



Original Research

Bovine Tuberculosis: Abattoir Prevalence, Species Identification and Its Economic Impact Assessment of Assam and Meghalaya

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Rec. Date:	Feb 09, 2018 09:50
Accept Date:	Mar 19, 2018 17:20
DOI	10.5455/ijlr.20180209095048

Abstract

To determine the prevalence of tuberculous lesions, species identification of the organism and associated direct economic losses due to the condemnation of organs/tissues of slaughtered cattle and buffaloes in Assam and Meghalaya, India. Over three years (September 2014 to October 2017), a total of 6053 carcasses (4985 cattle and 1068 buffalo) has been examined for tuberculous like lesions. Species identification was confirmed by biochemical test (viz. Nitrate reduction test, Pyrazinamidase test and Niacin detection test) and PCR targeting *oxyR* gene. Based on post-mortem examination the overall period prevalence was found to be 12.89%. Direct economic losses associated with the condemnation of 1742.94 Kg of meat/organs due to tuberculous lesions was valued as 339253.80 (\$ 5241.46). Our results indicate that bovine tuberculosis caused by *M. bovis* is prevalent in Assam and Meghalaya and accounts for high economic losses due to the condemnation of edible organs/meat only.

Key words: Bovine Tuberculosis, Economic Loss, Meat Condemnation, *Mycobacterium bovis*, North East India

How to cite: Barua, A., Raj, H., Goswami, C., Konwar, P., & Chutia, J. (2018). Bovine Tuberculosis- Abattoir Prevalence, Species Identification and Its Economic Impact Assessment of Assam and Meghalaya. International Journal of Livestock Research, 8(8), 204-213. doi: 10.5455/ijlr.20180209095048

Introduction

Bovine tuberculosis (BTB) caused by *Mycobacterium bovis*, is endemic in many developing countries with a negative impact on animal productivity as well as public health due to its zoonotic nature (Michel *et al.*, 2010; Torgerson and Torgerson, 2010). Bovine tuberculosis is a disease of zoonotic and economic significance worldwide especially in developing countries resulting in losses of human live, productive time on the part of farmers, and a huge sum of money from the condemnation of carcasses at slaughter (Ejeh *et al.*, 2014). Farmers, slaughterhouse workers, animal keepers at the zoo as well as veterinary





professionals are at high risk to the exposure of BTB infection through contact with infected livestock or their carcasses (Hambolu *et al.*, 2013; Elmonir and Ramadan, 2016). BTB has impact on economic loss due to the decrease in milk yield and from the increased condemnation of meat and edible organs (de la Rua-Domenech *et al.*, 2006).

Diagnosis of tuberculosis in cattle is mainly through tuberculin testing, culture and molecular genotyping (Brosch *et al.*, 2002; Warren *et al.*, 2006). Culture is the gold standard, however, in resource poor regions like North East (NE) India, monitoring of BTB by bacteriological study and IFN- γ assay are always not feasible because assays are costly and time consuming. Also, there is lack of organised slaughter house and routine post-mortem (PM) examination. However, meat inspection is an applicable tool for the detection of BTB in slaughtered animals in developing countries which provides useful insight into the prevalence of BTB and plays important role in both quality assurance and control (Youssef and Ahmed, 2014). There is also a paucity of information on the prevalence of bovine tuberculosis in NE India. In many slaughter houses in this region, the localized tuberculous lesions were trimmed and the remaining parts of the carcass were passed fit for human consumption and captive wild animal in zoo. Unfortunately, there is no official bovine slaughter house in Assam and Meghalaya. The objective of this study is to determine the prevalence of tuberculous lesions, species identification of the organism and associated direct economic losses due to the condemnation of organs/tissues of slaughtered cattle and buffaloes in Assam and Meghalaya in India.

