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Isolation and molecular characterization of non tuberculosis mycobacteria from skin positive reactors and pathological lesions of cattle at Bako, Western Ethiopia

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A cross-sectional study was conducted on 858 cattle (371 recruited from Bako Agricultural Research Center, BARC and 487 from Bako municipal abattoir) from October 2009 to April 2010 to isolate and characterize Mycobacterial species from Skin positive reactors and pathological lesion positive cattle. To this effect, comparative intradermal tuberculin (CIDT) test, post mortem examination, bacteriological culturing, and genus typing using multiplex polymerase chain reaction (PCR) were applied. On the basis of CIDT, prevalence of positive reactors was 1.3% (95% CI: 0.15; 2.45) at the cut-off point of ≥4 mm following the recommendation of the World Organisation for Animal Health (OIE), and 9.0% (95% CI: 6.0; 12.0) following the recently suggested cut-off of >2 mm. Apart from sex (P=0.047), there was no association between host related risk factors and positive reactors upon univariate analysis. However, animals in the age category of 2 to 5 years, those with medium body condition score and cattle born on farm were at high relative risk of infection than the other categories. Lesion positivity in slaughtered cattle was 9.3% (95% CI: 6.3; 11.8). Lesions were more frequent and severe in the mesenteric (60.9%), retropharyngeal (17.4%), and bronchial (17.4%) lymph nodes of slaughtered cattle. Culture positivity in suspicious tissues was 32.6% (29/89) out of which 69% (20 of 29) was confirmed to be acid-fast bacilli (AFB) on Ziehl Neelsen staining. Further characterisation of these isolates using genus typing (multiplex polymerase chain reaction) revealed that all of the isolates were members of the non-tuberculous mycobacteria (NTM). The isolation of NTM from tubercle lesions underlines their roles in causing lesions, which are similar with lesions caused by *Mycobacterium bovis*. Therefore, further investigations to identify the specific species, the source of infections, transmission route and the pathogencity of NTM in specific host is recommended.

Key words: Bovine tuberculosis, non-tuberculous mycobacteria, grazing cattle, genus typing, Ethiopia.

INTRODUCTION

Bovine Tuberculosis (BTB) is a chronic infectious disease characterised by progressive development of granulomatous lesions or tubercles in the lung tissue, lymph nodes, or other organs of infected animals.

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Mycobacterium bovis (*M. bovis*), the causative agent of BTB, together with other highly related bacteria, forms a complex, the *M. tuberculosis* complex (MTC), a group of genetically very closely related (but distinct) mycobacteria (Imaeda, 1995). The MTC comprises seven members consisting of *M. bovis*, *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium canetti Mycobacterium caprae, and Mycobacterium pinnipedii* (Aranaz et al., 2003; Cousins et al., 2003; Smith et al., 2006). Among MTC, *M. bovis* has a wide range of target organs (lungs, gastrointestinal tract, mammary gland, kidney and reproductive organs) as well as mammalian hosts (wild animals, domestic animals and humans) (Phillips et al., 2002; Rupp et al., 2007).

Infection of cattle with *M. bovis* constitutes a human health hazard and economic implications in terms of trade restrictions and productivity losses (Phillips et al., 2002; Villarreal-Ramos et al., 2003). In low-income countries, particularly in Africa, the epidemiology and public health significance of BTB is largely unknown for politico-economic reasons, including the high costs of a sustainable testing programme, social unrest due to political instability and ethnic wars, resulting in the displacement of large numbers of human and animal populations, scarcity of veterinary expertise and communication networks. In addition, there are few or no laboratories that are capable of isolating and characterising mycobacteria from animal and human beings (Ayele et al., 2004).

