demonstrates that existing necropsy procedures should be improved.

Keywords

Abattoir – Bacteriology – Bovine tuberculosis – Detailed examination – Lymph node – Mycobacteria – Routine inspection – Sensitivity.

Introduction

Bovine tuberculosis (BTB) is a prototype granulomatous inflammatory disease caused by an intracellular bacillus, *Mycobacterium bovis*, classically identified as acid-fast bacillus (AFB) in histopathological section stains (15). Although cattle are considered the true hosts, *M. bovis* has one of the broadest host ranges of all known pathogens (20, 27).

The prevalence of *M. bovis* in the developed world is low (27, 35), but the extent to which the disease affects the developing world is unknown and could be high due to a lack of control programmes (25). In Ethiopia, BTB is considered endemic based on abattoir inspection (4, 31, 36) and tuberculin test surveys (1, 3). However,

there are no records of nationwide distribution because of inadequate disease surveillance and a lack of good diagnostic facilities.

Detection of tuberculosis by slaughter surveillance requires that infected cattle present with visible lesions at the inspected sites. However, results of various studies have indicated that in some cattle infected with *M. bovis* gross lesions are not visible in the tissues examined at slaughter. Therefore, there is a possibility that when very large numbers of carcasses are inspected daily, some of those with incipient lesions remain undetected (10, 13, 35). Furthermore, meat inspection can lead to the classification of non-tuberculous lesions as tuberculous due to

similarities in macroscopic appearances. Hence, use of bacteriological techniques is always recommended to confirm tuberculous lesions (9).

Bovine tuberculosis remains one of the most devastating diseases of cattle in developing countries (7), with implications both for the economy of farming communities and for public health (17, 19), especially in societies where animals and humans cohabit (25). Currently, the human immunodeficiency virus/acquired immune deficiency syndrome pandemic has made *M. bovis* a serious public health threat (12).

Eradication of BTB was a major component of the anti-tuberculosis strategy in all the nations that eliminated the disease (6). Since abattoir inspection is an essential element in BTB campaigns (11), meat inspection procedures must detect the disease with a high level of sensitivity. This paper describes BTB in slaughtered cattle and routine meat inspection procedures at the municipal abattoir in Hossana, southern Ethiopia.

Materials and methods

Setting

The study was conducted in the municipal abattoir in Hossana, the capital of Hadiya Zone, southern Ethiopia. The abattoir is the only source of inspected beef for an estimated 100,000 inhabitants. The perimeter of the abattoir is not well fenced, and hence, dogs and other animals have easy access to the compound. The overall hygiene of the abattoir, including the drainage and refrigeration systems, is poor and there is an acute shortage of water.

The animals are routinely bled by cutting the jugular vein after casting; only adult cattle were killed in the abattoir during the study period and a junior meat inspector performed both the ante-mortem and routine post-mortem inspections. There is no cooling system in place for the transportation of carcasses to the market and sanitary conditions are therefore poor. Since backyard slaughtering is a common practice in Ethiopia, the number of animals slaughtered at the abattoir was relatively small, averaging about sixteen each working day.

Study population

A total of 751 animals, slaughtered at the abattoir between November 2002 and February 2003, were examined for tuberculous lesions. This figure comprises 679 males originating from feedlots and 72 females culled because of old age or other reproductive problems.

Although Gimma, Wolayta Soddo, Gurage Zone and Hossana were the major sources of cattle for slaughter, the exact area of origin of the animals could not be traced since they were not individually identified. As a result, none of the cattle examined had a history of tuberculosis and they were therefore not tested for tuberculosis before slaughter. However, all the cattle brought to the abattoir were found to be apparently normal at the ante-mortem examination.

Routine abattoir inspection

Routine inspection for the detection of tuberculous lesions at the abattoir is conducted according to the method developed by the Meat Inspection and Quarantine Division of the Ministry of Agriculture (31, 36). The method involves palpation and incision of the bronchial, mediastinal and prescapular lymph nodes, as well as visual inspection and incision of the lungs, liver, kidneys and udder. Other lymph nodes are incised only if a lesion is recorded in the above tissues. The whole carcass is condemned if generalised tuberculosis with involvement of carcass lymph nodes is diagnosed, otherwise only the infected organ, or infected section of the organ, is rejected.