Materials and Methods

Study Area

Different slaughter houses in Byrnihut, Killing, Umsning, Shillong, Jowai areas of Meghalaya and six mile, Hatigaon, Kharupetia, Nagaon, Morigaon, Borpetta, Dhuburi and Laluk areas of Assam were selected for the study (Fig. 1). More than 20000 animals were slaughtered annually in these unorganised abattoirs which served more than 5 million consumers and captive wildlife species. This study was carried out over 3 years from September 2014 to October 2017 and different abattoirs were visited several times by authors during this period for active survey.

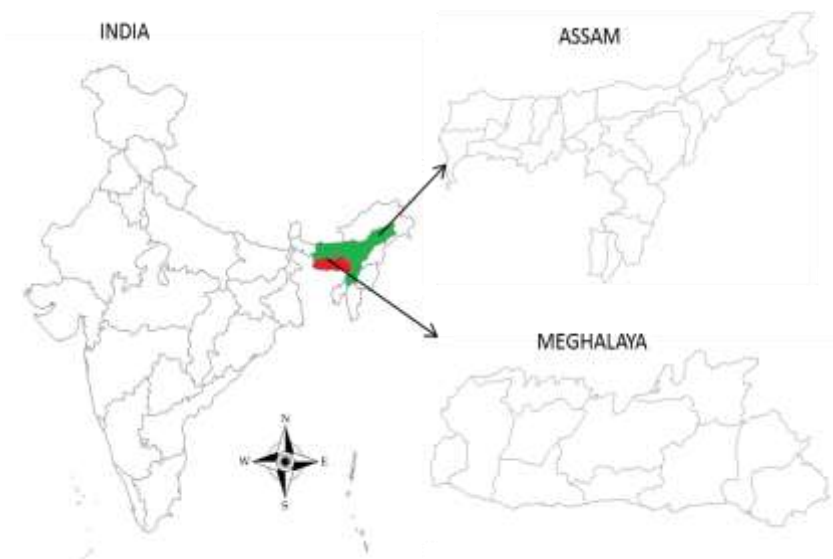


Fig. 1: Maps of Assam and Meghalaya, the two north eastern states of India. (Map is not to scale)

Sample Size Determination

The total number of carcasses for the study was calculated based on the formula given by Thrusfield (2007).

$$N = \frac{Z^2 P(1 - P)}{D^2}$$

Where N is the required sample size, Z is the Z -score at different confidence interval, P is the expected prevalence and D is the desired absolute precision.

A 10% expected prevalence with 99% confidence interval and 1% desired absolute precision was used to determine the sample size required for the study. Hence, substituting these values the required sample size was determined to be 5972 carcasses. But to increase the precision of our estimates, the total numbers of animals included in the present study were 6,053. The sample units were selected using simple random sampling method from those animals brought for slaughter on the day of PM examination.

Post-Mortem Inspection and Prevalence Estimation

All animal carcasses were inspected by qualified, well-trained veterinarians. Mainly necrotic calcified, proliferative granulomatous nodules, milliary nodules were selected as tuberculous like lesions (Fig. 2). Visual examination, palpation and knife incision were performed for PM examination. Lymph nodes and organs including lungs, liver, kidneys, spleens, intestines, uterus and mammary glands were examined for the presence of tuberculous like lesions. The suspected lesions were collected into a clean, sterile container

to avoid contamination and transported through ice box to the laboratory. Prevalence was estimated as per Elmonir *et al.* (2015):

$$\frac{\text{Total number of infected carcasses}}{\text{Total number of slaughtered animals}} \times 100$$



Fig. 2: Tuberculous lesions cattle carcass A) Lung; B) Liver

Culture and Species Specification

The lesion samples were further confirmed by Ziehl-Neelsen staining for detection of acid fast bacteria and subsequently, culture was done in Lowenstein-Jensen (LJ) media (one incorporating glycerol and the other pyruvate) (Fig. 3).