In Ethiopia, the endemic nature of BTB in cattle has long been reported (Hailemariam, 1975). Most of the BTB surveys carried out in Ethiopia have been based on tuberculin skin testing and post mortem inspection of carcasses of slaughter animals in a particular locality. These limited and fragmented surveys show that the prevalence of BTB ranges from less than 1 to 50% depending on the production systems under which cattle are kept (Ameni et al., 2006, 2010; Berg et al., 2009; Tschopp et al., 2010a). Most of these studies did not involve the identification and characterisation of the causative agent. As a result, although the primary cause of BTB is assumed to be *M. bovis*, the scenario could be different in grazing cattle. In the present study, skin testing, pathological and bacteriological examinations and molecular characterisation of the isolates were used to establish the etiologic agent of tuberculosis-like lesions in grazing cattle.

MATERIALS AND METHODS

Study site

The study was conducted at Bako Agricultural Research Center(BARC) and Bako Municipal Abattoir, which are located in and around Bako town. Bako town is the center of Bako-Tibe district in Oromia National Regional State of Ethiopia. The town is located 250 km in the west of Addis Ababa, the capital of Ethiopia,

at an altitude of 1650 m above sea level on 37° 09' E and 9° 06' N. The town has hot and humid climate with average relative humidity of 60%. It gets a bimodal pattern of rainfall with the main rainy season extending from June to September and a short rainy season that extends from March to May with an average annual rainfall of 1300 mm. Mean monthly maximum and minimum temperatures are about 28 and 14°C, respectively, with an average monthly temperature of 21°C. The mean daily minimum and maximum temperatures are 9.4 and 31.1°C, respectively (Gebregziabher and Mulugeta, 2006).

Study animals and management

The study cattle were recruited from Bako Agricultural Research Center (BARC) farm and from cattle kept by the traditional mixed crop-livestock farming communities inhabiting around Bako. For the CIDT test, all the apparently healthy cattle above six months of age from the research farm were used. Among the 371 tuberculin tested cattle, 253 (68.2%) were Horro breed and 118 (31.8%) were crossbred (Horro x Holstein) cattle. Horro cattle are described as an intermediate Sanga-Zebu type and are dominant cattle breed in western Ethiopia (Alberro and Hailemariam, 1982). The study cattle from the research farm were managed by semi-intensive production system. They were allowed to graze on natural pasture (in herds of 30 to 40 cattle) during the day for 8 h. Thereafter, they were housed (in herds) in a barn with concrete floor and corrugated iron roof. The barn was cleaned daily and disinfected with 5% formalin at monthly interval. All animals were routinely monitored for any health problem and those above six months of age were annually vaccinated against Blackleg, Anthrax, Bovine Pasteurellosis, Lumpy Skin Disease (LSD) and Foot and Mouth disease (FMD). All the animals were de-wormed with anthelmintics and sprayed with acaricide regularly depending on the degree of parasite infestation and season of the year.

For Abattoir based survey, cattle slaughtered at Bako municipal abattoir were used. The slaughtered animals were purchased from local livestock markets by butchers and were brought to the abattoir for slaughter. All the slaughtered animals were indigenous Horro cattle managed under integrated extensive production system where cattle are kept primarily for draught power.

Study design and sampling

A cross-sectional study design with census sampling method was applied both in the research farm and Abattoir. Using this design, 371 cattle were recruited from the research farm and 487 were sampled from the Abattoir during the four months study period.

Comparative intradermal tuberculin (CIDT) test

While testing the study cattle by CIDT, body condition of each of the study animal was scored using the established guidelines for body condition scoring (Nicholson and Butterworth, 1986). For the CIDT test, two sites on the right side of the mid-neck, 12 cm apart in a horizontal line were shaved and the initial skin thicknesses were measured with calipers. One site was injected intradermally with an aliquot of 0.1 ml containing 2500 IU/ml bovine purified protein derivative (PPD) (Veterinary Laboratories Agency, Addlestone, Surrey KT15 3NB, UK).Similarly, 0.1 ml of 2500 IU/ml avian PPD (Veterinary Laboratories Agency, Addlestone, Surrey KT15 3NB, UK) was injected into the second site. After 72 h, the skin thicknesses at the injection sites were measured and registered. Test results were interpreted according to the recommendations of the Office International des Epizootics (OIE, 2009) for all animals and the recommendation for local animals (Ameni et al., 2008).