Detailed laboratory examination

The following lymph nodes –parotid, mandibular, sublingual, medial retropharyngeal, tracheobronchial, mediastinal, prescapular, prefemoral, mesenteric, superficial inguinal– and other tissue specimens such as the lungs, liver, kidneys and mammary gland were subjected to a detailed laboratory examination in the abattoir under a bright light source. The tissues were cut into slices of 2 cm using separate surgical blades. The slices were then examined for the presence of abscesses and tubercles (16, 22). Tissues with suspected lesions were submitted for culture. Specimens from 32 randomly selected cattle with no visible lesions were also examined (the samples were taken from the medial retropharyngeal, tracheobronchial, mediastinal and mesenteric lymph nodes and pooled separately for each individual animal) (24).

Microscopic examination

A direct smear was prepared from the centrifuged sediments of 18 tissues with tuberculous lesions before decontamination and stained using the Ziehl-Neelsen acid-fast staining technique (14, 34). The heat-fixed smears were stained with carbol-fuchsin, heated gently and allowed to stand for about 10 min. The stain was then poured off and the smears washed with tap water and decolourised with 25% sulphuric acid, then 96% ethyl alcohol for 1 min. each, with the slides being washed under tap water between each step. The smears were then counter-stained with methylene blue for 3 min.,

air-dried and examined for the presence of AFB under a light microscope employing a 100×oil-immersion objective.

Isolation of the mycobacteria

Samples of lymph nodes/tissues were collected individually into sterile universal bottles (multipurpose bottles used for transporting or keeping specimens for short periods) and kept in a deep freezer (−20°C) at the Hossana Veterinary Clinic before being transported to the Tuberculosis Laboratory of the Ethiopian Health and Nutrition Research Institute, Addis Ababa, for mycobacterial culturing. Using sterile blades, individual tissues were macerated in sterile Petri dishes to obtain fine pieces, then placed in a Stomacher bag containing approximately 5 ml of distilled water and homogenised for 10 min. (18). The homogenate was then decontaminated using 2 ml of 4% NaOH for 15 min., centrifuged at 3,000 g for another 15 min. and neutralised by 1% (0.1N) HCl using phenol red as the indicator. The sediment was inoculated onto a set of Löwenstein-Jensen slants supplemented with 0.4% sodium pyruvate (L-J pyruvate) and glycerol (standard L-J). Cultures were incubated aerobically at 37°C for up to 12 weeks, with weekly observation for discernible growth (32, 34). Growth considered as mycobacterial was examined using the Ziehl-Neelsen technique for confirmation of AFB (14, 21).

Identification of the mycobacteria

Initial identification of the mycobacterial species was based on the rate of growth and colony morphology. Species belonging to the *M. tuberculosis* complex show a slow growth rate. For further identification of the mycobacterial species, positive cultures were sub-cultured onto another set of Löwenstein-Jensen media supplemented with pyruvate or glycerol and incubated for another three to four weeks. Growth of *M. bovis* was enhanced by pyruvate. In addition, the reaction of the mycobacteria to conventional biochemical tests, namely niacin production, nitrate reduction and catalase activity, was used to distinguish *M. bovis* from other members of the *M. tuberculosis* complex (14, 34). Accordingly, the mycobacteria were considered to be *M. bovis* if they grew slowly, showed small colony size, if their growth was enhanced by pyruvate (and not glycerol) and if they produced negative results for niacin production, nitrate reduction and catalase activity.

Data collection and analysis

The sex of each animal was recorded at slaughter, age was estimated as previously described (2) and a body condition score was given (26) based on anatomical structures such as the tail, head, brisket, transverse processes of lumbar

vertebrae, the ribs and hips. The range and frequency of anatomical sites with tuberculous lesions were recorded for each carcass examined. Statistical analysis included a comparison of proportions, an χ^2 -test and a test of agreement (Kappa) (33). For all the analyses performed, the confidence level was 95% and P was ≤ 0.05 .

