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Epidemiology of bovine tuberculosis in Butajira, Southern Ethiopia: A cross-sectional abattoir-based study

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A cross-sectional study was conducted at Butajira Municipality abattoir from December, 2009 to April 2010 to investigate the epidemiology of bovine tuberculosis (bTB) in Butajira, Southern Ethiopia. Postmortem examination, mycobacterial culturing and multiplex genus typing techniques were used. An overall prevalence of 9% (40/446) of the animals examined harbor gross tuberculous lesions up on detailed post-mortem examination. Statistically significant difference was observed in the prevalence of bTB between different age groups (χ^2 = 11.441, p= 0.003) and body condition scoring (χ^2 = 10.384, p = 0.006). Higher prevalence of bTB was observed in older animals and animals with poor body condition. Bacteriological culture of the 40 samples gave growth on 13 with 9 of them acid fast Bacilli (AFB) positive. Genus typing of the AFB positive isolates by multiplex polymerase chain reaction (m-PCR) revealed seven non-tuberculous mycobacterium (NTM) and 1 *Mycobacterium tuberculosis* complex (MTBC) isolates. Further characterization of the isolates at specific species and investigation of the disease is recommended for controlling it in livestock and safeguard public health.

Key words: Abattoir, bovine tuberculosis, Butajira, multiplex genus typing, epidemiology, Ethiopia, postmortem examination, prevalence.

INTRODUCTION

Bovine tuberculosis (bTB) is a chronic infectious disease of animals characterized by the formation of granulomas in tissues and organs. It is caused by slowly growing non-photochromogenic bacilli members of the *Mycobacterium tuberculosis* complex (MTBC): *M. tuberculosis, Mycobacterium africanum Mycobacterium microti, Mycobacterium bovis, Mycobacterium canetti* and *Mycobacterium caprae* species (Radostits et al., 2000; Thoen et al., 2006).

bTB has been significantly widely distributed

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Abbreviations: AFB, Acid fast Bacilli; **bTB,** bovine tuberculosis; **m-PCR**, multiplex polymerase chain reaction; **MTBC,** *Mycobacterium tuberculosis* complex; **NTM,** non-tuberculous mycobacterium.

throughout the world and has been a cause for great economic loss in animal production and the most frequent cause of zoonotic TB in man (Tenguria et al., 2011). Moreover; in developing countries, still constitutes a major threat to public health where surveillance and control activities are often inadequate or unavailable (Ayele et al., 2004). bTB in humans is becoming increasingly important in developing countries, as humans and animals are sharing the same micro-environment and dwelling premises, especially in rural areas. Moreover, due to the association of mycobacteria with the high prevalence of HIV/AIDS in the developing world and susceptibility of HIV/AIDS patients to tuberculosis in general, the situation change is most likely (Amanfu, 2006).

bTB is one of the endemic infectious diseases that have long been recorded in Ethiopia (FAO, 1967; Hailemariam, 1975). Reports of abattoir based surveys showed varied prevalence that range from 1.48 to 15.4% in different municipal and export abattoirs of the country (Asseged et al., 2004; Teklu et al., 2004; Shitaye et al., 2006). However, still, there is lack of knowledge on the actual prevalence and distribution of the disease at a national level. Accurate and sound scientific baseline prevalence data of bTB across a range of eco-epidemiological settings is needed to ensure public health policies and disease control strategies. Thus, the study was carried out in Butajira Manucipital abattoir, Meskan district of the Southern Ethiopia to determine the epidemiology of bTB in slaughtered cattle.

MATERIALS AND METHODS

Study area and animals

The study was conducted from December 2009 to April 2010 in Butajira municipality abattoir, Meskan Woreda, Southern Nations Nationalities and Peoples Regional State (SNNPR) in Ethiopia. The area is located 130 km south of Addis Ababa, with Butajira being the main town. It has varying climates zones from arid dry lowland areas around 1500 m.a.s.l altitude to cool mountainous areas above 2000 m.a.s.l. The area is rich in livestock population; 123,495 bovine, 2,532 ovine, 19,231 caprine and 10,475 equine (CSA, 2004). According to the available logistics and time, a total of 446 apparently normal animals slaughtered in the abattoir from December 2009 to April 2010 were included in the present study. The major sources of cattle to this abattoir were Sulte, Enceno, Makecho, Threeamba and Draama.

Study design and sampling techniques

A cross-sectional study was designed to determine the prevalence and assess the potential risk factors of bTB. Tissue lesion samples suspected to be positive for bTB were sampled aseptically from suspicious organs.

Individual animal identification number, place of origin, breed, sex, ante-mortem examination findings were recorded at the animal quarantine stations before submission to slaughter houses. Age categorization was made using dental eruption and wear as described by Amstutz (1998). Physical examination of animals including body condition scores, history of animals, age groups were carried out before they were slaughtered. Body condition scoring was done using the method developed for zebu cattle (Nicholson and Butter worth, 1986). Parameters like sex, age, origin and body condition score were assessed for the presence of possible association with the presence of TB lesion.