Post mortem examination and pathology scoring

The lungs, lymph nodes and liver were removed by the investigator for the investigation of tubercle lesions. Each of the seven lobes of the two lungs were thoroughly inspected and palpated for any suspicious gross tuberculous lesions. Each lobe was sectioned into 2 cm thick slices to facilitate the detection of lesions. Similarly, mandibular, retropharyngeal, cranial and caudal mediastinal, left and right bronchial, hepatic, and mesenteric lymph nodes were sliced into thin sections and inspected for the presence of visible lesions (FAO, 2000). When gross lesions suggestive of BTB were found in any of the tissues, the animal was classified as having lesions. Furthermore, the severity of the gross lesions was scored by the semi-quantitative procedures described earlier (Ameni et al., 2007a, Vordermeier et al., 2002). Briefly, lesions in the lobes of the lungs were scored separately as follows: 0 = no visible lesions; 1 = no gross lesions but lesions apparent on slicing of the lobe; 2 = fewer than five gross lesions; 3 = more than five gross lesions; 4 = gross coalescing lesions. The scores for the individual lobes were added up to calculate the lung score. Similarly, the severity of gross lesions in individual lymph nodes was scored as follows: 0 = no gross lesions; 1 = small lesion at one focus (just starting); 2 = small lesions at more than one focus; 3 = extensive necrosis.

Isolation of mycobacteria

Lesions suspicious of tuberculosis upon postmortem examination were collected and placed in a 50 ml tubes with screw caps containing 5 ml of sterile 0.9% saline. A total of 89 specimens were collected from 46 cattle and transported in ice box to Aklilu Lemma Institute of Pathobiology (ALIPB, Addis Ababa). In the laboratory, the specimens were sectioned using sterile blades and then homogenised with a mortar and pestle. The homogenate was decontaminated by adding an equal volume of 4% NaOH by centrifugation at 3,000 rpm for 15 min. The supernatant was discarded, and the sediment was neutralised by 1% (0.1 N) HCl using phenol red as an indicator. Neutralisation was achieved when the color of the solution changed from purple to yellow (OIE, 2009). Next, 0.1 ml of suspension from each sample was spread onto a slant of Lowenstein-Jensen (LJ) medium. Duplicate slants were used, one enriched with sodium pyruvate and the other enriched with glycerol. Cultures were incubated aerobically at 37°C for about 10 weeks with weekly observation for growth of colonies. Cultures were considered negative if no visible growth was detected after 10 weeks of incubation. In the presence of visible growth of colonies, microscopic examination of cultures using the Ziehl–Neelsen (ZN) staining method was performed to select acid-fast bacilli (AFB) positive isolates.

Molecular typing

AFB positive isolates were heat-killed by mixing approximately 2 loopfuls of colonies in 200 μl distilled H_2O followed by incubation at 80°C for 45 min. Thereafter, Multiplex polymerase chain reaction (m-PCR) was used to confirm the presence of genus *Mycobacterium* in the isolate and to differentiate *M. tuberculosis* complex from *M. avium* complex, and other mycobacterial species following the standard operating procedure developed previously (VLA, 2005). The PCR targets the sequence of the genus *Mycobacterium* within the 16S rRNA gene (G1, G2), sequences within the hyper-variable region of 16S rRNA that is known to be specific to *M. avium* (MYCAV-R), and the MTB70 gene specific for *M. tuberculosis* complex. Heat killed AFB positive samples were used as source of DNA template. The reaction was carried out using Thermal Cycler (Applied Biosystems, GeneAMP 9700). Each PCR tube consisted of 5.2 μl H_2O, 8 μl HotStarTaqMasterMix, 0.3 μl