Results

Detailed laboratory examination at the abattoir revealed that 34 (4.5%) of the 751 carcasses had visible lesions and were therefore classified as tuberculous. Samples from these 34 carcasses were sent for culture and *Mycobacterium bovis* was recovered from six of the them. Furthermore, mycobacteriological examination of 32 randomly selected carcasses without visible lesions revealed one culture positive for *M. bovis* (Table I). Additionally, one tuberculous and one apparently normal carcass yielded fast growing mycobacteria, which produced a positive result for catalase activity, but negative results for both niacin production and nitrate reduction.

In contrast, routine abattoir inspection classified only ten of the 751 carcasses examined as tuberculous (Table II). Similarly, as can be seen in Table III, of the 66 specimens examined by culture, routine abattoir inspection classified only ten as tuberculous and 56 as non-tuberculous. Of the ten specimens identified as tuberculous by routine abattoir

Table I
Evaluation of detailed laboratory examination to detect cattle infected with *Mycobacterium bovis*

Detailed laboratory examination	Culture		Total
	Positive	Negative	
Positive	6	28	34
Negative	1	31	32
Total	7	59	66

Sensitivity = 85.7% (42.6-99.2)
Specificity = 52.5% (39.2-65.5)
Kappa (κ) = 0.13

Table II
Comparison of routine abattoir inspection and detailed laboratory examination to detect tuberculous lesions

Routine abattoir inspection	Detailed laboratory examination		Total
	Positive	Negative	
Positive	10	0	10
Negative	24	717	741
Total	34	717	751

Sensitivity = 29.4% (15.7-47.7)
Kappa (κ) = 0.44

inspection, culture confirmed *M. bovis* in only two, and of the 56 identified as non-tuberculous, five of these tested positive for *M. bovis* (Table III).

In a comparison of the efficacy of direct microscopy using 18 well-processed tuberculous specimens, only four AFB were recovered. Two of the positive smears proved negative upon bacteriological examination (Table IV).

The frequency of tuberculous lesions according to the anatomical sites involved is shown in Table V. About 84% of the lesions were found in the thoracic lymph nodes and the lungs. Tuberculous lesions were not detected in the tissues of the liver, kidneys and mammary gland or in the mandibular, sublingual, prefemoral and supramammary

Table III
Evaluation of routine abattoir inspection to detect cattle infected with *Mycobacterium bovis*

Routine abattoir inspection	Culture		Total
	Positive	Negative	
Positive	2	8	10
Negative	5	51	56
Total	7	59	66

Sensitivity = 28.6 (5.1-69.7)
Specificity = 86.4% (74.5-93.6)
Kappa (κ) = 0.13

Table IV
Comparison of direct microscopic examination and bacteriological examination to confirm mycobacterial infection

Direct microscopy	Culture		Total
	Positive	Negative	
Positive	2	2	4
Negative	2	12	14
Total	4	14	18

Kappa (κ) = 0.36

Table V
Distribution of tuberculous lesions in infected cattle

Tissue	Number of positive specimens	Percentage	<i>Mycobacterium bovis</i> positive	Percentage
Tracheobronchial lymph node	26	42.6	8	30.8
Mediastinal lymph node	21	34.4	6	28.6
Medial retropharyngeal lymph node	6	9.8	3	50
Lungs	4	6.6	3	75
Mesenteric lymph node	2	3.3	1	50
Parotid lymph node	1	1.6	1	100
Prescapular lymph node	1	1.6	1	100
Total	61		23	37.7

lymph nodes. The mean number of lesions per infected animal was 1.8 and about 56% of animals possessed only a single lesion.

A sex-dependent study revealed that 0.7% of males and 2.8% of females were tuberculous upon detailed laboratory examination. The difference between these results is not statistically significant. Similarly, no statistically significant differences in infection rates were found for the various age and different body score categories (Table VI).

