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Slaughter house surveillance for tuberculosis among cattle in Ri-Bhoi district of Meghalaya

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Abstract
The study was carried out to investigate bovine tuberculosis by necropsy inspection in different abattoirs of Meghalaya. Gross visual lesions were found in 26 carcasses. Suspected 39 tissue samples were collected from 26 carcasses. Pre-culture stain revealed 30 (76.92%) and culture growths were from 31 (79.49%) tissue samples. Highest lesions were recorded in lymph nodes (48.71%) followed by lungs (28.20%) and liver (23.07%). Pre-scapular (47.37%) and retropharyngeal (31.58%) contribute more lesions than other lymph nodes. Visual inspection may serve as good screening method for tuberculosis infected carcasses although pre culture staining and culture of bacteria from suspected lesion samples is necessary to have a concluding remark about the infection. The study also confirms the endemic status of bovine tuberculosis in these areas.

Keywords: Mycobacterium, Necropsy, culture, abattoir

Introduction
In many developing countries bovine tuberculosis (BTB) is a major infectious disease among domesticated animals and certain captive wild animals. It is estimated that *M. bovis* is responsible for about 5% of all TB infection in human (Michel *et al.* 2010) [5]. India possesses more than 16% of world cattle population. Cattles are also considered as natural host of *M. bovis*. Milk from healthy lactating cows had been reported to shed *M. bovis* bacilli (Danbirni *et al.* 2010) [3]. Ingestion of beef from infected cattle can be a major threat to public health as cooking may not always be an effective against *M. bovis* infection (van der Merwe *et al.* 2009) [7].

Abattoirs, butcher shops provide an ideal environment as a monitoring point for the screening of carcasses for BTB. Aerosol exposure to *M. bovis* is considered to be the most frequent route of infection of cattle, but infection may be occurred by contaminated material. The present study was undertaken for the purpose to investigate the infection of Bovine TB in abattoirs in Ri-Bhoi district of Meghalaya. In Meghalaya cattle are reared mostly for milk and livelihood. Predominantly local indigenous cattle constitutes more than 70% and others are jersey crossbred in Ri-Bhoi district.

Materials and Methods
Gross necropsy
All the carcasses were inspected for any gross visible lesion suspected of tuberculosis. Organs and tissue samples were collected from all the carcasses for further analysis. In this study, an animal was considered positive on necropsy if 1 or more lymph nodes or other tissues contained focal or multifocal abscesses or granulomas.

Pre-culture staining (PCS)
Ziehl-Neelson (ZN) staining for the detection of acid-fast bacteria (AFB) was performed on all tissue samples. A sample was considered positive for tuberculosis if there was evidence of granulomatous inflammation associated with focal necrosis or mineralization and/or if there was identification of AFB on the ZN stain.

Mycobacterial culture and species identification
Fresh and stored tissue samples were macerated and decontaminated using NALC and

inoculated on to Lowenstein Jensen (LJ) media. Briefly, approximately 1g of tissue exhibiting gross visible lesions was sliced and homogenized and then subjected for decontamination. The supernatant was discarded and the pellet formed re-suspended in 300µl of phosphate buffered saline (140mM NaCl, 26mM KCl, 10.0 mM Na2HPO4 and 1.7 mM KH2PO4). Then the re-suspended pellets were inoculated in duplicates onto LJ slants (one incorporating glycerol and the other pyruvate). LJ slants were incubated at 37 ºC and observed weekly for eight weeks. Using a sterile 0.1 µl plastic loop, the re-suspended pellets were spread and fixed at 80 ºC (for 10 min) onto a labelled slide. The slides were subjected for staining with modified ZN stain.

Results and Discussion

A total of 120 animals were pooled from slaughter house based on their debilitating health condition. Suspected visual lesions were observed in 20 carcasses, of which 16 were positive for mycobacteria in pre culture ZN stain and 18 positive in subsequent culture (Table 1). However, because a few of the animal each had more than one organ presenting lesions, samples of suspicious organs were obtained from 26 animals. In terms of organ involvement, the majority lesions were found in lymph nodes (48.71%) followed by lungs (28.20%) and liver (23.07%) (Table 2). Out of 19 lymph nodes, prescapular lymph nodes contribute more (Table 3). The high proportion of these lymph nodes with lesions suggestive of tuberculosis indicates that most routes of infection could be through aerosol infection or injuries or ulcer in the neck, shoulder, thorax or chest cavity (Atiadeve *et al*. 2014) [2]. Prevalence rate was found to be 15% in this area based on slaughter house surveillance.

The apparent animal prevalence with lesions suggestive of tuberculosis is comparable with Stefan *et al*. (2009) [6]. A distribution of lesions by organs shows that lymph nodes were the most infected followed by lung tissue and liver. Even though Bovine TB lesions are often found in the pulmonary region, other organs can equally be affected (Guitierrez *et al*. 1993) [4].

In 5 animals, although lesions are found but did not show any stain and culture growth, which may be due to parasitic infestation. The presence of visible lesions in any organs may not always be linked to mycobacterial infections as lesions may also cause by parasitic infestation or intracellular agents and this may lead to erroneous judgement by the meat inspector (Asseged *et al*. 2004) [1].

In the context of slaughter surveillance or carcass inspection, isolation of mycobacteria from bovine tissues can be difficult because there should first exist gross visible lesions. It also be noted that not all infected bovines may exhibit gross lesions.

Preparation of sample by manual maceration and homogenisation of tissue before decontamination may also affect the culture condition.

Due to economic loss most farmers sell infected/sick animals or send to slaughter house. These areas are considered as endemic for BTB which also reflected in this study. Microscopic examination of specimens taken from the abattoir were consistently scored highest for the presence of AFB implying a greater mycobacterial load, probably emanating from very sick animals (Atiadeve *et al.* 2014) [2]. This might be the reason of high incidence of tuberculosis in these areas. Transmission to neighbouring herd at market area and contamination through slaughter utensils are also recorded.

Proficient visual inspection may serve as good screening method for tuberculosis infected carcasses although pre culture stain and/or culture of bacteria may give brief idea about the infection.

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Table 1: Results of different screening methods for detection of mycobacteria infection in 120 carcasses.

	Total	Positive carcasses
Visual lesions (suspected)	120	26 (21.67%)
pre culture stain		16 (13.33%)
Culture		18 (15.0 %)

Table 2: Organs wise distribution of mycobacteria infection. (n = 39).

Organs	Suspected lesions	PCS + ve	culture +ve
Lung	11 (28.20%)	8 (20.51%)	8 (20.51%)
Lymph node	19 (48.71%)	16 (41.02%)	17 (43.59%)
Liver	9 (23.07%)	6 (15.38%)	6 (15.38%)
Total	39	30 (76.92%)	31(79.49%)

Table 3: Different lymph nodes involved in mycobacteria infection. (n = 19).

lymph nodes	lesion + ve	pcs + ve	culture + ve
Retro pharyngeal	6 (31.58%)	5 (26.31%)	6 (31.58%)
Messenteric	4 (21.05%)	2 (10.53%)	2 (10.53%)
Prescapular	9 (47.37%)	9 (47.37%)	9 (47.37%)
Total	19	16 (84.21%)	17 (89.47%)

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