of each of the primers, 5 μl of DNA templates of samples or controls making the total volume 20 μl. *M. avium*, and 2122/97 (*M. bovis* strain) were used as positive controls while H_2O (Qiagen), was used as a negative control. The mixture was heated for 15 min at 95°C, followed by 35 cycles of 1 min denaturation at 95°C, 1 min annealing at 61°C, and 1 min extension at 72°C; and final extension was done for 10 min at 72°C. The PCR product was electrophoresed in 1% agarose gel in 1XTAE running buffer. Ethidium bromide at a ratio of 1:200000 in 1% agarose gel, 100 bp DNA ladder, and orange 6x loading dye were used during gel electrophoresis. The gel was visualised in Multi–imageTM light cabinet using Alpha Innotech version 1.2.0.1 (Alpha Innotech Corporation). All members of the Genus *Mycobacterium* produced a band of 1030 bp, *M. avium* or subspecies such as *M. avium* subspecies *paratuberculosis* produces a band of 180 bp, while members of *M. tuberculosis* complex produce a band with 372 bp.

Data management and analysis

The data were coded and analysed using SPSS for Windows (Version 14.0; Statistical Package for Social Sciences Inc, Chicago, Illinois, USA). Proportions were used to summarise categorical exposure and outcome measures. Similarly, descriptive statistics such as mean and standard error of the mean were used to summarise pathology scores. Univariate and multivariate logistic regression analysis were conducted to examine the relationship between the outcome variable and selected animal risk factors. Odds Ratio (OR) with 95% confidence intervals (CI) was used to assess the level of association of prevalence to the different host factors. A statistically significant association between the different variables were said to exist if the estimated P-value is less than 0.05 (P<0.05) and the 95% confidence interval (CI) for OR doesn't include 1.0.

RESULTS

Prevalence of bovine tuberculosis

An apparent positive reactor at cut-off point of ≥4 mm following the recommendation of OIE was 1.3% (95% CI: 0.15; 2.45). On the other hand, the prevalence of non-specific reactors was 3.5% (95% CI: 1.6; 5.40). On the basis of the newly defined cut-off value of ≥2 mm (Ameni et al., 2008), the apparent positive reactors was increased to 9.0% (95% CI: 6.0; 12.0). Apart from sex (P=0.047), there was no association between breed, age, body condition, origin, pregnancy, and parity and positive reactors upon univariate analysis. However, animals in the age category of 2-5 years, those with medium body condition score and cattle born on farm were at high risk of infection than the other categories (Table 1).

Lesion prevalence was calculated for the 5 tuberculin reactor cattle from research farm and 487 slaughter cattle recruited from Bako municipal abattoir. Overall lesion prevalence was 9.3% (95% CI: 6.3; 11.84). Suspected TB lesions were observed in different tissues of the study animals (Table 2). There was a significant difference (x^2=91.174; P=0.000) in occurrence of lesions in different tissues of the study animals. Of the five skin test positive cattle killed for post mortem examination, tubercle lesions were detected in 3 (60%) of the animals for which lesion

Table 1. Logistic regression analysis of host related risk factors with tuberculin test positivity using the >2 mm cut-off point.

Variables	Number examined	Number positive (%)	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Sex				
Male	83	12(14.5)		
Female	288	21(7.3)	0 .46(0.22-0.99)	0.48(0.21-1.11)
Age group				
≤2	87	7(8.0)		
2-5	109	17(15.6)	2.11(0.83-5.35)	0.79(0.23-2.63)
5-8	72	4(5.6)	0.67(0.19-2.39)	0.17(0.03-0.94)
8-12	48	3(6.3)	0.76(0.19-3.09)	0.37(0.07-1.93)
>12	55	2(3.6)	0.43(0.08-2.15)	0.11(0.01-0.86)
Breed				
Local	253	27 (10.7)		
Cross	118	6(5.1)	0.45(0.18-1.12)	0.27(0.08-0.89)
Body condition				
Thin	36	2(5.6)		
Medium	299	29(9.7)	1.83(0.41-7.99)	1.73(0.35-8.61)
Good	36	2(5.6)	1(0.13-7.51)	1.95(0.21-18.30)
Origin				
Purchased	120	10(8.3)		
Born on farm	251	23(9.2)	1.11(0.5104-2.41)	0.52(0.18-1.49)

Table 2. Proportion and severity of lesioned tissues of bovine tuberculosis in cattle slaughtered at Bako municipal abattoir, Western Oromia, Ethiopia.