Discussion

In this study, detailed laboratory examination found that 4.5% (n = 34) of animals were tuberculous (Table I). This finding is consistent with previous reports (4, 13, 36) and may indicate a high tuberculosis infection rate in the general population. Only 29.4% (n = 10) of cattle with

Table VI
Association of *Mycobacterium bovis* infection with various risk factors

Variable	Number of animals examined	Number (percentage positive)	95% CI	χ^2
Sex				
Male	679	5 (0.74)	0.2 - 1.7	2.94
Female	72	2 (2.80)	0.3 - 9.7	
Age				
< 2 years	161	0	0.0 - 2.3	1.95
2-5 years	154	2 (1.30)	0.25-1	
> 5 years	436	5 (1.10)	0.4 - 2.7	
Body condition score				
Lean	302	2 (0.70)	0.1 - 2.4	3.01
Medium	278	5 (1.80)	0.6 - 4.1	
Fat	171	0	0.0 - 2.1	

CI: confidence interval

visible lesions (Table II) were detected by routine abattoir inspection, which means that the abattoir necropsy procedure failed to detect an estimated 70.6% of tuberculous animals. This finding is consistent with earlier reports (4, 36) and indicates that abattoir inspection procedures should be improved, given that the sensitivity of gross post-mortem examination is affected by the method employed and the anatomical sites examined (10). In line with this, several studies have proved that the prevalence of tuberculosis infection increases with enhanced meat inspection (multiple slicing of organs and glands) (4, 10, 13, 35).

When the results of detailed laboratory examination and routine abattoir inspection were compared to the gold standard test (culture of *M. bovis*), the sensitivity of the laboratory examination proved to be superior to that of routine inspection (85.7% versus 28.7%). In the detailed laboratory examination, lymph nodes were trimmed of superficial fat before visual examination. Additionally, in the laboratory, the lymph nodes and organs were sliced at 2 cm intervals for visual examination. Corner (10) and de Kantor *et al.* (13) reported similar differences between the levels of sensitivity of laboratory examination and routine abattoir inspection. Detailed laboratory examination alone detected 85.7% of all the lesions (Table I). A previous study in Australia (11) similarly reported that a detailed necropsy procedure alone detected 84.7% of all lesions. Detailed laboratory examination can therefore be considered a satisfactory procedure to detect tuberculous lesions.

The low specificity (and lower Kappa value of 0.13) of detailed laboratory examination (Table I) may indicate misclassification of non-tuberculous lesions but might also possibly be due to the absence of viable mycobacteria in calcified tuberculosis lesions. In completely calcified lesions, tubercle bacilli are dead and therefore no growth will be obtained upon culture (16, 29).

The low agreement ($k = 0.36$) between direct microscopy and bacteriology (Table IV) complies with a previous report from Ethiopia (36) and indicates that AFB other than mycobacteria (e.g. *Nocardia*) may have produced the spurious results. The mycobacteria may also have been killed during decontamination or the smear may have been contaminated by environmental mycobacteria during staining. Irrespective, direct microscopy is considered to be the least sensitive of all diagnostic methods for the detection of AFB (5, 8).

In the present study, gross tuberculosis lesions were found most frequently in lymph nodes of the thoracic cavity (77%), then in lymph nodes of the head (11.5%), followed

by the lungs (7%). This finding is consistent with previous reports (28, 35), and may indicate infection by the aerosol route. However, other studies (23, 31) reported lymph nodes of the head as being the most frequently infected tissue. Another important finding in this study was the relatively higher *M. bovis* isolation rate from tuberculous lesions in the lungs and lymph nodes of the head (Table V), probably indicating active infection. Similarly, McIlroy *et al.* (21) confirmed *M. bovis* in 73% of samples from tuberculous lungs.