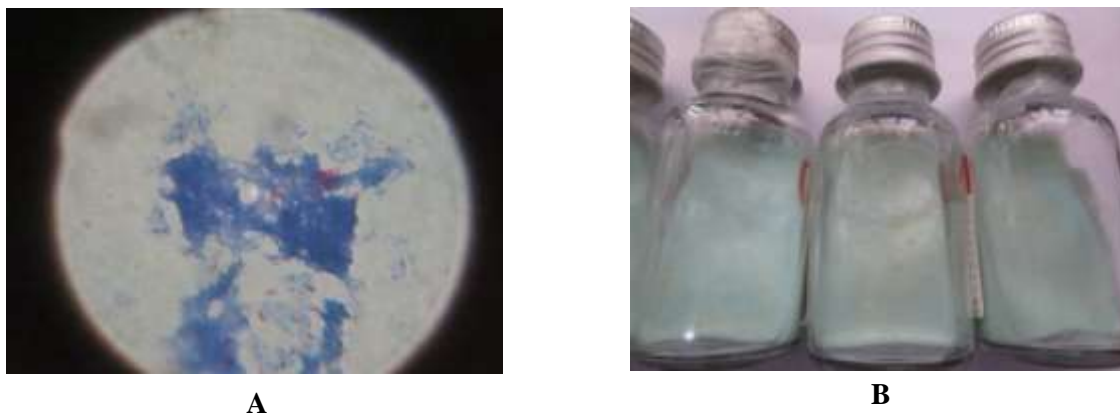


Fig. 3: a) Ziehl-Neelsen staining for detection of Acid fast bacteria from suspected organ samples collected from abattoir. b) Culture of *M. Bovis* in Lowenstein-Jensen media (supplemented with pyruvate).

Species specification was done according to Barua *et al.* (2017). Briefly, culture positive isolates were further subjected for the different biochemical test (*viz.* nitrate reduction test, pyrazinamidase test and niacin detection test) and PCR targeting *oxyR* gene. PCR was performed with forward primer oxyRMTB-12F (5-

TGGCCGGGCTTCGCGCGT-3) for both the species; however the reverse primers were different. In one reaction, reverse primer oxyRMT1R (5'-GCACGACGGTGGCCAGGCA-3) specific for *M. tuberculosis* while, in another reaction primer oxyRMB2R (5'-TGACGACGGTGGCCAGGTA-3) specific for *M. bovis* were used, which amplify a product size of 280 base pair (Barua *et al.*, 2017).

Economic Losses Estimation

Direct economic losses due to the condemnation of muscle or organs with tuberculous lesions were estimated based on total weight loss of meat in kilogram (kg) and average market price per kg. During the study period, butchers and slaughterhouse personnel were interviewed for data of the average weight and market price for edible (muscles, lungs, heart, liver, kidney and spleen) and non-edible (intestine, uterus) organs/tissues of cattle and buffaloes carcasses. The average meat yield for the whole carcass of cattle and buffaloes was 237 kg (178-296 kg). The average price for 1 kg of meat was Rs/-220.00. The average weight for lung (with heart attached) and liver was around 1.8 Kg and marketed price for liver was Rs. 220.00 and lung was Rs. 180.00 per Kg. The price of intestine was Rs. 100.00 per unit and that of uterus was Rs. 50.00. Indirect annual economic loss (IAEL) was estimated based on annual average slaughter in the abattoirs of these regions (AAS), average price of 1Kg condemned meat/organ (AP) and prevalence rate (P), *i.e.* $IAEL=AAS \times AP \times P$ (Kwaghe *et al.*, 2015).

Results and Discussion

In this study, the overall prevalence of tuberculous lesions (Fig. 2) among all the slaughtered animals in different abattoirs in these regions during the period from March 2014 to April 2017 was 12.89% (Table 1). The overall prevalence rate of BTB in slaughtered cattle and buffaloes was 13.09% and 11.89% respectively. The yearly prevalence rate was recorded as 12.41%, 13.35% and 12.58% during 2014-15, 2015-16 and 2016-17 respectively (Table 1).