Tissues	Number examined	Number positive (%)	Lesion severity (Mean±SEM)
Lung lobes			
Left apical	46	1(2.2)	0.04±0.04
Left cardiac	46	2(4.3)	0.13±0.09
Left diaphragmatic	46	2(4.3)	0.15±0.11
Right cardiac	46	1(2.2)	0.04±0.04
Right accessory	46	1(2.3)	0.04±0.04
Right diaphragmatic	46	2(4.3)	0.17±0.12
Lymph nodes			
Mandibular			
Retropharyngeal	46	1(2.2)	0.02±0.02
Left bronchial	46	8(17.4)	0.33±0.12
Cranial mediastinal	46	8(17.4)	0.28±0.10
Caudal mediastinal	46	3(6.5)	0.07±0.03
Hepatic	46	4(8.7)	0.13±0.07
Mesenteric	46	6(13.0)	0.22±0.09
Liver	46	28(60.9)	1.11±0.15
	46	4(8.7)	0.15±0.08

Figure 1. Tuberculous lesion detected in mesenteric lymph node of one of the tuberculin skin test positive cattle at Bako Agricultural Research Centre.
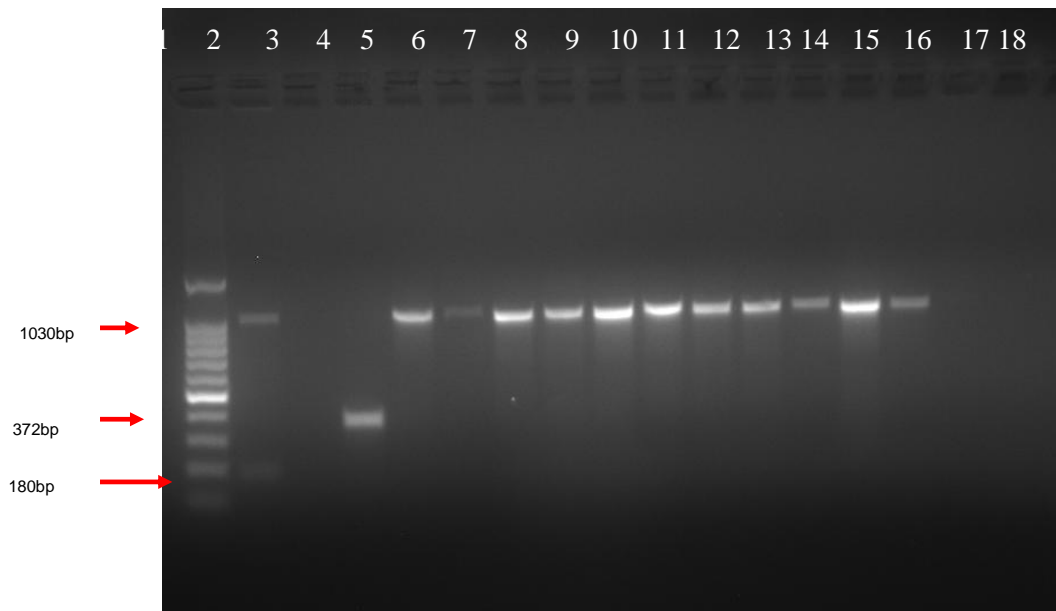
Figure 2. Gel electrophoresis separation of PCR products by multiplex PCR genus typing of mycobacteria isolated from cattle of western Ethiopia. Lane 1= 100 bp Ladder; Lane 2=*M. avium* complex (positive control); Lane 3= Qiagen H$_2$O (negative control); Lane 4= *M. tuberculosis* complex (positive control); Lane 5-18 were isolates from individual cattle with suspected tuberculous lesions; Lane 5-15 were positive for genus Mycobacterium; Lane 16-18 were negative for genus Mycobacterium.

distribution and severity was established. All the three tuberculin reactor cattle had lesions in mesenteric lymph node (Figures 1 and 2); 66.7% had lesions in retropharyngeal and cranial mediastinal lymph nodes. Lung lesion was observed in only one of the tuberculin

reactor cattle.