In this study, the average number of infected tissues per infected carcass was 1.8 (Table V) and about 56% of animals possessed only a single lesion. This finding is consistent with a previous report (11) and emphasises the possibility of missing a tuberculous carcass during routine activities. The failure to detect a lesion during abattoir inspection has greatest significance in cattle with a single lesion since if the lesion is missed, there is no further chance of detecting the disease in that animal. As opposed to earlier findings (11, 36), tuberculous lesions were not found in the mandibular and sublingual lymph nodes or in the mammary gland. This may be the result of a lower level of generalised tuberculosis in this environment compared to other environments, such as the abattoir in Addis Ababa which was the subject of a previous report by the authors (4).

The absence of significant differences in infection rates based on age and sex and body condition scores was consistent with previous reports (4, 36). This indicates the presence of other factors that may play a significant role in the spread of tuberculosis in this environment. Possible risk factors include management (as related to confinement), herd size and location, in relation to proximity to already infected farms (3). However, a higher proportion of females were infected, possibly due to their longer productive life and other stressful factors (such as pregnancy, parturition, lactation, etc.) associated with female animals (23, 30).

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Lésions tuberculeuses non détectées par les inspections systématiques pratiquées dans les abattoirs : l'expérience de l'abattoir municipal d'Hossana, sud de l'Éthiopie

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Résumé

L'efficacité des procédures d'inspection des viandes utilisées pour la détection des bovins tuberculeux a été évaluée en recherchant la tuberculose bovine chez 751 animaux. L'étude comportait l'inspection systématique à l'abattoir, la collecte de tissus pour un examen détaillé au laboratoire et l'étude bactériologique en vue d'identifier la présence de *Mycobacterium bovis*.

Sur les 751 carcasses examinées, 34 (4,5 %) présentaient des lésions tuberculeuses. L'inspection systématique à l'abattoir n'a permis de détecter que 29,4 % des carcasses ayant des lésions visibles. On a trouvé 84 % des lésions tuberculeuses dans les poumons et les ganglions lymphatiques thoraciques, 11,5 % dans les ganglions lymphatiques de la tête et les 4,5 % restants dans les ganglions mésentériques et autres des carcasses. De plus, on a isolé *M. bovis* dans une carcasse qui ne présentait aucune lésion tuberculeuse à l'examen macroscopique. En raison de la faible sensibilité des inspections systématiques à l'abattoir, il est nécessaire d'améliorer les procédures d'autopsie existantes.

Mots-clés

Abattoir – Bactériologie – Examen détaillé – Ganglion lymphatique – Inspection systématique – Mycobactérie – Sensibilité – Tuberculose bovine .



Lesiones tuberculosas inadvertidas al efectuar inspecciones de rutina en el matadero: la experiencia del matadero municipal de Hossana (sur de Etiopía)

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Resumen

Los autores describen un estudio destinado a evaluar la eficacia de los procedimientos de inspección de la carne que se utilizan para detectar ganado tuberculoso. A tal efecto se analizaron los despojos de 751 animales en busca de indicios de tuberculosis bovina. Además de la inspección de rutina practicada en el momento del sacrificio, el estudio comprendía la extracción de tejidos para su examen detallado en laboratorio y el estudio bacteriológico para identificar colonias de *Mycobacterium bovis*.

Se encontraron lesiones tuberculosas en 34 de las 751 canales examinadas (un 4,5%). En las inspecciones de rutina practicadas en el matadero se detectaron sólo un 29,4% de las canales con lesiones visibles. Un 84% de las lesiones se encontraban en los pulmones y nódulos linfáticos torácicos, un 11,5% en nódulos

craneales y el restante 4,5% en otros nódulos linfáticos, principalmente los mesentéricos. Además se aisló *M. bovis* a partir de una canal que no presentaba lesiones tuberculosas macroscópicas. La baja sensibilidad de las inspecciones rutinarias practicadas en el matadero hace imprescindible mejorar los actuales procedimientos de necropsia.

Palabras clave

Bacteriología – Examen detallado – Inspección de rutina – Matadero – Micobacteria – Nódulo linfático – Tuberculosis bovina – Sensibilidad.



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