A total of 1692 suspected organ and tissue samples were collected from 780 carcasses. In the year 2014-2015 out of 816 cattle carcasses were screened, where 106 carcasses were suspected for BTB based on PM examination. However, during Ziehl-Neelsen staining and culture by Lowenstein-Jensen (LJ) media 108 carcasses revealed positive TB lesions. It was found that lymph nodes and lungs contributed highest tuberculous lesions with a percentage of 39.01% and 29.49% respectively followed by liver (22.39%), muscle (7.62%), intestine (1.06%) and uterus (0.41%) (Table 1). All the culture isolates were found to be *M. bovis* based on biochemical tests and PCR assay (Fig. 3& Fig. 4).

Table 1: Year-wise distribution of screening of cattle and buffaloes carcasses for tuberculous lesions

Study period	Organs	Total carcasses screened		Animal suspected (Prevalence)		TB lesions	
		Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
2014-2015	Muscle	816	296	106 (12.99%)	32 (10.81%)	21	8
	Liver					73	26
	Lung					108	29
	Lymph nodes					96	51
	Intestine					3	2
	Uterus					2	2
Total (2014-15)						303	118
2015-2016	Muscle	2173	463	298 (13.71%)	54 (11.66%)	34	17
	Liver					86	53
	Lung					124	63
	Lymph nodes					169	96
	Intestine					4	4
	Uterus					2	1
Total (2015-2016)						419	234
2016-2017	Muscle	1996	309	249 (12.47%)	41 (13.27%)	38	11
	Liver					102	39
	Lung					110	65
	Lymph nodes					164	84
	Intestine					4	1
	Uterus					0	0
Total (2016-2017)						418	200
Total (2014-2017) species wise		4985	1068	653 (13.09%)	127 (11.89%)	1140	552
Overall (2014-2017)		6053		780 (12.89%)		1692	

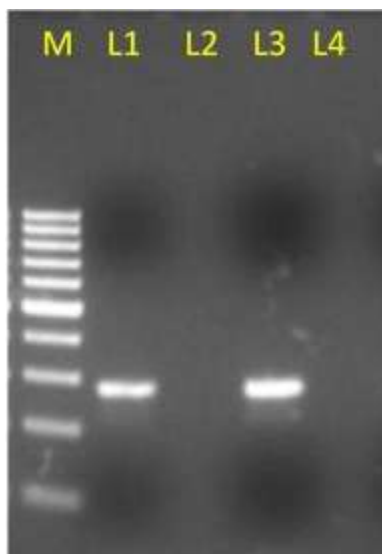


Fig. 4: Agarose gel electrophoresis image showing presence of *oxyR* (280bp) gene in *M. bovis* (L1 and L3) but absent in *M. tuberculosis* (L2 and L4). Lane M indicates 100bp

The overall weight losses of organs in Kg from all the slaughtered carcasses were 1195.08 and 547.86 in cattle and buffaloes respectively (Table 2). A total of Rs. 233313.60 (\$ 3604.69) in cattle and Rs. 105940.00 (\$ 1636.77) in buffaloes was lost due to condemnation of organs affected by tuberculous lesions Based on an oral interview with the slaughter house workers, it was estimated that annually an average of 29200 animals were slaughtered in these abattoirs. The indirect economic loss was calculated considering prevalence rate of 12.89% and the average price of Rs. 220.00 per Kg condemned meat/organ. Indirect annual economic loss (IAEL) was found to be Rs. 963600.00 (\$ 14887.60) due to the condemnation of meat.

Table 2: Economic loss due to the condemnation of various organs

Study period	Organs	Total weight (Kg)		Total Loss (₹)	
		Cattle	Buffalo	Cattle	Buffalo
2014-2015	Muscle	15.96	6.08	3511.2	1337.6
	Liver	131.4	46.8	28908	10296
	Lung	194.4	52.2	34992	9396
	Lymph nodes	0	0	0	0
	Intestine	9	6	900	600
	Uterus	3	3	150	150
Total (2014-15)		353.76	114.08	68461.2	21779.6
2015-2016	Muscle	25.84	12.92	5684.8	2842.4
	Liver	154.8	95.4	34056	20988
	Lung	223.2	113.4	40176	20412
	Lymph nodes	0	0	0	0
	Intestine	12	12	1200	1200
	Uterus	3	1.5	150	75
Total (2016-2017)		418.84	235.22	81266.8	45517.4
2016-2017	Muscle	28.88	8.36	6353.6	1839.2
	Liver	183.6	70.2	40392	15444
	Lung	198	117	35640	21060
	Lymph nodes	0	0	0	0
	Intestine	12	3	1200	300
	Uterus	0	0	0	0
Total (2016-2017)		422.48	198.56	83585.6	38643.2
Total (2014-2017)		1195.08	547.86	233313.6	105940
Species wise					
Overall (2014-2017)		1742.94		339253.6	