On the other hand, 8.83% (43 of 487) of slaughter cattle from Bako municipal abattoir had tubercle lesions in one or more tissues/organs of the body. As indicated in Table 2, mesenteric lymph node was found to be the

most severely affected tissue (mean pathology score±SEM, 1.11±0.15). The characteristic lesions observed upon gross pathological examination in the mesenteric and other lymph nodes had distinct enlargement and upon sectioning, the lymph nodes had greenish confluent caseous necrosis (Figure 1) with some calcification of the necrotized tissue, different from the usual yellowish or grey white caseous necrosis which is commonly observed in lesions caused by *M. bovis* infection.

Isolation of mycobacteria

Of the 89 tissue specimens cultured on primary culture media, 29 specimens showed growth, constituting a culture positivity of 32.6%. Colonies of culture positive isolates were processed to demonstrate acid-fast bacilli (AFB) under a microscope. Accordingly, 69% (20 of 29) isolates showed acid-fast bacilli on ZN staining.

Molecular typing

AFB positive samples were further characterised by *Mycobacterium* genus typing. Multiplex polymerase chain reaction (m-PCR) analysis revealed the presence of the genus *Mycobacterium* in 80% (16 of 20) of the isolates of which 11 isolates were depicted by Figure 2. None of the isolates belonged to the *M. tuberculosis* complex. However, all the isolates including those from BTB reactors were identified as NTM.

DISCUSSION

This study provides the first data on tuberculosis in the study area and study animals (Horro breed and their crosses). The overall prevalence of 1.3% (≥4 mm cut-off) found in this study is similar with the prevalence reported by other workers in the traditional farms of Ethiopia in zebu breed (Shihun, 2008; Tschopp et al., 2010a, 2010b). Moreover, in Uganda and Tanzania, different researchers reported comparable animal prevalence in indigenous cattle in different districts (Cleaveland et al., 2007; Inangolet et al., 2008; Oloya et al., 2006; Shirima et al., 2003). However, higher prevalence was recorded in intensive farms in Ethiopia (Ameni et al., 2010; Regassa et al., 2010).

The low positive reactors in the present study could, among others, be due to the fact that the study animals were kept in small herds and widely exercise free grazing in the field, which minimises the transmission of mycobacteria contrary to the intensive farms which favors the spread of mycobacteria because of confinement and overcrowding. Moreover, all the cattle enrolled into the present study were zebu breed, and their crosses which

are relatively resistant to tuberculosis (Ameni et al., 2006; Radostits et al., 2000).

Our study shows apparent prevalence of 8.9% using a cut-off for skin test reaction of ≥2 mm as suggested by Ameni et al. (2008), who stated that maximum sensitivity can be achieved in central Ethiopia using a ≥2 mm cut-off without affecting specificity. A similar finding was reported by Gumi et al. (2011). However, a lower prevalence were reported by other researchers (Tschopp et al., 2010a, 2010b; 2010c; Tschopp et al., 2009) from different areas of Ethiopia. The use of appropriate cut-off point of a diagnostic test is critically important in disease surveillance and hence disease control and eradication.