Post-mortem examination

Post mortem examination was done as described by Corner (1994). Briefly, detailed inspection of lymph nodes and organs that are reported to be frequently affected were done under bright light source. Lymph nodes were incised into slices of 2 cm using separate surgical blades in order to facilitate detection of tubercular lesions. The slices were then examined for the presence of tubercular pathological lesions (Gracey and Collins, 1992). Tissues with suspected lesions were collected separately in sterile universal bottles with phosphate buffer saline solution and kept at +4°C at the Butajira District Veterinary Clinic before being transported once per week to the TB laboratory of AHRI (Neill et al., 1992). Type and stage of tuberculosis lesion, frequency of infection of anatomical sites were also recorded for individual tuberculous suspected cattle.

Isolation and identification of mycobacteria

Specimens collected from tuberculosis suspected slaughtered cattle were processed according to OIE established standard protocols (OIE, 2004). The specimens were sectioned into pieces using sterile blades, and homogenized by pestle and mortar for 10 min. The homogenate was decontaminated by adding an equal volume of 4% NaOH for 15 min followed by centrifugation at 3000 rpm for another 15 min. The supernatant was discarded while the sediment was neutralized by 1% (0.1N) HCl using phenol red as an indicator. Neutralization was achieved when the color of the solution changed from purple to yellow (WHO, 1998). Thereafter, 0.1ml of suspension from each sample was spread onto a slope of Lowenstein-Jensen (LJ) medium. Duplicates of LJ were used; two enriched with sodium pyruvate while the other two was enriched with glycerol. Cultures were incubated aerobically at 37°C for about 5-8 weeks with weekly observation for growth of colonies (Vestal, 1998). Identification of the mycobacterial species was performed based on the rate of growth and colony morphology and growth on pyruvate or glycerol supplemented LJ media. Growth was considered when mycobacterial colony was observed and examined using the Ziehl-Neelsen technique for confirmation of AFB (de Kantor et al., 1998).

Molecular typing of isolates

AFB positive isolates were heat-killed by mixing approximately 2 loopful of colonies in 200 µl distilled H_2O followed by incubation at 80°C for 45 min. Following the standard procedure by Wilton and Cousins (1992), multiplex polymerase chain reaction (m-PCR) was used to confirm the presence of genus Mycobacterium in the isolate and to differentiate MTBC from *Mycobacterium avium* complex, and other mycobacterial species.

Data collection, management and statistical analysis

Data related to age, breed, body condition and origin of each animal were recorded on a data sheet during the ante mortem examination. Presence or absence of TB-like lesions and affected tissue(s) were recorded on postmortem examination. The recorded data were entered into Microsoft Excel data sheets and analyzed

Table 1. Association of animal risk factors with tuberculous lesions.

Risk factor	Number of examined	Number positive (%)	95% CI	χ^2	P Value
Age (years)				11.44	
<5	70	8(11.4%)	5.07-21.28		0.003
5-8	325	25(7.7%)	4.54-10.43		
>8	51	7(13.7%)	5.70-26.26		
Sex				0.32	
Male	329	29 (8.8%)	5.48-11.76		0.57
Female	117	11 (9.4%)	4.79-16.19		
Body condition				10.38	
Poor	62	9(14.5%)	6.86-25.78		0.006
Medium	284	24(8.5%)	5.49-12.31		
Good	100	7(7%)	2.86-13.89		
Origin of animals				2.26	0.68
Sulte	88	9 (10.2%)	4.78-18.53		
Inceno	119	8(6.7%)	2.95-12.82		
Draama	101	11(10.9%)	5.56-18.65		
Makecho	79	5(6.3%)	2.08-14.16		
Threeamba	59	7(11.9%)	4.91-22.93		

Table 2. Distribution of lesions in lymph nodes with their respective frequency of occurrence.

Anatomical site	Organ affected	Frequency (%)
Head	Sub-mandibular lymph nodes	3 (7.5)
	Retropharyngeal lymph nodes	1 (2.5)
Thoracic	Tracheo-bronchial Lymph nodes	14 (35)
	Mediastinal Lymph nodes	11 (27.5)
Abdomen	Mesenteric Lymph nodes	11 (27.5)
	Total	40 (100)

using SPSS 17.0 statistical software. Descriptive statistics was used to determine the proportion of cattle carcass harboring tuberculous lesions. The difference between the effects of different risk factors on prevalence was analyzed. A statistically significant association between variables was said to exist if the calculated P<0.05. The range and frequency of anatomical sites with tuberculous lesions were recorded for each carcass examined. For all the analysis performed, P ≤ 0.05 was taken as statistically significant.

RESULTS

Prevalence and analysis of associated risk factors

The prevalence of animals with suspicious tuberculous lesions was 9% (95% CI: 6.48-12.01). The association of different risk factors responsible for the occurrence of the disease is depicted in Table 1. Accordingly, statistical significant differences were observed between tuberculosis lesion prevalence and age and body.

Distribution and location of pathological lesions

The frequency and distribution of lesions according to organ level and anatomical sites is indicated in Table 2. 62.5% (25/40) of the total gross lesions observed was from the lymph nodes of thoracic region followed by 27.5% (11/40) in the mesenteric lymph nodes and 10% (4/40) of gross lesions were detected in the lymph nodes of the head.