There are limited reports available from India and many epidemiological and public health aspects of BTB remain largely unknown (Baqir *et al.*, 2014; Neeraja *et al.*, 2014a, b; Verma *et al.*, 2014a,b). Thakur *et al.* (2010) recorded 14.31 to 34.42% prevalence rate of BTB in India. In another surveillance study in Assam and Meghalaya, Barua *et al.* (2017) recorded 16.58 to 19.59% prevalence of BTB based on gamma interferon (IFN- γ) assay and single intradermal comparative tuberculin test (SICTT). Based on slaughter house surveillance the prevalence rate of bovine TB in Ri-Bhoi district of Meghalaya was 15% (Barua *et al.*, 2016). The overall prevalence of tuberculous lesions among all slaughtered bovines in these abattoirs

was approximately 12.89%. The prevalence in this study was higher than Egypt (0.21-0.6%) (Ramadan *et al.*, 2012; Youssef and Ahmed, 2014) and African countries as 9% in Chad (Milian *et al.*, 2000), 6.1% in Ethiopia (Aylate *et al.*, 2013) and 1.9% in Nigeria (Ejeh *et al.*, 2014). However, lower prevalence (0.052%) was detected by Shitaye *et al.* (2006) in ten years' surveillance study in Ethiopia. From all the slaughtered carcasses, the predominant organs showing tuberculous lesions were the lungs and its associated lymph nodes. This finding was inconsistent with many studies (Shitaye *et al.*, 2006; Aylate *et al.*, 2013; Youssef and Ahmed, 2014; Barua *et al.*, 2016). Furthermore, it was recorded that the prevalence of pulmonary tuberculous lesions was higher in cattle than buffalo's carcasses, which agreed with findings of Youssef and Ahmed (2014). The variation of BTB prevalence in this study with the previously reported studies in other developing countries might be attributed to many factors that include animal husbandry measures (production system, ventilation, hygienic conditions, etc.), animal breeds, grazing habits, climatic changes and national regulations for BTB combating (such as, comparative tuberculin test and slaughter policy). In NE India, dairy cattle and buffaloes are usually raised by semi-intensive production where animals kept indoors in large numbers.

Majority of reports on economic impact due to TB were from African countries. Comparatively lower economic losses were recorded in Rwanda (\$ 4810) by Habarugira *et al.* (2014), in Nigeria (\$ 18200) by Ejeh *et al.* (2014) and in Ghana (\$ 18693.1) by Atawalna *et al.* (2016). In Nigeria, Kwaghe *et al.* (2015) recorded direct loss of \$ 24209.396 and indirect loss of \$ 1723691.6. The variation in economic losses might be due to the variation of BTB prevalence rate, the economic situation of the country as well as market price and demand for meat.

Conclusion

Based on these findings, it is highly recommended to strengthen surveillance and control measures against BTB in livestock of the study area. Strict measures and interventions should be taken towards preventing unauthorized slaughtering. PM examination measures and awareness programme regarding knowledge of occupational zoonoses and risks factors might be helpful to diminish the incidence of BTB.

Acknowledgements

Authors are thankful to Indian Council of Agricultural Research, New Delhi for funding the project "Outreach project on zoonotic diseases" and Director of Research (Veterinary), Assam Agricultural University for providing necessary facilities to carry out research work.

Conflict of Interest

The authors declare no conflict of interest.

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