In the present study, detailed post mortem examination of carcasses revealed an apparent lesion prevalence of 9.3% which is in agreement with the report by Biffa and others (Biffa et al., 2009). In contrast, it is higher than the some reports from Ethiopia (Ameni et al., 2010, Berg et al., 2009; Regassa et al., 2010) although it is lower than other reports from Ethiopia and Tanzania (Cleaveland et al., 2007; Mamo et al., 2011). The frequency and severity of tubercle lesion in slaughter cattle was higher in the mesenteric lymph node than the thoracic lymph nodes. Similarly, few studies reported higher lesion prevalence in alimentary tract of animals managed under similar settings (Cleaveland et al., 2007; Mamo et al., 2011). The localisation of the majority of tuberculosis lesions in mesenteric lymph nodes in free grazing cattle indicates that the ingestion route might be the principal route of transmission (Radostits et al., 2000; Whipple et al., 1996). In a comparative BTB study in grazing and housed cattle, Ameni and others observed a significant difference in lesion distribution between animals farmed intensively and those kept on pasture (Ameni et al., 2007b). Accordingly, digestive tract lesions predominated in animals kept on pasture whereas animals in intensive farms were presented with lesions predominantly in the respiratory tract.

Molecular characterisations of AFB positive isolates using m-PCR revealed that all of the isolates were NTM. The characterisation of these NTM from traditionally free grazing cattle with gross tuberculous-like lesions might indicate the role of NTM in epidemiology of animal tuberculosis in the study area. However, a definitive conclusion about the role of NTM in the epidemiology of animal tuberculosis in the whole breeding region of western Ethiopia is not possible because of the small number of animals examined in this study. On the other hand, the characterisation of NTM from tuberculous tissues of cattle from the research farm and abattoir could indicate their role in hindering the detection of TB in cattle in the area and the possible underestimation of the prevalence of TB in studies that depend on tuberculin testing. To this effect, the low prevalence of TB in cattle from the research farm in our study might be due to the effect of NTM.

The characterisation of NTM from TB reactors in our

study may be due to mixed-infection of the animals with *M. bovis* and NTM. As NTM infection is widespread in grazing cattle in the area, tuberculin reactor cattle from the research farm (80% of reactor animals from the research farm were those purchased from local market and introduced into the herd) may be infected with both NTM and *M. bovis*. It is known that NTM tends to outgrow the slowly growing *M. bovis* in culture. In contrary, animals that showed positive result for both tuberculin test and NTM could possibly be due to false positive reaction to *M. bovis* as a result of cross-reaction with the antigens contained in the NTM.

In the present study, we demonstrated a high prevalence of environmental mycobacteria in grazing cattle. Previous studies in Ethiopia and Tanzania revealed the association of NTM with granulomatous lesions in cattle and humans (Berg et al., 2009). An extensive study on Ethiopian cattle confirmed that about one third of the isolates from tuberculous lesions were NTM (Berg et al., 2009; Shihun, 2008). Moreover, NTM were associated with tuberculous camel and wildlife populations in Ethiopia (22, 38).

Similar studies in Uganda and Chad reported the isolation of NTM strains from more than 40% of the animals with tuberculous lesions (Diguimbaye-Djaïbe et al., 2006; Oloya et al., 2006). In the present study, the NTM isolates were not characterised to the species and strain level due to lack of laboratory facilities particularly a sequencer to sequence the 16S rRNA locus (Han et al., 2002). It would have been very useful to compare our results with other works where NTM were isolated in the frame work of TB studies in other countries.

In our study, most of the NTM were isolated from mesenteric lymph nodes, suggesting the environmental exposure of the animals through ingestion with fodder or water. Moreover some of the NTM were isolated from bronchial and medisatinal lymph nodes, suggesting aerosol transmission. So far, there are no studies demonstrating the entry route and tissue tropism for NTM in domestic livestock, humans, and wildlife. In this study, the detection of majority of the lesions and NTM in mesenteric lymph nodes could be inherent to the NTM infection route and their tissue tropism as it is the case for digestive tract in Jones disease due to *M. paratuberculosis*.

Although *M. bovis* has been known to be the major cause of BTB in cattle, mycobacteria other than *M. tuberculosis* complex members were isolated from the suspicious lesions in the present study. The isolation of these NTM from tubercle lesions underlines their roles in causing lesions, which are milder as compared to the lesions caused by *M. bovis*. Further investigation for the identification of specific species, the source of infections, transmission route and their pathogencity in specific host is needed. Moreover, the role and public health significance of NTM in human population of the region should be investigated.

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