Mycobacteriology and microscopy

Outof thetotal40tuberculous lesions mycobacteriologically processed and cultured, growth was observed in 13 with nine of them acid fast bacilli (AFB) positive.

Figure 1. Gel electrophoresis separation of polymerase chain reaction products of multiplex genus typing of the genomic DNA of mycobacteria isolated from cattle with grossly suspicious TB lesions. Lane: 1 = a ladder of band at an interval of 100 bp DNA; 2 = *Mycobacterium avium* (positive control); 3 = Qiagene-water (negative control); 4 = *Mycobacterium tuberculosis* complex (positive control); Lanes 5-13 are isolates from individual cattle with tuberculous lesions; Lanes 5-7, 9-11 and 13 are positive samples for genus Mycobacterium (1080 bp); Lane 8 is positive for *Mycobacterium tuberculosis* complex (372 bp); Lane 12 is negative for the genus mycobacterium.

Genus typing of AFB isolates

Genus specific m-PCR typing of 9 AFB positive isolates showed a PCR product size of 1030 bp for 7 isolates and 372 bp for 1 isolate which is specific for NTM and MTBC, respectively. While 1 isolate did not show a signal at all (Figure 1).

DISCUSSION

The proportion of slaughtered cattle that harbor tubeculous lesions up on detailed abattoir inspection were 9%. Comparable findings were recorded by Biffa et al. (2009) and Ameni et al. (2001) that reported a prevalence of 10.1 and 8.8%, respectively. The study result was higher than the previous prevalence reports by various authors: 4.5% in Hosanna (Teklu, 2003), 5.2% in Nazreth (Ameni and Wudie, 2003), 1.48% in Addis Ababa (Asseged et al., 2004), 2.4% in Jimma (Jemale, 2005), 3.46% in Addis Ababa (Shitaye et al., 2006) and 5.8 in Setit-Humera (Romha et al., 2013). In contrarst, it was lower the finding by Mamo (2007) who reported a prevalence of 24.7%. These variations could be due to differences origin, type of production system and breed of animals slaughtered in the abattoirs (Romha et al., 2013).

In parallel to previous reports (Corner, 1994; Neill et al., 1994; Collins, 1996, Whipple et al., 1996), large proportions of tuberculous lesions (62.5%) were detected in the lymph nodes of the thoracic region. This suggests that respiratory route is the primary route of transmission and infection (O'Reilly and Daborn, 1995; Ameni and Wudie, 2003; Teklu et al., 2004).

Statistically significant difference was observed in the prevalence of bTB between different age groups (χ^2 = 11.441, p= 0.003) and body condition scoring (χ^2 = 10.384, p = 0.006) up on analysis of different risk factors. Higher prevalence of bTB had been recorded in old aged and poor body conditioned animals. As the age of the cattle increase owning to increased chances of exposure and infection with bTB, Humblet et al. (2009) explicated that those stressors, malnutrition and immuno-suppressants increases with age; thus, older animals are more likely to have been exposed than younger ones. It has been suggested that increased incidence of bTB in older animals can be explained by a declining of protective capability in aging animals (O'Reilly and Daborn, 1995). Similarly, the high prevalence of bTB in poor conditioned cattle could be due to the fact 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 (Collins and Grange, 1994). Moreover, previous studies confirmed that animals with poor body conditions and in nutritional deficiency have reduced immune resistance to bTB

(Doherty et al., 1995).

The culture result of bTB suggestive pathologic lesions was low as compared to other study reports (Ameni et al., 2007, 2010). Failure to grow the major portions of the specimens could have been due to misclassification of non tuberculous lesions (Teklu et al., 2004) caused by other granuloma-causing organisms (Radostits et al., 2000). Fully calcified lesions without viable tubercle bacilli could also give the low recovery of mycobacteria (Pritchard, 1988). Multiplex genus typing of the isolates revealed that out of 9 AFB positive isolates 7 isolates showed signals for the genus mycobacterium (NTM) and 1 showed signal for mycobacterium tuberculosis complex respectively. The isolation of large number of NTM showed the importance of NTM in the epidemiology of bTB to cause tuberculous like lesions. Similar study results from different part of Ethiopia (Shimelis, 2008; Berg et al., 2009; Romha et al., 2013) and other African countries also shows the isolation of several NTM strains from animals with tuberculous lesions (Diguimbaye-Djaïbe et al., 2006; Oloya et al., 2006). Moreover, NTM had been isolated from milk and nasal swab of tuberculin reactor animals in Chifra pastoral district of Afar region, Norht eastern Ethiopia (Ashenafi et al., 2013).

The study supports the endemic nature of bTB in cattle and the potential zoonotic risk of bTB to humans in the study area. Further investigation to reveal the epidemiological significance for public health in the region and to identify the potential risk factors for infection and transmission of bTB among the livestock and at the interface of animals and humans is suggested. Education and awareness creation among community about the economic and public health significance of bTB is also important to design a feasible community-based control program.

Conflict of Interests

The authors have not declared any conflict of interests.

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